ISSN 0035-7715
eISSN 2451-2311

ROCZNIKI PAŃSTWOWEGO ZAKŁADU HIGIENY

ANNALS OF THE NATIONAL INSTITUTE OF HYGIENE

Quarterly
2022
Volume 73
Number 3 - SEPTEMBER

EDITOR and PUBLISHER:
NATIONAL INSTITUTE OF PUBLIC HEALTH NIH
– NATIONAL RESEARCH INSTITUTE
Warsaw, Poland

ROCZNIKI PAŃSTWOWEGO ZAKŁADU HIGIENY
(ANNALS OF THE NATIONAL INSTITUTE OF HYGIENE)

Published since 1950

Quarterly, 4 issues in 1 volume per year (No 1 - March, No 2 - June, No 3 - September, No 4 - December)
The journal is devoted to research studies on food and water safety, nutrition, environmental hygiene, toxicology and health risk assessment, public health and other areas related to health sciences

Available at http://wydawnictwa.pzh.gov.pl/roczniki_pzh

Edited and published by the National Institute of Public Health NIH - National Research Institute, Warsaw, Poland

Indexed/abstracted in: PubMed/MEDLINE, Scopus, Clarivate Web of Science (all data bases), EMBASE, EBSCO, Agro, DOAJ, Food Science and Technology Abstracts, Global Health, NISC SA Databases, Index Copernicus Int., Polish Medical Bibliography/Central Medical Library, Polish Ministry of Education and Science (MEiN), CNKI Scholar

Full articles are freely accessible on the journal's website: http://wydawnictwa.pzh.gov.pl/roczniki_pzh/annals
The printed version of the journal is identical to the on-line version.

Editorial office:
National Institute of Public Health NIH - National Research Institute
24 Chocimska Street, 00-971 Warsaw, Poland
e-mail: roczniki.pzh@pzh.gov.pl
Editor-in-Chief phone: +48 22 54 21 266; fax +48 22 849 35 13, e-mail: cwludwicka@pzh.gov.pl

ISSN 0035-7715
eISSN 2451-2311

ROCZNIKI PAŃSTWOWEGO ZAKŁADU HIGIENY

ANNALS OF THE NATIONAL INSTITUTE OF HYGIENE

Quarterly
2022
Volume 73
Number 3 - SEPTEMBER

EDITOR and PUBLISHER:
NATIONAL INSTITUTE OF PUBLIC HEALTH NIH
– NATIONAL RESEARCH INSTITUTE
Warsaw, Poland

Printing house:
Agencja Reklamowa TOP
Chocimska 4, 87-800 Włocławek
tel.: + 48 54 427 09 70
http://www.agencjatop.pl

ROCZNIKI PAŃSTWOWEGO ZAKŁADU HIGIENY
[ANNALS OF THE NATIONAL INSTITUTE OF HYGIENE]

Volume 73 **2022** **Number 3**

Rocz Panstw Zakl Hig 2022;73(3):247-258
http://wydawnictwa.pzh.gov.pl/roczniki_pzh/ https://doi.org/10.32394/rpzh.2022.0218

REVIEW ARTICLE

THE IMPORTANCE OF NUTRITIONAL MANAGEMENT AND EDUCATION IN THE TREATMENT OF AUTISM

Anna Mandecka[1], *Bożena Regulska-Ilow*[2]

[1]Wroclaw University of Environmental and Life Sciences, Faculty of Biotechnology and Food Sciences, Department of Functional Food Products Development, Chełmońskiego 37, 51-630 Wroclaw, Poland
[2]Wroclaw Medical University, Faculty of Pharmacy, Department of Dietetics, Borowska 211, 50-556 Wrocław, Poland

ABSTRACT
Autism spectrum disorders (ASDs) are an early-onset neurodevelopmental disorders. The key symptoms of ASD include social deficits, verbal and non-verbal communication deficits, and restricted, repetitive patterns of behaviour, interests, or activities. Dietary patterns have been evidenced to be related to maternal nutritional status that might lead to different metabolic conditions, and maternal metabolic dysfunction has been observed to be associated with ASD. Furthermore growing evidence suggests that the gut microbiota has a role in the pathophysiology of ASD. Differences in composition of the gastrointestinal (GI) microbiota in children with ASD compared to unaffected siblings and/or healthy unrelated controls have been reported in various studies. The above-mentioned ASD factors and symptoms can be regulated by proper nutrition. The importance of nutrition and its possible impact on ASD patients is key to integral therapy. According to numerous research studies, various nutritional approaches succeeded in reducing the severity of patients' core ASD symptoms. The numerous options for diet that is used in the ASD therapy, as described in the scientific literature, are related to the problem of choosing an appropriate nutritional treatment. Each nutrition programme needs to be personalised and tailored to an individual patient. The aim of the paper is to review the available literature on dietary interventions in children with ASD and provide up-to-date evidence.

Key words: *autism spectrum disorder, gut microbiota, nutritional models, special diet, nutritional education*

STRESZCZENIE
Zaburzenia ze spektrum autyzmu (ASD) są zaburzeniami neurorozwojowymi o wczesnym początku. Kluczowe objawy ASD obejmują deficyty społeczne, deficyty komunikacji werbalnej i niewerbalnej oraz ograniczone, powtarzalne wzorce zachowań, zainteresowań lub czynności. Udowodniono, że wzorce żywieniowe są powiązane ze stanem odżywienia matki, co może prowadzić do różnych stanów metabolicznych, a zaburzenia metaboliczne u matki są powiązane z ASD. Coraz więcej dowodów sugeruje, że mikroflora jelitowa odgrywa rolę w patofizjologii ASD. W różnych badaniach donoszono o różnicach w składzie mikroflory przewodu pokarmowego (GI) u dzieci z ASD w porównaniu ze zdrowym rodzeństwem i/lub zdrowymi, niespokrewnionymi grupami kontrolnymi. Czynniki i objawy ASD można regulować poprzez odpowiednie odżywianie. Znaczenie żywienia i jego możliwy wpływ na pacjentów z ASD jest kluczem do integralnej terapii. Zgodnie z licznymi badaniami naukowymi, różne podejścia żywieniowe odniosły sukces w zmniejszaniu nasilenia podstawowych objawów ASD u pacjentów. Strategie postępowania dietetycznego stosowane w terapii ASD, opisane w literaturze naukowej, wiążą się z problemem wyboru odpowiedniego leczenia żywieniowego. Każdy program żywieniowy musi być spersonalizowany i dostosowany do indywidualnego pacjenta.
Celem pracy jest przegląd dostępnej literatury na temat interwencji dietetycznych u dzieci z ASD i dostarczenie aktualnych danych naukowych.

Słowa kluczowe: *autyzm, mikrobiota jelitowa, modele dietetyczne, dieta, edukacja żywieniowa*

Corresponding author: Anna Mandecka, Wroclaw University of Environmental and Life Sciences, Faculty of Biotechnology and Food Sciences, Department of Functional Food Products Development, Chełmońskiego Str. 37, 51-630 Wroclaw, Poland. Tel.: +48 667421235; e-mail: anna.mandecka@upwr.edu.pl

INTRODUCTION

Autism spectrum disorders (ASDs) are a set of severe developmental disorders manifesting up to 30th month of life and they are associated with congenital anomalies of the nervous system. This disorder is highly variable and multiform. Difficulties in verbal and nonverbal communication and in understanding social phenomena are a common feature of ASDs [1].

In the last decade, the number of patients diagnosed with ASD doubled, rising from 6.7 to 14.7 per 1,000 children aged 8 years. It is currently estimated that approximately 1% of the world population has ASD, with a male-to-female ratio of 2.5:1 [2]. Other authors indicate that one in 88 children may exhibit developmental disorders that are characteristic of ASD, and rates describing the prevalence of the condition may be much higher, especially among males. The prevalence rate of autism among men and women can be as high as approx. 5:1 [3].

Given the epidemiologic data indicating a higher incidence of ASDs, specialists treating ASD patients attempt to understand the aetiology of the disease and they consider various factors that may affect its pathogenesis. A particular attention is drawn to the role of epigenetic, neurobiological, genetic, neurological and hormonal factors underlying this complex condition. It is emphasised that interactions between genetic and environmental factors contribute to ASDs, where genetic factors are only 10-20% of ASD cases [1, 2, 3]. According to recent epidemiological data, up to 40–50% of ASD-related symptoms may be caused by environmental factors [4].

Due to such a significant role of environmental factors in the development of ASDs, more and more specialists treating ASD patients are paying attention to the role of diet and nutrition in the treatment of ASDs, searching for the optimal model of nutritional treatment [1].

Specific selected dietary nutrients (sulphur-containing amino acids such as cysteine and methionine, folic acid, vitamins B_{12} and B_6) are of critical importance for the nutrition of ASD patients, already at the cellular level [5]. Deficiencies in antioxidants and methylation metabolites in the ASD population, which provide key epigenetic regulation of gene expression, were documented during neurodevelopment [6]. Concentrations of glutathione and S-adenosyl methionine, which are major intracellular antioxidants and methyl donors for many metabolic reactions in the body, are significantly lower in ASD patients and this is related to oxidative stress [7].

Therefore, nutrition interventions receive extraordinary attention as a complementary and unavoidable component of autism therapy, however, they are frequently omitted and treated without due consideration. Figure 1 shows an optimal procedure model in which important components of a health

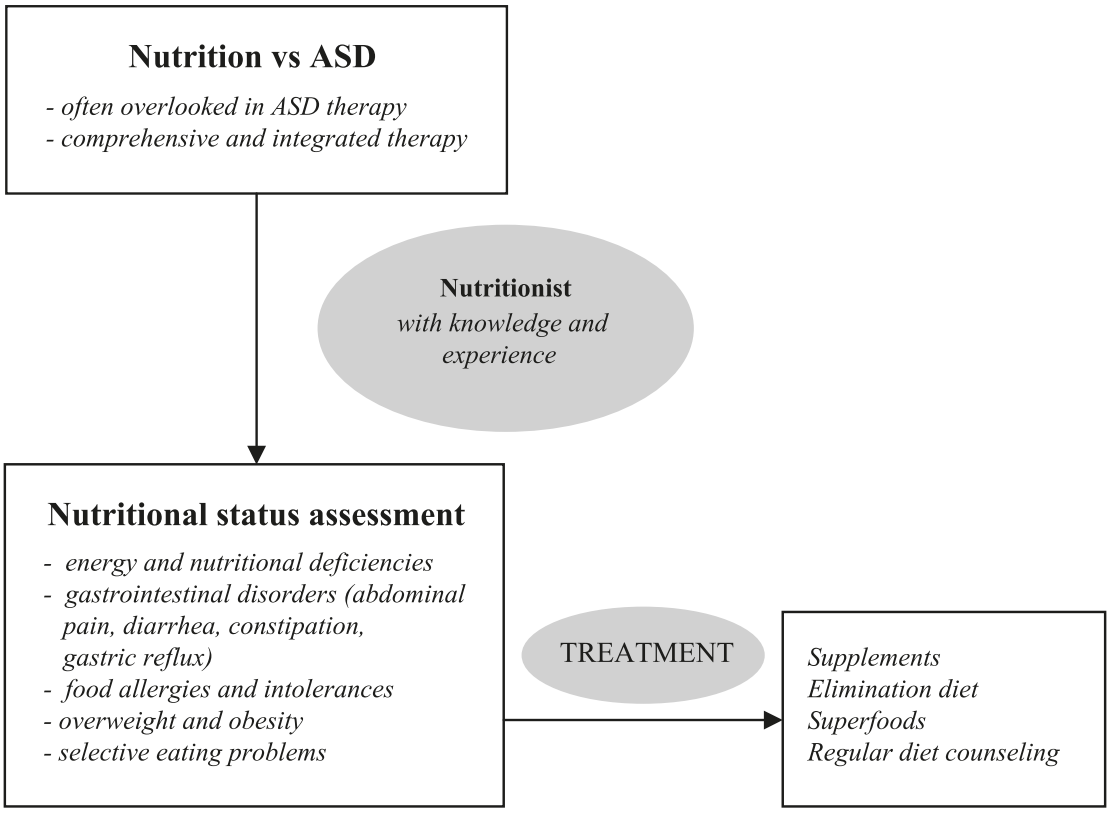

Figure 1. Integration nutrition ASD care, and the possible model of treatment [5, 8]

assessment cannot be omitted when implementing a diet therapy in ASD patients [5, 8].

The prenatal nutritional programming and prevalence of ASDs

A women's nutrition during pregnancy is crucial for fetal brain development. A pre-pregnancy diet is key to optimise the nutritional status, which plays a significant role in maintaining a healthy pregnancy and supporting a developing fetus. Nutrition at the time of conception is key to gamete function and placental development [9].

Approximately 2-3 weeks after fertilisation, the embryo undergoes organised processes of neuronal proliferation and migration, synapse formation, myelination and apoptosis for fetal brain development. During this period of rapid development, the brain exhibits increased sensitivity to the environment; its dysfunctions may predispose the fetus to postnatal neurodevelopmental disorders [10)] Provision of nutrients during the pre-conceptional and prenatal periods not only provides essential building blocks for the brain, but they may also "program" the brain through epigenetic mechanisms to confer a risk or resistance to neurological states later in life [9, 10]. The modifiable nature of nutrition during sensitive periods potentially provides opportunities for intervention. The quality of prenatal nutrition was identified as a potential risk factor for the development of ASDs.

According to the gut-brain axis (GBA) theory, early-life nutritional programming – covering pregnancy – may affect cognitive functions and predispose to the development of ASDs in genetically susceptible individuals [6]. Several studies attempted to clarify the role of nutrition in the development of ASDs. It was reported that maternal obesity might lead to fetal encephalitis [7]. The studies also stress the role of nutritional composition and dietary balance of mainly polyunsaturated fatty acids (PUFAs), whose deficiencies during pregnancy are associated with reduced learning and memory abilities and reduced cognitive functions in the offspring if PUFA dietary content has not been adjusted at an early stage of the fetal development [5].

PUFAs are structural components of cells, especially arachidonic acid (AA) of the omega-6 (n-6) family and docosahexaenoic acid (DHA) of the omega-3 (n-3) family. PUFAs rapidly accumulate in the brain from the third trimester of pregnancy until early postnatal life, and their composition in individual membranes depends mainly on the supply of DHA in the maternal diet [9, 11]. According to the Nurses' Health Study II (NHSII) [12], the total dietary n-6 PUFA content and supplementation among women prior to pregnancy was significantly and inversely associated with the risk of ASD diagnosis. Both n-3 and n-6 PUFAs are fatty acids that are essential for prenatal brain development, however, the elevated ratio of n-6 PUFAs to n-3 PUFAs, which is characteristic of the modern Western diet, may increase inflammatory processes [13]. The main dietary sources of n-3 PUFAs, especially DHA, are oily sea fish (salmon, halibut, herring, mackerel, tuna). According to the researchers there is a significant effect of adequate fish consumption by a mother on the development of ASD in her offspring. The Spanish INMA study [14] found that higher consumption of fish and seafood during the first trimester of pregnancy was associated with lower autistic traits, as measured by the Childhood Asperger Syndrome Test.

Fish and seafood are recommended dietary components; they provide complete proteins, vitamins, mineral salts and valuable omega-3 fatty acids, however, they can also be a source of dietary methylmercury. According to the authors of some studies there may be a risk of excessive mercury accumulation in ASD patients as one of the causes of ASD development [15]. Methylmercury easily crosses the blood-brain and blood-placental barriers. It also passes into breast milk, contributing to the exposure of the infant, whose body may accumulate mercury in blood cells and brain. This causes damage to the central nervous system (CNS) [16].

In many European Union countries, especially those with high consumption of fish and seafood, dietary recommendations have been implemented to limit the consumption of certain species of fish, mainly by women planning their pregnancy, pregnant women, breastfeeding mothers and children. These countries include France, Denmark, Finland, Spain, Ireland, Italy and the United Kingdom. In Poland, the consumption of fish, especially seafood, is relatively low compared to other EU countries. The European Commission has developed a special note concerning this issue, which is addressed to the most vulnerable groups of consumers, to limit the consumption of predatory fish such as a swordfish, shark, marlin and pike by pregnant women, women planning their pregnancy, breastfeeding mothers and children to one serving (approx. 100 g) per week. It is also recommended to limit tuna consumption to no more than two servings per week [17].

The U.S. Environmental Protection Agency (EPA) and the U.S. Food and Drug Administration (FDA) have recommended to limit the fish consumption by pregnant women, breastfeeding mothers and women planning their pregnancy to a serving size of 340 g per week, including no more than 170 g of tuna per week, and to completely avoid the consumption of predatory fish, i.e. a shark, swordfish, king mackerel and tilefish [17].

Although there are recommended restrictions on the consumption of selected fish species, specialists

believe that benefits for the offspring that are associated with the consumption of fish rich in omega-3 acids by women of childbearing age are more important than risks associated with their lack in the diet [15, 17]. According to *Vecchione* et al. study [15] there was no significant association between consumption of fish in the prenatal period and traits related to ASDs in children. *Vecchione* et al. noted, however, that traits associated with ASD, as measured by the Social Responsiveness Scale (SRS), were more frequently observed in children whose mothers had higher consumption of fish in the second half of pregnancy.

Moreover, a pregnant woman should pay special attention to the dietary content of folic acid and vitamin D; in case of their deficiency it should be consider to supplementation in appropriate doses. Folic acid is an essential cofactor of one-carbon metabolism that is involved in DNA and RNA synthesis and DNA methylation – processes that are particularly significant during periods of rapid growth and development. Insufficient folic acid intake is associated with altered DNA methylation and impaired fetal brain development. Supplementation with folic acid, a synthetic form of folate, before pregnancy and in early pregnancy was found to be effective in preventing neural tube defects [9, 18]. According to several studies, taking folic acid and a set of vitamins during first few months of pregnancy was associated with a lower risk of ASD. *Schmidt* et al. [19] observed that folic acid supplementation of ≥ 600 mcg/d during first months of pregnancy was associated with a reduced risk of ASD (RR=0.38; 95% Cl: 0.16; 0.90). Similar findings were obtained by other authors [20, 21].

Vitamin D deficiency is widespread worldwide, including pregnant women who are a particularly vulnerable group. It is speculated that vitamin D deficiency in a woman's body during pregnancy may be involved in the development of autism. Furthermore, there is a hypothesis that vitamin D supplementation during pregnancy and early childhood will reduce the incidence of autism recurrence in new-born's [22].

The role of the gut microbiota in ASD

Diet is one of the most influential environmental factors in determining the composition of the gastrointestinal (GI) microbiota. Intestinal dysbiosis, manifested by the appearance of intestinal disorders (intestinal gases, bloating, diarrhoea, reflux symptoms), and the influence of certain bacterial taxa on ASD symptoms are very common in ASD patients. In recent years, the GI microbiota has been identified as a potential pathway affecting symptom manifestation in cognitive and neurodevelopmental disorders such as anxiety, depression and ASD. Abnormalities in the GI microbiota of ASD children and associations between specific types of microorganisms and some ASD symptoms were observed [23, 24]. The gut microbiota of ASD patients differs in terms of composition and diversity from that of healthy developing individuals. The most diverse gut microbiome was in patients with co-occurring GI disorders, compared to the ASD group without such co-occurring disorders and healthy controls [25, 26].

The studies focusing on the evaluation of intestinal states in ASD children reported various degrees of chronic inflammatory bowel disease (IBD) with purulent secretion as well as fecal mass impaction. Moreover, there were enlarged lymph nodes that substantially occluded the intestinal lumen. The clinical image resembled inflammation in the course of *Crohn's* disease or it was atypical. The aforementioned inflammation was described as inflammation associated with ASD. Abnormalities in the intestinal microflora – dysbiosis – were identified as the cause of the development of that inflammation [27, 28].

Frequent intestinal dysfunctions and co-morbidities with microbial dysbiosis cause GI complaints that occur in up to 90% of ASD cases and thus they may play a key role in the pathogenesis of this disorder [5]. Moreover, children with ASD and GI symptoms also have immune imbalances in the gut, which may be related to an abnormal host response to microbial dysbiosis and impaired intestinal barrier integrity [29]. ASD children have a high prevalence rate of atopic diseases, including food allergy. Therefore, interventions to treat gut dysbiosis may not only help to reduce the incidence and severity of GI symptoms in ASD children, but they may also help to balance the immune system function and potentially improve some behavioural symptoms [30].

According to *Berding* et al. study [23], stool samples, food diaries from 3 days and the "Youth and Adolescent Food Frequency Questionnaire" (YAQ) were taken from ASD children. The microbiota composition of the patients was examined in relation to eating behaviours, nutrient and food group intake, as well as dietary patterns (DPs) obtained from YAQ. In ASD children, two different DPs were associated with unique microbial profiles. DP1, with a higher intake of vegetables, legumes, nuts and seeds, fruit, refined carbohydrates and starchy vegetables but a lower intake of sweets, was associated with lower abundance of *Enterobacteriaceae, Lactococcus, Roseburia, Leuconostoc and Ruminococcus.* DP2, with a low intake of vegetables, legumes, nuts, seeds and starchy vegetables, was associated with higher levels of *Barnesiellaceae, Alistipes* and lower levels of *Streptophyta,* as well as higher concentrations of propionate, isobutyrate, valerate and isovalerate. Higher concentrations of isobutyrate and isovalerate may suggest microbial metabolic changes and

increased energy food extraction from the microbiota in ASD children

In contrast, the presence of *Peptostreptococcaceae* and *Faecalibacterium* affected the development of social skills deficits in ASD children. Diet-related microbial profiles were associated with GI symptoms, however, there was no significant interaction between nutrition and microbiota in the assessment of outcomes concerning the social skills deficit. In summary, DPs associated with fecal microbiota composition and volatile chain fatty acids (VFA) concentrations were identified in ASD children [23, 31].

Creating conditions for the development of habitual, long-term (>6 months) and favorable DPs significantly improves the microbiological profile of the gut and its microbiological stability in children aged 4-8 years [23]. Adverse diet-induced changes in microbiota composition can lead to an increased risk of developing certain diseases (e.g. IBD), while a healthier long-term feeding scheme may be more beneficial for promoting a microbial profile that could potentially protect against diseases [32]. A lower ratio of *Bacteroidetes* to *Firmicutes* and higher abundance of *Clostridium* and *Desulfovibrio,* in particular, are associated with increased ASD symptoms in children [26]. There is currently growing evidence of the relationship between particular bacteria and ASD symptoms [23, 27].

The therapeutic effect of fecal microbiota transplant (FMT)

The gut microbiome is a significant environmental factor that may influence ASD symptoms, and several studies found that ASD children had distinctive gut microbiomes compared to neurotypical children [33, 34].

Modification of the gut microbiome is a potential route to improve gastrointestinal and behavioural symptoms in ASD children, and microbiota transfer therapy (MTT) may convert a dysbiotic gut microbiome into a healthy one by providing a large number of commensal microorganisms from a healthy donor. Given the connection between the gut and brain (gut-brain axis), modulating the gut microbiome using antibiotics, probiotics, prebiotics and/or FMT may be a viable therapeutic option [35].

A large diversity and number of commensal microorganisms from a healthy donor are used in FMT for converting the dysbiotic gut microbiome into a healthy microbiome. In fact, FMT is the most effective therapy for treating recurrent infections such as *Clostridium difficile* [36].

K*ang* et al. [30] conducted a pioneering trial of FMT. The MTT plan for ASD children with chronic GI problems consisted of a fortnight's treatment with vancomycin followed by intestinal cleansing, then a high-dose FMT for 1-2 days and 7-8 weeks of daily maintenance doses along with a gastric acid suppressant. After a 10-week MTT and an 8-week follow-up period (18 weeks in total), the researchers observed an 80% reduction in GI symptoms and a slow but steady improvement in baseline ASD symptoms. At the same time, the microbial diversity of the gut, including the number of potentially beneficial microbes, increased significantly after MTT.

Two years after the original clinical trial, the authors of the same study re-evaluated the participants' health status to determine whether there was continuation of observed improvements in behaviour and GI symptoms and to determine the long-term effects of MTT on the gut microbiome. The obtained results are promising because after 2 years of repeated follow-ups it was found that the improvements persisted and the composition of the beneficial gut microbiota maintained and remained altered. There was a significant reduction in GI symptoms in the patients under study, and the median of relative abundance of *Bifidobacteria* and *Prevotella* compared to baseline values increased 4-fold and 712-fold, respectively, at 10th week of the study and 5-fold and 84-fold, respectively, after two years of the study [35].

A particular attention should be drawn to an increase in abundance of *Prevotella* after MTT as its lower abundance in the faeces of ASD children, compared to neurotypical children, was confirmed in other studies. The authors of another study also showed reduced amounts of *Prevotella* in the oral microbiome of ASD children [37, 38].

Although human studies concerning the impact of the microbiome on the development of ASD are extensive, cause-and-effect relationships have not been clearly defined and it has not been determined whether changes in the gut microbiome are a consequence of ASD or they contribute to ASD symptoms. *Sharon* et al. [39] investigated whether altered human microflora might promote ASD-like behaviours in mice. Faecal samples from human donors were selected based on the Autism Diagnostic Observation Schedule (ADOS) and the GI severity index (GSI) and they were transplanted into mice. The researchers found that animals that received microbiota transplants from ASD patients developed repetitive behaviours and less interest in social behaviours. According to the transplanted material (ASD *vs.* healthy), the animals had microbiome of different composition and diversity. It was concluded that the gut microbiota regulates behaviour of mice by producing neuroactive metabolites, suggesting that gut-brain connections contribute to the pathophysiology of ASD. Factors such as altered host genetics and perinatal events, combined with altered microbiota, may together influence the aetiology of ASD by combining risks that increase the severity of symptoms.

Dietary models in ASD

The importance of nutrition and its possible impact on ASD patients is key to integral therapy. According to numerous research studies, various nutritional approaches succeeded in reducing the severity of patients' core ASD symptoms [5, 40, 41]. However, the information to date concerning the relationship between different dietary models and ASD symptoms is frequently complex, while at the same time it does not provide any specific information regarding how to initiate the nutritional therapy. Specialists attempt to explain the relationship between ASD and nutrition through various mechanisms, but the ideal diet still does not exist. However, there is lack of a practical feeding algorithm for ASD children and the presentation of some dietary models that could be implemented in therapy, immediately after an ASD diagnosis.

One of the early dietary intervention in the first stage of implementing nutritional changes may be the introduction of the principles of the Mediterranean diet (MD) in the daily diet of ASD patient. Although there are no significant studies on the relationship between ASD and the MD but it has been reported to be beneficial against the cardiovascular system, metabolism and mental diseases. MD includes fruits and vegetables, legumes, nuts, cereals, olive oil and fish. Moreover, low intake of saturated fat, red meat and sugar is also involved in MD [42]. In the study conducted by *House* et al. [43], the behaviour of the offspring of 325 pregnant women was examined concerning the mother's degree of the rate of Adherence to the Mediterranean Diet (AMD). The offspring of mothers with high AMD were found to be less likely to have depression than the offspring of other mothers. It was observed that the offspring of mothers with the lowest degree of AMD had more ASD behaviours than the offspring of other mothers with higher levels of adherence. Furthermore, the mother's high AMD has been associated with decreased methylation of the IGF2 and SGCE/PEG10 locus and increased methylation of MEG3 locus, which result in reduced adverse behaviour, and increased social relations criteria, respectively.

The numerous options for diet that is used in the ASD therapy, as described in the scientific literature, are related to the problem of choosing an appropriate nutritional treatment. Each nutrition programme needs to be personalised and tailored to an individual patient. Specialists mostly focus on several dietary models, i.e.: gluten-free, casein- free and sugar -free diets (GFCFSF), ketogenic diet (KD),special carbohydrate diet (SCD), FOODMAP diet. When choosing a specific dietary model, the following factors should be included: nutritional status of the patient, type of GI complaints, recommended supplementation, food selectivity, type of food allergies and intolerances, severity of the disease.

GFCFSF diet is the most commonly selected diet that support the ASD therapy. It is normocaloric diet, individually balanced in terms of the content of macronutrients, vitamins and minerals. From the diet should be excluded: products containing gluten, casein, sugar and highly processed products with added sugar. Hypothetical mechanisms of action of the GFCF diet in ASD are: excess opioid activity, increased autoimmunity, oxidative stress and inflammation, reactivity of antibodies to gluten products and gut microbiota perturbations [44]. GFCFSF diets are used largely due to opioid peptides released by the digestion of both gluten and casein. High concentrations of opioid peptides lower the methylation index, leading to altered patterns of DNA methylation and gene expression. Opioid peptides, derived from gluten and casein, reduce cysteine uptake by cells; because cysteine reduces the rate of glutathione synthesis (GSH), their activity results in reduced GSH levels. Moreover, lower levels of GSH in the GI tract may promote inflammation and contribute to symptoms of GI discomfort and dysfunction [5]. Although there is a belief that the GFCF diet is completely harmless, there is no clear conclusion that it has no risk at all, especially the nutrient deficiencies that may arise are of great concern. Inadequate consumption of dairy products and GFCF diets have been found to be associated with high levels of homocysteine [45, 46]. The meta-analysis, conducted by *Quan* et al. [47], showed that a GFCF diet can reduce stereotypical behaviors and improve the cognition of children with ASD. Though most of the included studies were single-blind, the benefits of a GFCF diet that have been indicated are promising.

The excessive supply of sugar-containing carbohydrate products increases intestinal inflammation and contributes to the development of adverse intestinal microflora and growth of pathological fungi. *Tarnowska* et al. study [48] aims to identify factors influencing the purchasing decisions of families with ASD children on gluten-free and/or casein-free and/or sugar-free diets and the difficulties associated with such diets. The study included a group of 40 families with ASD children (32 boys and 8 girls) aged between 3–10 years. It was found that the factors that had the greatest influence on parents' decisions concerning the purchase the products included the composition of products, the presence of a certificate confirming the absence of gluten and/or milk, and taste qualities. Furthermore, following the elimination diet was a significant obstacle when traveling or socialising, causing conflicts with family and community. In addition, the limited range of healthy gluten-free, casein-free, and sugar-free foods, poor quality of taste and unsatisfactory quality of food made it difficult to purchase and prepare varied meals.

The ketogenic diet (KD) is another type of diet that was proposed as a nutrition intervention to alleviate symptoms in ASD patients. This is a high-fat, protein-sufficient and low-carbohydrate diet, resulting in the body using fatty acid metabolites as a primary source of energy [5]. Specialists should remember to individually balanced that diet in terms of the content of macronutrients, vitamins and minerals and also constantly monitoring the concentration of ketones in the patient's body. The classic model of the ketogenic diet assumes that for 4 grams of fat there is 1 gram of protein and carbohydrates in total (distribution of the daily supply of macronutrients: fats - 90%; protein - 8%; carbohydrates - 2%). The modified Atkins diet is less restrictive than the classic ketogenic diet model, and the proportions of macronutrients are arranged as follows: fats - 60%; protein - 10%; carbohydrates - 30%. The MCT (medium chain triglycerides) diet is a variant of the ketogenic diet based on medium-chain MCT fatty acids, and the distribution of macronutrients is as follows: fats - 73% (including 30-60% MCT fatty acids); protein - 10%; carbohydrates - 17% [49, 50, 51].

The researchers of animal model studies proved that KD might be an effective intervention in alleviating ASD symptoms [52, 53]. Furthermore, larger cohort studies found that the use of KD results in noticeable improvements in: learning, social behaviour, speech, cooperation and reduction of hyperactivity in ASD patients [5, 40, 54].

Lee et al. [40] investigated the effect of a 3-month ketogenic, gluten-free diet (GF) combined with MCTs in ASD children aged between 2–17 years. The main dietary restrictions were based on: - limiting the supply of carbohydrates to 20-25 g/day, - protein requirements were determined individually, taking into account age and body weight (RDA standard), - contents of MCT was set up to 20% of energy from the daily food ration (dietary source: coconut oil or pure MCT oil), - exclusion of all gluten-containing products. After the study had ended, patients showed improved behaviour and aggression control, as well as improved social behaviours. This type of dietary intervention was identified as a potentially beneficial therapeutic option regarding ASD to improve underlying autistic disorders.

Following the SCD diet protocol can also have a positive effect on alleviating ASD symptoms. The goal of this diet is to alleviate symptoms of carbohydrate malabsorption and reduce the proliferation of pathogenic intestinal microflora resulting in dysbiosis. In SCD, it is aimed to prevent malabsorption of foods that are more difficult to digest, prevent the formation of undigested residual nutrients and, as a result, prevent the growth of pathogenic bacteria in the intestine [55]. The main principle of the SCD diet is to limit the intake of complex carbohydrates, di-, oligo- and polysaccharides, including starch. The diet should include sources of simple carbohydrates, i.e. glucose, fructose, galactose. Furthermore, an important element of the SCD diet are also milk, fermented, lactose-free probiotic products (natural yoghurts) [56].

Abele et al. [57] conducted the study to investigate the potential of a specific carbohydrate diet and selected dietary supplements in reducing some autistic spectrum disorder (ASD) symptoms in children. This was a quantitative, non-blinded, non-randomized three-month pilot study of a dietary and nutritional intervention. The intervention group received a specific carbohydrate dietary plan - Specific Carbohydrate Diet/ Gut and Psychology Syndrome diet (SCD / GAPS) – and a few dietary supplements (omega-3 essential fatty acids, ascorbyl-palmitate, probiotics, vitamin D, and vitamin C). The analyzed diet (SCD/GAPS) enriched with some specific supplements were found to be a safe and effective approach for reducing some symptoms of ASD in children.

Studies confirm that the GI symptoms in ASD children are related to behavioural problems. FODMAP-restricted diets are used for alleviating irritable bowel syndrome (IBS) symptoms such as abdominal pain, bloating, constipation and diarrhoea. The diet should be followed by a 6-8 week to relieve inflammation in the gut, then expanding the diet to include foods with increasingly higher amounts of FODMAPs [41, 58]. *Nogay* et al. pilot study [41] aims to evaluate the effect of the diet of low fermentable oligosaccharides, disaccharides, monosaccharides and polyols (FODMAPs) on gastrointestinal and behavioural problems in ASD children. The researchers found that the study group following the FODMAP diet experienced significant relief in terms of some GI problems compared to baseline in this group and the control group. Fermentable oligo-di-monosaccharides and polyols (FODMAPs) form a heterogeneous group of poorly absorbed short-chain carbohydrates that are later fermented in the small or large intestine. FODMAPs include fructose (e.g. fruit and high-fructose corn syrup), lactose (e.g. milk and dairy products), fructans (e.g. cereals, vegetables and fruit), galactans (e.g. legumes and vegetables) and sugar alcohols (polyols such as sorbitol, fruit and vegetables) [58].

Other types of diets or product eliminations are selected based on specialised tests recommended by a doctor and dietician. This may include such diets as a Feingold, anti-inflammatory, oxalate-free, low-histamine or purine-free diet, according to the child's status of the organism, for example, problems with fat digestion or elevated serum ammonia levels. When following a specific diet, highly processed foods that contain food additives and preservatives should not be consumed. Food needs to be as natural as possible,

preferably cooked at home using simple products. The diet should be light and varied, so as to relieve the affected intestine while providing the body with essential nutrients [59].

Moreover, the elimination-rotation diet, which consists of excluding products that cause (delayed) IgG food allergies, may improve behaviour of ASD patients. Therefore, a food allergy test needs to be performed to indicate harmful food products. Self-monitoring can be difficult because the body's reaction may occur as long as 3 days after eating a particular food product. Moreover, symptoms typical of an IgE allergy, such as rash, are not present in IgG food allergies. However, such symptoms as abdominal pain, bloating, intestinal gases and headache may indicate IgG food allergies. In the first stage of the diet, harmful food products should be eliminated based on the test results, then they should be reimplemented into the diet after a set period of time, in rotation (as a rule, every 4th day). The elimination-rotation diet needs to be followed under the supervision of a dietitian to ensure the provision of all nutrients for the child, which are necessary for their growth and development [3].

Food selectivity in ASD *vs.* nutrition education

ASD patients may exhibit higher food selectivity (i.e. eating a narrow range of food products and/or rejecting one or more food groups), which limits the intake of certain foods and it may lead to nutritional deficiencies. Mild to severe food selectivity affects up to 95% ASD children [60]. Children with mild food selectivity may not require treatment. However, children with moderate to severe food selectivity will require intervention to promote dietary diversity and reduce the risk of nutritional deficiencies, bone density loss and constipation [60, 61].

Food selectivity also increases the challenges of parenting the ASD child. Parental attempts to introduce new, previously unfamiliar food products or dishes into a child's diet may result in crying, aggression, self-injury, throwing objects, spitting and pushing food away as a result of self-defence and protest [62]. Such destructive behaviours may also occur at the mere sight or smell of unfamiliar and unwanted foods. Parents may also be concerned that the persistent introduction of new foods and dishes into their child's diet may lead to further dietary restrictions and worsening of nutritional deficiencies [63].

In ASD patients, achieving adequate food intake is a challenge, and some nutrient deficiencies were identified. The authors of a meta-analysis, which determines the differences in terms of nutrition between ASD children and a group of typically developing children, found that participants with ASD consumed less protein, phosphorus, selenium, vitamin D and calcium, thiamine, riboflavin and vitamin B12. Moreover, ASD children consumed less omega-3 PUFAs and more fruit and vegetables, compared to controls [64].

Combined behavioural and medical interventions, in highly organised inpatient units or day treatment programmes, are well supported in the case of ASD children with strong food selectivity. These intensive programmes, however, are expensive and thus they are not available to all social groups [65]. Furthermore, ASD children with moderate food selectivity may not require such advanced treatment. Parent training appears to be useful for moderate food selectivity in ASD children [66]. The high prevalence of food selectivity in ASD and associated health risks determine the need to develop and test therapies tailored to the severity of the condition.

Sharp et al. [67] developed a meal plan specifically designed for ASD children with moderate food selectivity. A key component of this programme was the development of a multidisciplinary model of care that combines behavioural intervention and nutrition education. The dietitian assessed anthropometric measurements and dietary needs of each child based on a food diary assessment. The dietitian also participated in food selection to increase meal variety. Moreover, dietitians provided nutrition education for participants to promote principles of healthy eating and reduce the risk of nutritional deficiencies due to food selectivity. The implemented educational programme was also aimed at improving the mealtime behaviour. Parents of ASD children, who participated in the study, learnt how to select meal portions and how to give praise/attention to their child to shape target mealtime behaviours. The combination of behaviour management strategies and nutrition education was designed to promote health and a healthy, balanced diet.

It was found that the implemented educational programme was acceptable to both parents and ASD children. Parents expressed great satisfaction with the programme. Dietitians individually prepared food plans for each patient, which further demonstrated the high reliability of the programme. The authors of the study concluded that such educational programmes may improve the mealtime behaviour and promote dietary expansion in ASD children with moderate food selectivity [68].

Matching symptom severity to intervention based on a specific criterion, such as the degree of dietary restriction, meets the call for designing educational programmes that address patient-specific requirements. This is particularly significant in patients with multiple diseases, which is frequently a characteristic of ASDs [68].

Park et al. [69] conducted an analysis of the relationship between age and BMI as well as the mealtime behaviours and food preferences of ASD

students to identify problematic mealtime behaviours. The researchers proved that students with ASD could be divided according to the degree of problematic eating behaviours, and food preferences were not significantly different from those of typically developing students. The conclusion that age and BMI values mediated the differences in meal behaviours of students with ASD seems to be particularly important, suggesting that personalised programmes of nutrition education are necessary. It was found that younger children were choosier about food and they needed a separate educational programme to experience a wider variety of foods, while older children needed an educational programme focused on obesity prevention and treatment.

A particular attention should be drawn to frequently overlooked fact that autism, although observed in early childhood, is not an exclusively childhood disorder. Typically, autistic adults are significantly less frequent subject of interest than autistic children. Meanwhile, children with autism grow and change, becoming autistic adolescents and adults who, in many cases, are able to compensate for their deficits [70].

The high prevalence of food selectivity of varying intensity in ASD patients is a flagrant need to develop and test methods of treatment that can be implemented on a broader scale and be age-appropriate and appropriate to severity of the disease. Early childhood, multilevel education, including nutrition education that may influence the way the ASD symptoms manifest themselves in later developmental periods, is one of the most significant factors.

CONCLUSIONS

The ASDs definition are used to group disease entities that are neurodevelopmental abnormalities. Many biological and environmental factors have been identified, potentially leading to the development of ASD.

Maternal nutrition is a potentially modifiable factor important for fetal neurodevelopment. Understanding the sensitive window of exposure and gene-diet interactions may help inform precise intervention and prevention. Future studies that comprehensively quantify maternal nutrient intake from both food and supplements and integrate more objective measures of biomarkers reflecting intake and metabolism are warranted.

The gut microbiota is believed to play a crucial role in human health and disease through involvement in physiological homoeostasis, immunological development, glutathione metabolism, amino acid metabolism, etc., which in a reasonable way explain the role of gut-brain axis in autism. Based on the studies conducted so far, it is estimated that the incidence of gastrointestinal disorders among patients with autistic disorders is much higher than in the population of healthy people, i.e. without the presence of neurodevelopmental disorders.

Gastrointestinal problems that are seen associated with most of the autism cases suggest that it is not just a psychiatric disorder as many claim but have a physiological base, and alleviating the gastrointestinal problems could help alleviating the symptoms .

The importance of nutrition and its possible impact on ASD patients is key to integral therapy. According to numerous research studies, various nutritional approaches succeeded in reducing the severity of patients' core ASD symptoms. The diet of ASD patients is also a key factor for the worsening of ASD symptoms. Children with autism have food selectivity and limited diets due to smell, taste, or other characteristics of foods.

Various dietary interventions are tried to alleviate the symptoms of ASD. Furthermore, utrition plays an important role in healing gastrointestinal problems that patients with ASD suffer from. Accordingly, individual-specific arrangements can be made on existing dietary protocols. Overall, more studies are needed for proving the effectiveness of the proposal diets in individuals with ASD.

Conflict of interest

Authors declare no conflict of interest.

REFERENCES

1. *Peretti S, Mariano M, Mazzocchetti C, Mazza M, Pino MC, Verrotti Di Pianella A, Valenti M.* Diet: the keystone of autism spectrum disorder? Nutr Neurosci. 2019;22(12):825-839. doi: 10.1080/1028415X.2018.1464819.
2. *Lai MC, Lombardo MV, Baron-Cohen S.* Autism. Lancet 2014;383:896-910. doi.org/10.1016/S0140-6736(13)61539-1.
3. *Cekici H, Sanlier N.* Current nutritional approaches in managing autism spectrum disorder: A review. Nutr Neurosci. 2019; 22(3): 145-155. doi: 10.1080/1028415X.2017.1358481.
4. *Modabbernia A, Velthorst E, Reichenberg A.* Environmental risk factors for autism: an evidence-based review of systematic reviews and meta-analyses. Mol Autism 2017;8:13. doi: 10.1186/s13229-017-0121-4.
5. *Karhu E, Zukerman R, Eshraghi RS, Mittal J, Deth RC, Castejon AM, Trivedi M, Mittal R, Eshraghi AA.* Nutritional interventions for autism spectrum disorder. Nutr Rev. 2020;78(7): 515-531. doi: 10.1093/nutrit/nuz092.
6. *Moody L, Chen H, Pan YX.* Early-life nutritional programming of cognition–the fundamental role of epigenetic mechanisms in mediating the relation between early-life environment and learning and memory process. Adv Nutr. 2017;8(2):337–350. doi:10.3945/an.116.014209.

7. *Vargas DL, Nascimbene C, Krishnan C, Zimmerman AW, Pardo CA.* Neuroglial activation and neuroinflammation in the brain of patients with autism. Ann Neurol. 2005;57(1):67-81. doi: 10.1002/ana.20315.
8. *Liu J, Amat M, Kong X.* Missing components in current management of Autism Spectrum Disorders (ASD): nutrition, dental care, and house-call programs. Rev J Autism Dev Disord. 2020;7:219-225. doi.org/10.1007/s40489-019-00182-x.
9. *Li M, Francis E, Hinkle SN, Ajjarapu AS, Zhang C.* Preconception and Prenatal Nutrition and Neurodevelopmental Disorders: A Systematic Review and Meta-Analysis. Nutrients 2019;11(7):1628. doi: 10.3390/nu11071628.
10. *Lyall K, Schmidt RJ, Hertz-Picciotto I.* Maternal lifestyle and environmental risk factors for autism spectrum disorders. Int J Epidemiol. 2014;43(2):443–464. doi: 10.1093/ije/dyt282.
11. *Lauritzen L, Brambilla P, Mazzocchi A, Harsløf LBS, Ciappolino V, Agostoni C.* DHA effects in brain development and function. Nutrients 2016;8(1):6. doi: 10.3390/nu8010006.
12. *Lyall K, Munger KL, O'Reilly EJ, Santangelo SL, Ascherio A.* Maternal dietary fat intake in association with autism spectrum disorders. Am J Epidemiol. 2013;178(2):209–220. doi.org/10.1093/aje/kws433.
13. *Patterson E, Wall R, Fitzgerald GF, Ross RP, Stanton C.* Health implications of high dietary omega-6 polyunsaturated fatty acids. J Nutr Metab. 2012;2012:539426. doi: 10.1155/2012/539426.
14. *Julvez J, Mendez M, Fernandez-Barres S, Romaguera D, Vioque J, Llop S, Ibarluzea J, Guxens M, Avella-Garcia C, Tardón A, Riaño I, Andiarena A, Robinson O, Arija V, Esnaola M, Ballester F, Sunyer J.* Maternal consumption of seafood in pregnancy and child neuropsychological development: A longitudinal study based on a population with high consumption levels. Am J Epidemiol. 2016;183(3):169–182. doi: 10.1093/aje/kwv195.
15. *Vecchione R, Vigna C, Whitman C, Kauffman EM, Braun JM, Chen A, Xu Y, Hamra GB, Lanphear BP, Yolton K, Croen LA, Fallin MD, Irva Hertz-Picciotto, Newschaffer CJ, Lyall K.* The Association Between Maternal Prenatal Fish Intake and Child Autism-Related Traits in the EARLI and HOME Studies. Rev J Autism Dev Disord. 2021;51(2):487-500. doi: 10.1007/s10803-020-04546-9
16. *Farina M, Rocha JBT, Aschner M.* Mechanisms of methylmercury-induced neurotoxicity: evidence from experimental studies. Life Sci. 2011;89(15-16):555-563. doi: 10.1016/j.lfs.2011.05.019.
17. FAO/WHO: Report of the Joint FAO/WHO Expert Consultation on the Risks and Benefits of Fish Consumption. Rome, Food and Agriculture Organization of the United Nations; Geneva, World Health Organization 2011, 50 pp.
18. *Lee HS.* Impact of maternal diet on the epigenome during in utero life and the developmental programming of diseases in childhood and adulthood. Nutrients 2015;7(11):9492–9507. doi: 10.3390/nu7115467.
19. *Schmidt RJ, Iosif AM, Angel EG, Ozonoff S.* Association of maternal prenatal vitamin use with risk for autism spectrum disorder recurrence in young siblings. JAMA Psychiatry 2019;76(4):391–398. doi: 10.1001/jamapsychiatry.2018.3901.
20. *Levine SZ, Kodesh A, Viktorin A, Smith L, Uher R, Reichenberg A, Sandin S.* Association of maternal use of folic acid and multivitamin supplements in the periods before and during pregnancy with the risk of autism spectrum disorder in offspring. JAMA Psychiatry 2018;75(2):176–184. doi: 10.1001/jamapsychiatry.2017.4050.
21. *Li YM, Shen YD, Li YJ, Xun GL, Liu H, Wu RR, Xia K, Zhao JP, Ou JJ.* Maternal dietary patterns, supplements intake and autism spectrum disorders: A preliminary case-control study. Medicine 2018;97(52):e13902. doi: 10.1097/MD.0000000000013902.
22. *Stubbs G, Henley K, Green J.* Autism: Will vitamin D supplementation during pregnancy and early childhood reduce the recurrence rate of autism in newborn siblings? Med Hypotheses 2016;88:74-78. doi: 10.1016/j.mehy.2016.01.015.
23. *Berding K, Donovan SM.* Diet can impact microbiota composition in children with autism spectrum disorder. Front Neurosci. 2018;12:515. doi: 10.3389/fnins.2018.00515.
24. *Tomova A, Husarova V, Lakatosova S, Bakos J, Vlkova B, Babinska K, Ostatnikova D.* Gastrointestinal microbiota in children with autism in Slovakia. Psychol Behav. 2015;138:179-187. doi: 10.1016/j.physbeh.2014.10.033.
25. *Coretti L, Paparo L, Riccio MP, Amato F, Cuomo M, Natale A, Borrelli L, Corrado G, Comegna M, Buommino E, Castaldo G, Bravaccio C, Chiariotti L, Berni Canani R, Lembo F.* Gut Microbiota Features in Young Children With Autism Spectrum Disorders. Front Microbiol. 2018;9:3146. doi: 10.3389/fmicb.2018.03146.
26. *Chaidez V, Hansen RL, Hertz-Picciotto I.* Gastrointestinal problems in children with autism, developmental delays or typical development. J Autism Dev Disord. 2014;44(5):1117–1127. doi: 10.1007/s10803-013-1973-x.
27. *Sanctuary MR, Kain JN, Chen SY, Kalanetra K, Lemay DG, Rose DR, Yang HT, Tancredi DJ, German JB, Slupsky CM, Ashwood P, Mills DA, Smilowitz JT, Angkustsiri K.* Pilot study of probiotic/colostrum supplementation on gut function in children with autism and gastrointestinal symptoms. PLOS ONE. 2019;14(1):e0210064. doi: 10.1371/journal.pone.0210064.
28. *Leader G, Tuohy E, Chen JL, Mannion A, Gilroy SP.* Feeding Problems, Gastrointestinal Symptoms, Challenging Behavior and Sensory Issues in Children and Adolescents with Autism Spectrum Disorder. J Autism Dev Disord. 2020;50(4):1401-1410. doi: 10.1007/s10803-019-04357-7.
29. *de Magistris L, Familiari V, Pascotto A, Sapone A, Frolli A, Iardino P, Carteni M, De Rosa M, Francavilla R, Riegler G, Militerni R, Bravaccio C.* Alterations of the intestinal barrier in patients with autism spectrum disorders and in their first-degree relatives. J Pediatr Gastroenterol Nutr. 2010;51(4):418–424. doi: 10.1097/MPG.0b013e3181dcc4a5.

30. *Kang DW, Adams JB, Gregory AC, Borody T, Chittick L, Fasano A, Khoruts A, Geis E, Maldonado J, McDonough-Means S, Pollard EL, Roux S, Sadowsky MJ, Lipson KS, Sullivan MB, Caporaso JG, Krajmalnik-Brown R.* Microbiota transfer therapy alters gut ecosystem and improves gastrointestinal and autism symptoms: an open label study. Microbiome 2017;5(1):10. doi: 10.1186/s40168-016-0225-7.
31. *Wang L, Christophersen CT, Sorich MJ, Gerber JP, Angley MT, Conlon MA.* Elevated fecal short chain fatty acid and ammonia concentrations in children with autism spectrum disorder. Dig Dis Sci. 2012; 57(8): 2096-2102. doi: 10.1007/s10620-012-2167-7.
32. *Albenberg LG, Wu GD.* Diet and the intestinal microbiome associations, functions, and implications for health and disease. Gastroenterology 2014;146(6):1564-1572. doi: 10.1053/j.gastro.2014.01.058.
33. *Gondalia SV, Palombo EA, Knowles SR, Cox SB, Meyer D, Austin DW.* Molecular characterization of gastrointestinal microbiota of children with autism (with and without gastrointestinal dysfunction) and their neurotypical siblings. Autism Res. 2012;5(6):419-427. doi: 10.1002/aur.1253.
34. *Son JS, Zheng LJ, Rowehl LM, Tian X, Zhang Y, Zhu W, Litcher-Kelly L, Gadow KD, Gathungu G, Robertson CE, Ir D, Frank DN, Li E.* Comparison of Fecal Microbiota in Children with Autism Spectrum Disorders and Neurotypical Siblings in the Simons Simplex Collection. PLoS One. 2015; 10(10): e0137725. doi: 10.1371/journal.pone.0137725.
35. *Kang DW, Adams JB, Coleman D, Pollard EL, Maldonado J, McDonough-Means S, Caporaso JG, Krajmalnik-Brown R.* Long-term benefit of Microbiota Transfer Therapy on autism symptoms and gut microbiota. Sci Rep. 2019;9(1):5821. doi: 10.1038/s41598-019-42183-0.
36. *Bagdasarian N, Rao K, Malani PN.* Diagnosis and treatment of *Clostridium difficile* in adults a systematic review. JAMA 2015; 313(4):398–408. doi: 10.1001/jama.2014.17103.
37. *Kang DW, Ilhan ZE, Isern NG, Hoyt DW, Howsmon DP, Shaffer M, Lozupone CA, Hahn J, Adams JB, Krajmalnik-Brown R.* Differences in fecal microbial metabolites and microbiota of children with autism spectrum disorders. Anaerobe 2018; 49: 121-131. doi: 10.1016/j.anaerobe.2017.12.007.
38. *Qiao Y, Wu M, Feng Y, Zhou Z, Chen L, Chen F.* Alterations of oral microbiota distinguish children with autism spectrum disorders from healthy controls. Scient Rep. 2018;8(1):1-12. doi: 10.1038/s41598-018-19982-y.
39. *Sharon G, Cruz NJ, Kang DW, Gandal MJ, Wang B, Kim YM, Zink EM, Casey CP, Taylor BC, Lane CJ, Bramer LM, Isern NG, Hoyt DW, Noecker C, Sweredoski MJ, Moradian A, Borenstein E, Jansson JK, Knight R, Metz TO, Lois C, Geschwind DH, Krajmalnik-Brown R, Mazmanian SK.* Human Gut Microbiota from Autism Spectrum Disorder Promote Behavioral Symptoms in Mice. Cell 2019;177(6):1600-1618. doi: 10.1016/j.cell.2019.05.004.
40. *Lee RWY, Corley MJ, Pang A, Arakaki G, Abbott L, Nishimoto M, Miyamoto R, Lee E, Yamamoto S, Maunakea AK, Lum-Jones A, Wong M.* A modified ketogenic gluten-free diet with MCT improved behavior in children with autism spectrum disorder. Physiol Behav. 2018;188:205-211. doi: 10.1016/j.physbeh.2018.02.006.
41. *Nogay NH, Walton J, Roberts KM, Nahikian-Nelms M, Witwer AN.* The Effect of the Low FODMAP Diet on Gastrointestinal Symptoms, Behavioral Problems and Nutrient Intake in Children with Autism Spectrum Disorder: A Randomized Controlled Pilot Trial. J Autism Dev Disord. 2021;51(8): 2800-2811. doi: 10.1007/s10803-020-04717-8.
42. *Ventriglio A, Sancassiani F, Contu MP, Latorre M, Di Slavatore M, Fornaro M, Bhugra D.* Mediterranean Diet and its Benefits on Health and Mental Health: A Literature Review. Clin Pract Epidemiol Ment Health. 2020;16 (Suppl-1):156–164. doi: 10.2174/1745017902016010156.
43. *House JS, Mendez M, Maguire RL, Gonzalez-Nahm S, Huang Z, Daniels J, Murphy SK, Fuemmeler BF, Wright FA, Hoyo C.* Periconceptional maternal mediterranean diet is associated with favorable offspring behaviors and altered CpG methylation of imprinted genes. Front Cell Dev Biol. 2018;6:107. doi: 10.3389/fcell.2018.00107.
44. *Lange KW, Reissmann A.* Is the gluten-free and casein-free diet efficacious in the treatment of childhood autism spectrum disorder? JFB. 2021;15. doi.org/10.31665/JFB.2021.15277.
45. *Lutsey PL, Steffen LM, Feldman HA, Hoelscher DH, Webber LS, Luepker RV, Lytle LA, Zive M, Osganian SK.* Serum homocysteine is related to food intake in adolescents: the Child and Adolescent Trial for Cardiovascular Health. Am J Clinic Nutr. 2006;83(6):1380–1386. doi: 10.1093/ajcn/83.6.1380.
46. *Valente FX, Campos Tdo N, Moraes LF, Hermsdorff HH, Cardoso Lde M, Pinheiro-Sant'Ana HM, Gilberti FA, Peluzio Mdo C.* B vitamins related to homocysteine metabolism in adults celiac disease patients: a cross-sectional study. Nutr J. 2015;14:110. doi: 10.1186/s12937-015-0099-8.
47. *Quan L, Xu X, Cui Y, Han H, Hendren RL, Zhao L, You X.* A systematic review and meta-analysis of the benefits of a gluten-free diet and/or casein-free diet for children with autism spectrum disorder. Nutr Rev. 2022;80(5):1237-1246. doi: 10.1093/nutrit/nuab073.
48. *Tarnowska K, Gruczyńska-Sękowska E, Kowalska D, Kozłowska M, Majewska E, Winkler R.* Difficulties and factors influencing purchase decision. The perspective of families with children with autism spectrum disorders on a gluten-free and casein-free diet. Preliminary study. Rocz Panstw Zakl Hig. 2020;71(3):321-328. doi: 10.32394/rpzh.2020.0122.
49. *Ruskin DN, Murphy MI, Slade SL, Masino SA.* Ketogenic diet improves behaviors in a maternal immune activation model of autism spectrum disorder. PloS one. 2017;12(2):e0171643. doi: 10.1371/journal.pone.0171643.
50. *Castro K, Faccioli LS, Baronio D, Gottfried C, Schweigert Perry I, dos Santos Riesgo R.* Effect of a ketogenic diet on autism spectrum disorder: A systematic review. Res Autism Spectr Disord. 2015;20:31-38. doi.org/10.1016/j.rasd.2015.08.005.

51. *El-Rashidy O, El-Baz F, El-Gendy Y, Khalaf R, Reda D, Saad K.* Ketogenic diet versus gluten free casein free diet in autistic children: a case-control study. Meta. Brain Dis. 2017;32(6):1935-1941. doi: 10.1007/s11011-017-0088-z.
52. *Mychasiuk R, Rho JM.* Genetic modifications associated with ketogenic diet treatment in the BTBR+ Tf/J mouse model of autism spectrum disorder. Autism Res. 2017;10(3):456-471. doi: 10.1002/aur.1682.
53. *Newell C, Johnsen VL, Yee NC, Xu WJ, Klein MS, Khan A, Rho JM, Shearer J.* Ketogenic diet leads to O-GlcNAc modification in the BTBRt+Tf/J mouse model of autism. Biochim Biophys Acta Mol Basis Dis. 2017;1863(9):2274-2281. doi: 10.1016/j.bbadis.2017.05.013.
54. *Varesio C, Grumi S, Zanaboni MP, Mensi MM, Chiappedi M, Pasca L, Ferraris C, Tagliabue A, Borgatti R, De Giorgis V.* Ketogenic Dietary Therapies in Patients with Autism Spectrum Disorder: Facts or Fads? A Scoping Review and a Proposal for a Shared Protocol. Nutrients 2021;13(6):2057. doi: 10.3390/nu13062057.
55. *Suskind DL, Wahbeh G, Gregory N, Vendettuoli H, Christie D.* Nutritional therapy in pediatric Crohn disease: the specific carbohydrate diet. J Pediatr Gastroenterol Nutr. 2014;58(1):87-91. doi: 10.1097/MPG.0000000000000103.
56. *Obih C, Wahbeh G, Lee D, Braly K, Giefer M, Shaffer ML, Nielson H, Suskind DL.* Specific carbohydrate diet for pediatric inflammatory bowel disease in clinical practice within an academic IBD center. Nutrition 2016;32(4):418-25. doi: 10.1016/j.nut.2015.08.025.
57. *Ābele S, Meija L, Folkmanis V, Tzivian L.* Specific Carbohydrate Diet (SCD/GAPS) and Dietary Supplements for Children with Autistic Spectrum Disorder. In: Proceedings of the Latvian Academy of Sciences. Section B. Natural, Exact, and Applied Sciences 2021;75(6):417-425. doi.org/10.2478/prolas-2021-0062.
58. *Nanayakkara WS, Skidmore PM, O'Brien L, Wilkinson TJ, Gearry RB.* Efficacy of the low FODMAP diet for treating irritable bowel syndrome: the evidence to date. Clin Exp Gastroenterol. 2016;9:131–142. doi: 10.2147/CEG.S86798.
59. *Madzhidova S, Sedrakyan L.* The use of dietary interventions in pediatric patients. PMC. 2019; 7(1): 1-13. doi: 10.3390/pharmacy7010010.
60. *Sharp WG, Berry RC, McCracken C, Nuhu NN, Marvel E, Saulnier CA, Klin A, Jones W, Jaquess DL.* Feeding problems and nutrient intake in children with autism spectrum disorders: A meta-analysis and comprehensive review of the literature. J Autism Dev Disord. 2013;43(9):2159–2173. doi: 10.1007/s10803-013-1771-5.
61. *Sharp WG, Postorino V, McCracken CE, Berry RC, Criado KK, Burrell TL, Scahill L.* Dietary intake, nutrient status, and growth parameters in children with autism spectrum disorder and severe food selectivity: An electronic medical record review. J Acad Nutr Diet. 2018;118(10):1943–1950. doi: 10.1016/j.jand.2018.05.005.
62. *Curtin C, Hubbard K, Anderson SE, Mick E, Must A, Bandini LG.* Food selectivity, mealtime behavior problems, spousal stress, and family food choices in children with and without autism spectrum disorder. J Autism Dev Disord. 2015;45(10): 3308–3315. doi: 10.1007/s10803-015-2490-x.
63. *Schreck KA, Williams K.* Food preferences and factors influencing food selectivity for children with autism spectrum disorders. Res Dev Disabil. 2006;27(4): 353–363. doi: 10.1016/j.ridd.2005.03.005.
64. *Esteban-Figuerola P, Canals J, Fernández-Cao JC, Arija Val V.* Differences in food consumption and nutritional intake between children with autism spectrum disorders and typically developing children: A meta-analysis. Autism 2019;23(5):1079-1095. doi: 10.1177/1362361318794179.
65. *Sharp WG, Volkert VM, Scahill L, McCracken CE, McElhanon B.* A systematic review and meta-analysis of intensive multidisciplinary intervention for pediatric feeding disorders: How standard is the standard of care? J Pediatr. 2017;181:116–124. doi: 10.1016/j.jpeds.2016.10.002.
66. *Cosbey J, Muldoon D.* EAT-UP™ Family-Centered Feeding Intervention to Promote Food Acceptance and Decrease Challenging Behaviors: A Single-Case Experimental Design Replicated Across Three Families of Children with Autism Spectrum Disorder. J Autism Dev Disord. 2017;47(3): 564–578. doi: 10.1007/s10803-016-2977-0.
67. *Sharp WG, Burrell TL, Berry RC, Stubbs KH, McCracken CE, Gillespie SE, Scahill L.* The Autism Managing Eating Aversions and Limited Variety Plan vs Parent Education: A Randomized Clinical Trial. J Pediatr. 2019;211:185-192. doi: 10.1016/j.jpeds.2019.03.046.
68. *Smith SM, Bayliss EA, Mercer SW, Gunn J, Vestergaard M, Wyke S, Salisbury C, Fortin M.* How to design and evaluate interventions to improve outcomes for patients with multimorbidity. *J Comorb.* 2013;3:10–17. doi: 10.15256/joc.2013.3.21.
69. *Park HJ, Choi SJ, Kim Y, Cho MS, Kim YR, Oh JE.* Mealtime Behaviors and Food Preferences of Students with Autism Spectrum Disorder. Foods 2020;10(1):49. doi: 10.3390/foods10010049.
70. *Hyman SL, Levey SE, Myers SM.* Council on children with Disabilities, Section on Developmental and Behavioral Pediatrics. Identification, Evaluation, and Management of Children With Autism Spectrum Disorder. Peditarics 2020;145(1):e20193447. doi: 10.1542/peds.2019-3447.

Received: 15.06.2022
Accepted: 20.07.2022
Published online first: 05.08.2022

Rocz Panstw Zakl Hig 2022;73(3):259-264
http://wydawnictwa.pzh.gov.pl/roczniki_pzh/ https://doi.org/10.32394/rpzh.2022.0227

REVIEW ARTICLE

ACRYLAMIDE IN HUMAN BREAST MILK – THE CURRENT STATE OF KNOWLEDGE

Hanna Mojska[1]

[1]National Institute of Public Health NIH – National Research Institute, Department of Nutrition and Nutritive Value of Food, Chocimska 24, 00-791 Warsaw, Poland

ABSTRACT

Human milk is a first choice in infant nutrition. It not only provides all the nutrients necessary for the proper infant`s development but also contains bioactive factors that provide natural protection against infections. Unfortunately, chemical contaminants can pass to breast milk and pose a health risk for the breastfed infant's health. Acrylamide is a typical process contaminant and in food it is formed as a result of the Maillard reaction. Numerous studies have shown that acrylamide is a neurotoxic and carcinogenic compound. So far there have been published only three studies on the acrylamide content in human milk. In two of them, the acrylamide level in most of the tested samples did not exceed the value of 0.5 µg/L. In the third study, the authors assessed the circulation of acrylamide in the body of two breastfeeding women after consuming products with high acrylamide content. Depending on the time elapsed after the meal, the acrylamide content ranged from 3.17 µg/L to 18.8 µg /L. These studies show that the breastfeeding mothers' diet may have a significant influence on the level of acrylamide in their milk. However, it seems that the acrylamide content in breast milk is also influenced by the time of breast milk collection, including the time elapsed after the mother's meal. To assess the exposure of breastfed infants to acrylamide in human milk, more data is needed on the acrylamide content in human milk at different stages of lactation and using standard protocols for human milk sampling.

Key words: *acrylamide, human breast milk, breastfeeding mothers, diet, exposure*

INTRODUCTION

Breastfeeding is the best way to feed babies early in life. Human milk provides all the necessary nutrients in the amount that meets the needs at a given stage of baby's development. It is also a source of a wide range of bioactive factors that may prevent allergies and protect against infections. World Health Organization (WHO) recommends "exclusive breastfeeding for 6 months, followed by continued breastfeeding as complementary foods are introduced, with continuation of breastfeeding for 2 years or longer as mutually desired by mother and infant" [1]. The content of individual nutrients and bioactive compounds in breast milk is influenced by both maternal physiological factors and lifestyle, including eating habits during pregnancy and lactation [2]. Unfortunately, chemical contaminants derived from environmental pollution as well as generated during food processing may be transferred to breast milk and pose a risk for the breastfed infant's health.

Acrylamide (prop-2-enamide; CAS 79-06-1) is a vinyl monomer used on an industrial scale for the synthesis of polyacrylamide polymers which are widely used among others, as fillers for industrial and drinking water filters, in the petroleum, paper, textile and cosmetic industries [3]. In 2002, the Swedish National Food Agency in collaboration with scientists from the Stockholm University reported for the first time results with a high acrylamide content in heat-treated food [4]. Twenty years after the first reports, it is well known that acrylamide is a processing contaminant mainly formed in food as a product of the Maillard reaction between free asparagine and reducing sugars, especially glucose and fructose, under temperatures of more than 120°C [5, 6]. The main source of acrylamide in the human diet are thermally processed potato and cereal products as well as coffee and its substitutes. The content of acrylamide in food varies widely, from below 10 µg/kg in bread to even more than 7000 µg/kg in individual samples of coffee substitutes [7]. Recent studies by *Timmermann* et al. [8] show that the estimated median exposure to acrylamide from food worldwide varies from 0.02 µg/kg bw/day to 1.53 µg/kg bw/day. Another important source of human exposure to acrylamide

Corresponding author: Hanna Mojska, National Institute of Public Health NIH – National Research Institute, Department of Nutrition and Nutritive Value of Food, Chocimska 24, 00-791 Warsaw, Poland, e-mail: hmojska@pzh.gov.pl

is smoking [9, 10, 11]. In recent years, studies have been conducted on the fate of acrylamide during digestion in the human gastrointestinal tract [12] and the possibility of acrylamide formation from other intermediate products of the Maillard reaction, e.g. 5-hydroxymethylfurfural, released both during digestion in the gastrointestinal tract and present in thermally processed products [13, 14].

The International Agency for Research on Cancer classified acrylamide as „probable human carcinogen" (Group 2A) as early as 1994 [3], concluding that the carcinogenicity of acrylamide in animal studies is well documented, despite limited evidence for it in human studies. Acrylamide also demonstrates neurotoxic properties and can contribute to damaging the central and peripheral nervous system, both among experimental animals and people exposed to this compound at work [15, 16]. The margin of exposure (MOE) criterion is commonly used to assess the carcinogenic and neurotoxic risks of acrylamide [17]. The MOE is the ratio of the Benchmark Dose Lower Limit ($BMDL_{10}$) to the estimated human intake of the compound. Joint FAO/WHO Expert Committee on Food Additive (JECFA) [18] considered that 0.18 mg/kg bw /day (lowest value in the range of $BMDL_{10}$ values) for Harderian gland tumors in male mice and 0.31 mg/kg bw /day for breast tumors in female rats are appropriate for assessing the risk of carcinogenic effects of acrylamide and advises that a calculated MOE of less than 10 000, based on $BMDL_{10}$ from animal studies, may indicate that the compound poses a risk to human health.

In the human body, acrylamide is metabolized through two main metabolic pathways: epoxidation to glycidamide and glutathione conjugation to mercapturic acids. The conversion of acrylamide to glycidamide, its main metabolite, is catalyzed by an enzyme of cytochrome P450 (isoenzyme CYP2E1) [19] Both acrylamide and glycidamide form adducts with haemoglobin. Adducts of acrylamide and glycidamide with hemoglobin (AAVal and GAVal) are used to assess long-term exposure to acrylamide [20]. In turn, the assessment of recent exposure uses the metabolites of acrylamide and glycidamide in form of mercapturic acid derivatives, N-Acetyl-S-(2-carbamoylethyl)-L-cysteine (AAMA) and glycidamide to N-Acetyl-S-(2-carbamoyl-2-hydroxyethyl)-L-cysteine (GAMA), which are excreted in the urine [21, 21].

EFFECT OF DIETARY ACRYLAMIDE INTAKE DURING PREGNANCY ON OFFSPRING

Due to its very good solubility in water, acrylamide is quickly absorbed and transferred to various tissues. It is able to pass through the placenta barrier [23] posing a risk to the growing foetus. Several studies [24, 25, 26, 27] have confirmed the relationship between dietary intake of acrylamide by pregnant women and low birth weight, length and head circumference of newborns. They also demonstrated an increased risk of having a baby that is small for gestational age (SGA). On the other hand, it is worth noting, that *Nagata* et al. [28] did not confirm an inverse relationship between the consumption of acrylamide in the diet in a group of 204 Japanese pregnant women and the birth size of their newborns. At the same study, they found that higher acrylamide consumption was significantly positively associated with higher levels of umbilical cord estradiol during labor. The results of *Nagata* et al. [28] indicate other possible action of acrylamide.

TRANSFER OF ACRYLAMIDE FROM DIET INTO MILK AND EFFECTS ON OFFSPRING

Animal studies [29, 30] showed that oral administration of acrylamide to lactating female rats did not cause adverse effects in the offspring. The authors concluded that this is probably due to the limited transfer of acrylamide across the blood / milk barrier during lactation and the low level of acrylamide in the milk, which is insufficient to initiate toxic changes. In turn, *Pabst* et al. [31] determined the acrylamide content in cow feed and investigated the potential to carry-over of acrylamide from cattle feed to cow's milk. From the results obtained, they calculated that the mean carry-over of acrylamide was 0.24% of the amount taken from feed. The mean half-life of acrylamide was estimated to be 2.8 hours. The authors concluded that acrylamide was rapidly transformed in the cows. Considering the analytically determined levels of acrylamide in the commercial feed at 180, 145 and 140 µg/kg, they also estimated that the expected concentration of acrylamide in raw milk from cows fed with such feeds could be a maximum of approximately 0.2 µg/kg. It is worth noting that the metabolism of acrylamide in the human body is also described as fast, and the presence of AAMA and GAMA, the main metabolites of acrylamide, is found in the urine 2 hours after oral ingestion of acrylamide [32].

ACRYLAMIDE IN HUMAN BREAST MILK

So far there have been published three studies on the acrylamide content in human milk [33, 34, 35]. The first results was published by *Sörgel* et al. [33] already in 2002. They evaluated the transmission of acrylamide in case of two breastfeeding women who consumed potato chips (crisps) with the acrylamide

content at the level of 800 μg/kg and 1000 μg/kg. The determined acrylamide content in the breast milk samples of the subjects was high and ranged in the first subject from 4.86 μg/L after 4 h to 3.17 μg/L after 8 h from eating potato crisps and, respectively, 10.6 μg/L after 3 h to 18.8 μg/L after 4 h in the other subject. The authors found that the level of acrylamide in human milk correlated with the amount of this compound ingested by breastfeeding mothers and with the time elapsed after a meal. They concluded that acrylamide is transferred from mother's diet to her breast milk and could pose a risk to infant during breastfeeding.

On the other hand, *Fohgelberg* et al. [34], who determined acrylamide in breast milk samples taken from 15 women and in 4 pooled breast milk samples (10 mothers per pool) in Sweden found that in most of the samples acrylamide content was below the limit of quantification (LOQ = 0.5 μg/kg). Only in one sample, the acrylamide content was above LOQ and was equal to 0.51 μg/kg. According to the authors, this confirms the rapid digestion of acrylamide in the human body and the limitation of its penetration into breast milk. However, it is not described how the individual milk samples were taken and how much time elapsed between sample collection and the last meal of the breastfeeding mother.

In recently published study carried out in Poland [35] the results of determination of acrylamide content in human breast milk, in two lactation periods, were presented. As in the studies by *Sörgel* et al. [33] and *Fohgelberg* et al. [34] acrylamide content was determined by liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS). In Polish study, acrylamide content was determined in 47 samples of colostrum collected by healthy women in the 2nd – 3rd day after childbirth in the Obstetrics Ward and in 26 samples of mature breast milk collected by breastfeeding mothers at home. In the majority (77 %) of colostrum samples and in over 40% of the mature milk samples, the acrylamide level was below the limit of quantification (LOQ = 0.1 μg/L). Additionally, in the majority of the samples, both colostrum (93.6% of the samples) and mature milk (96.1% of the samples), the acrylamide level was not higher than 0.5 μg/L. The highest level of acrylamide was found in only one sample of human milk and it reached 1.0 μg/L. These results are similar to the data presented by *Fohgelberg* et al. [34] and are from several to several dozen times lower than found by *Sörgel* et al. [33]. Our study also showed for the first time a difference between the acrylamide content in different lactation periods. The median level of acrylamide in the mature milk was significantly ($p < 0.05$) almost three times higher than the median level of acrylamide in the colostrum (0.14 μg/L vs. 0.05 μg/L). Comparing acrylamide intake from hospital and home diets, we found that acrylamide intake from home diet was significantly ($p < 0.0001$) more than twice as high (16.9 μg/day vs. 7.3 μg/day). It seems to confirm the impact of the breastfeeding mother`s diet on the acrylamide level in their breast milk. A positive correlation, although modest and borderline significant, between the intake of acrylamide from the diet by breastfeeding women and the content in breast milk was also found but only in relation to colostrum. This is probably due to the small number of women (n = 26) who provided samples of mature milk. The results seem to confirm that acrylamide is transferred from the mother's diet into her milk. Additionally, clearly indicating that acrylamide levels in breast milk can be lowered by changing the breastfeeding mother`s diet. However, further studies are needed to corroborate this finding.

The results of the studies carried out so far indicate that the acrylamide content in breast milk is probably significantly influenced by the time elapsed after the mother's meal and the method of taking milk samples. This has been well demonstrated by *Sörgel* et al. [33], who took milk samples 3, 4 and 8 hours after eating foods high in acrylamide and found high levels of acrylamide in breast milk. In turn, in our research [35], milk samples were collected in the morning after the babies were first fed. Probably even before the first meal of the breastfeeding mothers. This means that the time that elapsed from the mother's last meal ranged from about 8 - 12 hours. As a result, in our research we found trace amounts of acrylamide in human milk samples. On the other hand, it should be remembered that the content of acrylamide in the diet of breastfeeding women participating in our study was several dozen times lower than in the studies by *Sörgel* et al. [33]. It also seems that the methodology of sampling has an influence on the analytically determined content of acrylamide in breast milk. Doing it after the baby is fed is often an ethical choice but the actual acrylamide content in breast milk appears to be higher. It should also be remembered that human milk is a water/fat emulsion. The initial phase is dominated by the water fraction and the final phase of feeding contains more fat. Acrylamide is a very good soluble in water and therefore there may be more of it in the initial phase of breast milk compared to the final phase. It is also worth remembering that babies consume different amounts of milk each time they are fed. It depends on their individual needs. All these factors could influence the variability of the acrylamide content in individual breast milk samples, which was clearly shown in the studies carried out in Poland [35]. It seems that more research is needed on the acrylamide content of human milk using standardized human milk sampling protocols.

EXPOSURE OF BREASTFED INFANTS TO ACRYLAMIDE FROM BREAST MILK

Infant exposure to acrylamide present in breast milk as assessed by *Sörgel* et al. [33], based on the determined acrylamide content in milk of two women after eating potato chips (crisps), was very high and ranged from 0.66 μg/kg bw/day to 3.3 μg/kg bw/day. A significantly lower exposure was estimated by *Fohgelberg* et al. [34]. Taking into account that in most of the tested milk samples the acrylamide content was below the LOQ (0.5 μg/kg), they used 0.25 μg/kg (half the LOQ value) to estimate the exposure of breastfed infants. They estimated that the mean acrylamide intake during the first six months for children who were exclusively breastfed was 0.04 μg/kg bw/day. Exposure to acrylamide in the group of Polish exclusively breastfed infants was estimated in two different lactation period based on a similar approach [35]. For the calculations, we used the actually determined values of acrylamide in breast milk for samples above the LOQ (0.1 μg/L) and half the LOQ value (0.05 μg/L) for samples below this value. In our study, estimated average (median) exposure to acrylamide ranged from 0.003 μg/kg bw/day to 0.018 μg/kg bw/day, depending on the lactation period. To assess the risk of carcinogenicity of acrylamide among breastfed infants, the above results were compared with Benchmark Dose Lower Limit ($BMDL_{10}$) derived for animals as a health reference value [17]. The calculated margins of exposure (MOEs) for neonates and infants at the 95th percentile were slightly below 10 000, indicating that in this study the acrylamide level in breast milk may be of concern for health [18] for 5 % of breastfed infants. It is worth noting that despite the presence of acrylamide in breast milk, the content of this compound found in studies in Sweden [34] and Poland [35] was considerably lower than in baby food [36, 37, 38]. The exposure arising from the presence of acrylamide in breast milk was also significantly lower than in case of infants fed with formulas [36, 37, 38].

CONCLUSIONS

In conclusion, it should be stated that the results of the studies on acrylamide content in human milk are limited by the different periods of sampling of milk for testing and the different sampling methodology. It seems that both the lactation period and the stage during a single feeding, as well as the time elapsed after the mother's meal, have a significant influence on the level of acrylamide in human milk. More research is needed on the acrylamide content in human milk using standardized milk sampling protocols. However, breastfeeding mothers should pay attention to the composition of their diet and avoid products that may contain acrylamide. It seems necessary to develop specific recommendations for breastfeeding women. The use of a metabolomic approach [39, 40] that takes into account the relationship between acrylamide intake by breastfeeding mothers and the content of acrylamide in breast milk and the level of its metabolites in other body fluids could also increase knowledge about the circulation of acrylamide in the human body.

Conflict of interest

Author declare no conflict of interest.

REFERENCES

1. WHO. Available online: https://www.who.int/health-topics/breastfeeding (accessed on 20 May 2022).
2. *Koletzko B, Godfrey KM, Poston L, Szajewska H, van Goudoever JB, de Waard M, Brands B, Grivell RM, Deussen AR, Dodd JM, Patro-Golab B, Zalewski BM; Early Nutrition Project Systematic Review Group.* Nutrition during pregnancy, lactation, and early childhood and its implications for maternal and long-term child health: the Early Nutrition Project recommendations. Ann. Nutr. Metab. 2019;74(2):93-106. https://doi.org/10.1159/000496471.
3. International Agency for Research on Cancer: Acrylamide. Summary of Data Reported and Evaluation. W: IARC Monographs on the Evaluations of Carcinogenic Risks to Humans; Some industrial Chemicals; IARC: Lyon, France, 1994; Volume 60, pp. 389–433. Available online: https://monografs.iarc.fr/iarc-monographs-on-the-evaluation-of-carcinogenic-risks-to-human-61/ (accessed on 20 May 2022).
4. SNFA. Swedish National Food Administration. Information about acrylamide in food 2002: http://www.slv.se/engdefault.asp.
5. *Mottram, D.S.; Wedzicha, B.L.; Dodson, A.T.*: Acrylamide is formed in the Maillard reaction. Nature 2002;419:448-449.
6. *Stadler, R.H.; Scholz, G.*: Acrylamide: an update on current knowledge in analysis, levels in food, mechanisms of formation, and potential strategies of control. Nutr. Rev. 2004;62(12):449-467.
7. European Food Safety Authority. Panel on Contaminants in the Food Chain: Scientific Opinion on acrylamide in food. EFSA J. 2015, 13, 4104. Available online: https://www.efsa.europa.eu/en/efsajournal/pub/4104
8. *Timmermann, C.A.G.; Mølck, S.S.; Kadawathagedara, M.; Bjerregaard, A.A.; Törnqvist, M.; Brantsæter, A.L.; Petersen, M.*: A review of dietary intake of acrylamide in humans. Toxics 2021;9:155. https://doi.org/10.3390/toxics9070155
9. S*mith, C.J.; Perfetti, T.A.; Rumple, M.A.; Rodgman, A.; Doolittle, D.J.* "IARC Group 2A Carcinogens" Reported in Cigarette Mainstream Smoke. Food Chem. Toxicol. 2000;38:371-383.

10. *Moldoveanu, S.C.; Gerardi, A.R.*: Acrylamide analysis in tobacco, alternative tobacco products, and cigarette smoke. J. Chromatogr. Sci. 2011;49:234–242.
11. *Mojska, H., Gielecińska, I.; Cendrowski, A.*: Acrylamide content in cigarette mainstream smoke and estimation of exposure to acrylamide from tobacco smoke in Poland. Ann. Agric. Environ. Med. 2016;23(3):456-461. https://doi.org/10.5604/12321966.1219187
12. *Sansano, M.; Heredia, A.; Peinado I.; Andres, A*. Dietary acrylamide: What happens during digestion. *Food Chem.* 2017;237:58-64. doi: 10.1016/j.foodchem.2017.05.104.
13. Hamzalioglu, A.; Gokmen, V. Investigation of the reactions of acrylamide during in vitro multistep enzymatic digestion of thermally processed foods. Food Funct**.** 2015;6(1):109-114.
14. *Hamzalioglu, A.; Gokmen, V.* Investigation and kinetic evaluation of the reactions of hydroxymethylfurfural with amino and thiol groups of amino acids. Food Chem. 2018;240:354-360. doi: 10.1016/j.foodchem.2017.07.131.
15. *Vikström, A.C.; Warholm, M.; Paulsson, B.; Axmon, A.; Wirfält, E.; Törnqvist, M.*:Hemoglobin adducts as a measure of variations in exposure to acrylamide in food and comparison to questionnaire data. Food Chem. Toxicol. 2012;50:2531–2539. https://doi.org/10.1016/j.fct.2012.04.004
16. *He, F.; Zhang, S.; Wang, H.; Li, G.; Zhang, Z.; Li, F.; Dong, X.; Hu, F.*. Neurological and electroneuromyographic assessment of the adverse effects of acrylamide on occupationally exposed workers. Scand. J. Work Environ. Health 1989;15:125-129. doi: 10.5271/sjweh.1878.
17. *Bolger, P.M.; Leblanc, J.-C.; Setzer, R.W.* Application of the Margin of Exposure (MoE) approach to substances in food that are genotoxic and carcinogenic. Example: acrylamide (CAS No. 79-06-1). Food Chem. Toxicol. 2010;48:S25-S33. DOI: 10.1016/j.fct.2009.11.040
18. JECFA. Evaluation of Certain Food Additives and Contaminants; 72nd Report of the Joint FAO/WHO Expert Committee on Food Additive; WHO Technical Report Series; World Health Organization: Rome, Italy, 2011; p. 959.
19. *Von Tungeln, L.S.; Doerge, D.R.; Gamboa da Costa, G.; Marques, M.M.; Witt, W.M.; Koturbash, I.; Pogribny, I.P.; Beland, F.A.*: Tumorigenicity of acrylamide and its metabolite glycidamide in the neonatal mouse bioassay. Int. J. Cancer 2012;131:2008–15.
20. *Vikström, A.C.; Warholm, M.; Paulsson, B.; Axmon, A.; Wirfält, E.; Törnqvist, M.* Hemoglobin adducts as a measure of variations in exposure to acrylamide in food and comparison to questionnaire data. Food Chem. Toxicol. 2012;50:2531–2539.
21. *Bjellaas, T.; Stølen, L.H.; Haugen, M.; Paulsen, J.E.; Alexander, J.; Lundanes, E.; Becher,* G. Urinary acrylamide metabolites as biomarker for short-term dietary exposure to acrylamide. Food Chem. Toxicol. 2007;45:1020–1026.
22. *Boettcher, M.I.; Schettgen, T.; Kütting, B.; Pischetsrieder, M.; Angerer, J.:* Mercapturic acids of acrylamide and glycidamide as biomarkers of the internal exposure to acrylamide in the general population. Mutat. Res. 2005;580:167–176.
23. *Annola, K.; Karttunen, V.; Keski-Rahkonen, P.; Myllynen, P.; Segerbäck, D.; Heinonen, S.; Vähäkangas K.* Transplacental transfer of acrylamide and glycidamide are comparable to that of antipyrine in perfused human placenta. Toxicol. Lett 2008;(182):50-56.
24. *Duarte-Salles, T.; von Stedingk, H.; Granum, B.; Gützkow, K.B.; Rydberg, P.; Törnqvist, M.; Mendez, M.A.; Brunborg, G.; Brantsæter, A.L.; Meltzer, H.M.; Alexander, J.; Haugen, M.:* Dietary acrylamide intake during pregnancy and fetal growth results from the Norwegian Mother and Child Cohort study (MoBa). Environ. Health Perspect**.** 2013;(121):374-379. doi: 10.1289/ehp.1205396.
25. *Pedersen, M., von Stedingk, H,; Botsivali, M., Agramunt, S., Alexander, J., Brunborg, G., Chatzi, L., Fleming, S,; Fthenou, E., Granum, B., Gutzkow, K.B., Hardie, L.J., Knudsen, L.E., Kyrtopoulos, S.A., Mendez, M.A., Merlo, D.F., Nielsen, J.K., Rydberg, P., Segerbäck, D., Sunyer, J., Wright, J., Törnqvist, M., Kleinjans, J.C,; Kogevinas, M., NewGeneris Consortium*: Birth weight, head circumference, and prenatal exposure to acrylamide from maternal diet: the European prospective mother-child study (NewGeneris). Environ Health Perspect. 2012;(120):1739-1745.
26. *Kadawathagedara, M.; Chan Hon Tong, A.; Heude, B.; Forhan, A.; Charles, M.-A.; Sirot, V.; Botton, J.* The EDEN mother-child cohort study group: Dietary acrylamide intake during pregnancy and anthropometry at birth in the French EDEN mother-child cohort study. Environ. Res. J.149 (2016), 189-196. DOI: 10.1016/j.envres.2016.05.019
27. *Hogervorst, J. Vesper, H.W., Madhloum, N., Gyselaers, W., Nawrot, T.*: Cord blood acrylamide levels and birth size, and interactions with genetic variants in acrylamide-metabolising genes. Environ. Health 2021;20:35 https://doi.org/10.1186/s12940-021-00715-0.
28. *Nagata, C., Konishi, K., Wada, K., Tamura, T., Goto, Y., Koda, S., Mizuta, F., Iwasa, S.:* Maternal acrylamide intake during pregnancy and sex hormone levels in maternal and umbilical cord blood and birth size of offspring. Nutrition and Cancer 2019;71(1):77-82. DOI: 10.1080/01635581.2018.1524018
29. *Friedman, M.A., Tyl, R.W., Marr, M.C., Myers, C.B., Gerling, F.S., Ross, W.P.*: Effects of lactational administration of acrylamide on rat dams and offspring. Reprod. Toxicol. 1999;13:511- 520.
30. *Takahashi, M., Shibutani, M., Nakahigashi, J., Sakaguchi, N., Inoue, K., Morikawa, T.; Yoshida, M.; Nishikawa, A.*: Limited lactational transfer of acrylamide to rat offspring on maternal oral administration during the gestation and lactation periods. Arch. Toxicol. 2009;83:785 -793.
31. *Pabst, K., Mathar, W., Palavinskas, R., Meisel, H., Bluthgen, A., Klaffke, H.*: Acrylamide-occurrence in mixed concentrate feed dairy cows and carry-over into milk. Food Addit Contam. 2005;22(3):210-213.

32. *Goempel, K,; Tedsen, L., Ruenz, M,; Bakuradze, T., Schipp, D., Galan, J., Eisenbrand, G.; Richling, E.*: Biomarker monitoring of controlled dietary acrylamide exposure indicates consistent human endogenous background. Arch. Toxicol. 2017;91:3551–3560.
33. *Sörgel, F.; Weissenbacher, R.; Kinzig-Schippers, M.; Hofmann, A., Illauer, M., Skott, A., Landersdorfer, C.*: Acrylamide: Increased concentrations in homemade food and first evidence of its variable absorption from food, variable metabolism and placental and breast milk transfer in humans. Chemotherapy 2002;48:267-274. DOI: 10.1159/000069715
34. *Fohgelberg, P., Rosén, J,; Hellenäs, K.-E., Abramsson-Zetterberg, L.*: The acrylamide intake via some common baby food for children in Sweden during their first year of life – an improved method for analysis of acrylamide. Food Chem. Toxicol. 2005;43:951-959. DOI: 10.1016/j.fct.2005.02.001
35. *Mojska H., Gielecinska I., Winiarek J., Sawicki W.*: Acrylamide Content in Breast Milk: The Evaluation of the Impact of Breastfeeding Women's Diet and the Estimation of the Exposure of Breastfed Infants to Acrylamide in Breast Milk. Toxics 2021;9:298-316. https://doi.org/10.3390/toxics9110298
36. *Basaran, B., Aydin, F.*: Determination of acrylamide levels in infant formulas and baby biscuits sold in Turkey. Letters in Applied NanobioScience 2021;11(1):3155-65. https://doi.org/10.33263/LIANBS111.31553165
37. *Esposito F., Nolasco A., Caracciolo F., Velotto S., Montuori P., Romano R., Stasi T., Cirillo T.*: Acrylamide in Baby Foods: A Probabilistic Exposure Assessment. Foods 2021;10(12):2900-2913. https://doi.org/10.3390/foods10122900
38. *Mojska, H.; Gielecińska, I.; Stoś, K.*: Determination of acrylamide level in commercial baby foods and an assessment of infant dietary exposure. Food Chem. Toxicol. 2012;50:2722-2728. DOI: 10.1016/j.fct.2012.05.023
39. *Fernandez S. F., Pardo O., Coscolla C., Yusa V.*: Exposure assessment of Spanish lactating mothers to acrylamide via human biomonitoring. Environmental Research 2022;203:111832, https://doi.org/10.1016/j.envres.2021.111832
40. *Delgado A.A., Esteve – Turrillas F. A., Fernandez S. F., Garlito B., Pardo O.*: Review of the state of the art of acrylamide human biomonitoring. Chemosphere 2022;295:133880. https://doi.org/10.1016/j.chemosphere.2022.133880.

Received:12.08.2022
Accepted:15.09.2022

Rocz Panstw Zakl Hig 2022;73(3):265-274
http://wydawnictwa.pzh.gov.pl/roczniki_pzh/ https://doi.org/10.32394/rpzh.2022.0216

REVIEW ARTICLE

PHYTONUTRIENTS OF BILBERRY FRUIT AND SASKATOON BERRY IN THE PREVENTION AND TREATMENT OF DYSLIPIDEMIA

Jana Kopčeková[1], *Jana Mrázová*[1,]

[1]Slovak University of Agriculture in Nitra, Faculty of Agrobiology and Food Resources, Institute of Nutrition and Genomics, Nitra, Slovak Republic

ABSTRACT

The epidemiologic studies from the recent years indicate that high consumption of foods rich in bioactive compounds has a positive effect on human health and could diminish the risk of numerous diseases, such as cancer, heart disease, stroke, Alzheimer's disease, diabetes, cataracts, and even diseases related to age. From all species of fruit, definitely consumption of berries due to its high content of bioactive constituents prevents the risk of cardiovascular disease, oxidative stress and diabetes. The primary phenolic compounds in berries are flavonoids, particularly the anthocyanins. They have potential preventative and therapeutic effects on many diseases such as cancers, inflammation and cardiovascular diseases, obesity, neurodegenerative pathologies, and muscular degeneration. Bilberry fruits have been an important part of local diets in many countries, including Slovakia. They are valued for their pleasant taste and aroma and are often processed into jams, preserves, juices, and alcoholic beverages. In the last two decades, the Saskatoon berry has been cultivated in many parts of the world for its suitability for various food products and due to its high content of nutrients and polyphenols. Cardiovascular disease (CVD) remains the world's leading cause of morbidity and mortality. Dyslipidemia, which results from one or more abnormalities of blood lipids metabolism, remains a major key factor for progression of CVD and leads to the development of atherosclerotic plaques. The aim of this review is to compare and summarize the research evidence on the potential of bilberries and saskatoon berries with an emphasis on recent studies in humans in improving cardiovascular risk factors especially dyslipidemia.

Key words: *cardiovascular disease, lipid profile, bilberry, saskatoon berry, anthocyanins*

INTRODUCTION

Phytonutrients or phytochemicals are natural bioactive compounds obtained from plants that perform specific biological activities and modify different physiological functions to improve general human health [10, 77]. The mechanisms of action for the various compounds, especially as related to reduced risk of disease in individuals, are not fully understood [8, 31, 90].

Fruits are rich sources of numerous classes of biologically active compounds [49, 89]. From all species of fruit, definitely consumption of berries due to its high content of bioactive constituents prevents the risk of numerous diseases, such as cancer, cardiovascular disease, Alzheimer's, diabetes, cataracts, and even diseases related to age [4, 5, 12, 32, 58, 65, 68, 75, 78, 30, 95, 96, 100]. They are considered to be a good source of phenolic compounds, especially flavonoids and phenolic acids, which mostly contribute to their high antioxidant activity [5, 30, 78, 96]. Many of the health benefits, associated with berry fruits, may be linked to their high content of anthocyanins [54].

Bilberry (*Vaccinium myrtillus* L.) fruits have been an important part of local diets in many countries, including Slovakia. They are valued for their pleasant taste and aroma and are often processed into jams, preserves, juices, and alcoholic beverages. They are rich in anthocyanins which make for the intense dark purple coloration of the fruit, as well as all processed foods made from the berries. Their high market value is caused by their relatively difficult availability - bilberry bushes only grow in wild, montane areas. It is not possible to cultivate them due to very specific soil demands and the fruit harvesting is a tedious, tiring work, as it is done using either hands or small harvesting rakes [71, 94, 106].

In the last two decades, the saskatoon berry (*Amelanchier alnifolia*) has been cultivated in many

Corresponding author: Jana Mrázová, Slovak University of Agriculture in Nitra, Faculty of Agrobiology and Food Resources, Institute of Nutrition and Genomics, Tr. A. Hlinku 2, 949 76 Nitra, Slovak Republic, phone: +421 37 641 4223, e-mail: jana.mrazova@uniag.sk

parts of the world [84] for its suitability for various food products and due to its high content of nutrients and polyphenols [59]. Up to now saskatoon berry has been used as an ornamental plant species in Slovak and Czech Republic. Recently, the cultivation of *Amelanchier* combining decorative quality and high biological value of fruit has been gaining in popularity [46].

The aim of this review is to compare and summarize the research evidence on the potential of bilberries and saskatoon berries with an emphasis on recent studies in humans in improving cardiovascular risk factors especially dyslipidemia.

BOTANICAL NOMENCLATURE AND DEFINITION

Bilberry (*Vaccinium myrtillus* L.)

Among the colorful berries, *Vaccinium corymbosum* (called American the blueberry), and the wild-growing blueberry *Vaccinium myrtillus* L. (called bilberry) are popularly used in the human diet either fresh or in processed forms [19]. Both bilberries and blueberries belongs to the family Ericaceae, subfamily Vaccinoideae, genus Vaccinium, which includes approximately 450 species [33, 76, 79, 84, 85]. The bilberry (*Vaccinium myrtillus* L.) is a low-growing shrub native to northern Europe, but is now also found in parts of North America and Asia. Bilberry is also known as European blueberry, whortleberry, huckleberry and blaeberry. Bilberries are sometimes also called as blueberries because both have similar appearance and are close relatives, but the true blueberry is native to the United States [28, 29, 93]. These berries are very similar, but considerably differ from each other in some qualitative parameters, market availability and price [104].

Saskatoon berry (*Amelanchier alnifolia* Nutt.)

Saskatoon berry plant (*Amelanchier alnifolia* Nutt.) also referred to as saskatoon, chuckley pear, juneberry, western juneberry, serviceberry, pacific serviceberry, western serviceberry, alder-leaf shadbush, dwarf shadbush, prairie berry, and pigeon berry, is a type of tall shrub naturally growing in western regions of North America [46, 57, 87, 101], cultivated in many regions of the world [64]. Saskatoon berry is a native species of the North American plains. In the last two decades, his cultivation has expanded from North America to various countries in Asia and Europe, including Finland, Poland, and Czech Republic [102].

Although the Saskatoon berry appears similar to the blueberry, they are more closely related to the apple family and belong to the family *Rosacea* [34, 50, 57]. Other fruits belonging to *Rosaceae* family include apples, pears, prunes, plums, cherries, apricots, strawberries, raspberries and blackberries [63]. The fruit is a berry-like pome, red to purple to nearly black at maturity, 5–15 mm in diameter, insipid to delectably sweet, maturing at the end of June/beginning of July [63, 87].

NUTRITIONAL VALUE OF BILBERRY FRUIT AND SASKATOON BERRY

The chemical composition and quality of berries are variable, qualitatively and quantitatively depending on numerous factors, e.g. cultivar, geographic origin, climatic conditions, maturity at harvest and storage conditions [23, 104].

Generally, 100 g of fresh Vaccinium berries contains water (84%), carbohydrates (9.7%), proteins (0.6%), fat (0.4%) and 0.3 g of ash [65, 78]. Similarly, chemical studies on saskatoon berries have shown that water is their major constituent followed by carbohydrates. They contain about 82–84% water, 15–20% sugar, small amounts of protein and fat [45, 46, 57, 59, 63, 64]. *Mazza* et al. [63] reported that saskatoon fruits are slightly lower in water content than blueberries (79.6% vs. 84.2%).

Dietary fibre content in blueberries and bilberries varies between 3-3.5% of fruit weight [48, 78], they contain pectin, hemicellulose, and cellulose, the hemicellulose being mostly xylan [35]. Likewise, saskatoon contains a fair amount of fiber [45, 46, 57, 59, 63, 64], fresh saskatoonberries contain more fiber than blueberries [21].

Vaccinium myrtillus contain relatively higher levels of organic acids (e.g., citric and ascorbic acids), [48, 68, 78]. Organic acids account for 0.5% of the fresh weight of ripe saskatoon berries [81]. A considerably high content of minerals (e.g., phosphorus, potassium, and magnesium) are also found in bilberries [48, 78]. Saskatoon contains relatively large amounts of potassium, iron, magnesium and phosphorous calcium, copper, manganese and potassium [45, 46, 57, 59, 61, 64]. On average, saskatoon berries have significantly higher iron, magnesium, potassium, calcium and phosphorus levels than blueberries [21].

The vitamins C, thiamin, riboflavin, pantothenic acid, nicotinamide and β-carotene have been reported in fresh bilberry fruit [69]. Vitamins found in saskatoon berries include ascorbic acid, thiamin, riboflavin, pantothenic acid, pyridoxine, folic acid, tocopherols [45, 57, 59, 63]. Although saskatoonberries are not one of the best sources of vitamins, their vitamin content is similar to that of blueberries [63]. In comparison with blueberries, saskatoon serviceberry fruits contain a higher content of thiamin, and riboflavin [63].

Many phenolic compounds have been identified in both bilberries and saskatoon berries and differences in their phenolic profiles have been observed and linked to numerous factors. [14, 64, 88]. The primary phenolic compounds in berries are flavonoids, particularly the anthocyanins (mainly cyanidins) [59, 63, 73, 88, 102]. Bilberry has higher anthocyanin content compared to other types of berries, such as strawberry, cranberry, elderberry and raspberry [2, 13, 15, 47, 51, 56]. The total anthocyanin concentration in saskatoon berry is comparable to that of wild blueberry and higher than that in other small fruited species such as raspberry, sea buckthorn, chokeberry, and strawberry [46, 102]. The total anthocyanin content of bilberry is generally in the range of 300 -700 mg per 100 g fresh fruit, although this range varies with cultivar, growing conditions and degree of ripeness of the berry [9, 92]. The European wild-type bilberry is generally known to have a higher content of anthocyanins than blueberries [28, 29]. Anthocyanin content of saskatoon berries ranges from 25 to 179 mg/100 g of berries [63]. On the other hand, the studies of *Zatylny* et al. [101] proved that the anthocyanin content in 16 cultivars of saskatoons was not to as high as in blueberries.

The anthocyanins extracted from blueberries and bilberries so far are not unique in their chemistry and are generally of the 3-O-glycoside derivatives of cyanidin, delphinidin, malvidin, and petunidin [28, 29]. Among berries, the blueberry fruit stands out due to the presence of different types of anthocyanins, including malvidin, delphinidin, petunidin, cyanidin and peonidin, with the sugar moieties of glucose, galactose and arabinose [65]. The most common encountered in blueberry are malvidin and delphinidin and might constitute almost 75% of all identified anthocyanins [28, 29, 65]. There are at least four anthocyanins in ripe saskatoon berries of which cyanidin 3-galactoside and 3-glucoside account for about 61% and 21% of the total anthocyanins, respectively. Saskatoonberries contain a distinct spectrum of anthocyanins that is different from blueberries, the major components being cyanidin-3-galactoside, cyanidin-3-glucoside, cyanidin-3-arabinoside, and cyanidin-3-xyloside [3, 73]. All major saskatoonberry anthocyanins are cyanidin glycosides [21, 102].

Other groups of phenolics identified in bilberries are flavonols, flavan-3-ols, and hydroxycinnamic acids [14, 42, 55, 66, 67, 88], considerable amounts of stilbenes (resveratrol) [80] and catechin [20, 92]. Other phenolic compounds of saskatoon characterized include phenolic acids, including 3-feruloylquinic, chlorogenic, and 5-feruloylquinic acids [45, 57, 59, 81], rutin [73, 101] and different quercetin glycosides [46, 73, 84, 101].

BILBERRY AND SASKATOON BERRY IN THE PREVENTION AND TREATMENT OF DYSLIPIDEMIA

Because of the fruit's composition, mainly concerning the content of bioactive compounds, especially polyphenols, the berries can play a very beneficial antioxidant, anti-inflammatory, antitumor, hypoglycemic, antidiabetic, antiradical [3, 24, 46, 63, 82, 101], antifungal, anti-hypertensive, anti-allergic and antiviral role [61]. They have potential preventative and therapeutic effects on many diseases such as cancers, inflammation and cardiovascular diseases, obesity, neurodegenerative pathologies, and muscular degeneration [45, 54, 73].

Cardiovascular disease (CVD) remains the world's leading cause of morbidity and mortality [7, 62]. Although multiple risk factors for progression of CVD, dyslipidemia, which results from one or more abnormalities of blood lipids metabolism, remains a major key factor for this pathology and leads to the development of atherosclerotic plaques [44, 99]. Dyslipidemia is the imbalance of lipids such as cholesterol, low-density lipoprotein cholesterol, (LDL-C), triglycerides, and high-density lipoprotein (HDL-C) [74]. High HDL-C, triglyceride (TG) and low LDL-C concentrations are risk factors for cardiovascular diseases [36, 86]. Hypercholesterolemia was reported as the highest attributable risk factor for atherosclerosis and subsequent coronary heart disease (CHD) in a given population [22, 43]. LDL-C has now largely replaced total cholesterol (T-C) as the primary lipid measurement for evaluation of risk due to atherogenic lipoproteins. LDL-C is a measure of the T-C content of LDL particles, reflecting both the number of LDL particles and their individual cholesterol content [39]. A reduction in serum cholesterol is strongly associated with a reduction in CVD risk [83]. From a public health perspective, lifestyle modification, including dietary changes, is considered a first step in controlling and treating CVD risk factors [41].

Food rich to polyphenols are beneficial in the prevention of the cardiovascular diseases, they are connected with lower risk of stroke, ischemic heart disease, inflammatory markers and oxidation stress, type 2 diabetes even the diseases related to age and with it related memory imparement [31].

Some epidemiological studies suggest that anthocyanins attenuate the development of atherosclerotic cardiovascular diseases [16, 37, 103]. Anthocyanins are powerful antioxidants that can neutralize free radicals [11, 25, 40, 91]. In addition to their antioxidant effects, anthocyanins have been reported to suppress lipid peroxidation, stabilize DNA, modify adipocyte gene expression, improve

insulin secretion and sensitivity, and have anti-carcinogenic, anti-inflammatory, and antibacterial effects [53, 56, 84]. The most common forms of anthocyanin are cyanidin-3-glucoside, cyanidin-3-galactoside and delphinidin-3-glucoside. These three compounds interfere at multiple points in the progression of cardiovascular disease [37, 103].

From all species of fruit, definitely berries shows according to its high content of anthocyanins significant cardio-protective effect [4, 56, 65, 92]. Consumption of fresh bilberry has an important influence on the prophylaxis and progression of CVD due to its antioxidant properties and antiplatelet activity [16, 72]. Acute consumption of berries ameliorates postprandial glycemic response, improves profile of circulating inflammatory markers and increases antioxidative capacity of plasma. Long-term intake of berries and berry products may improve plasma lipid profile, reduce chronic inflammation and support cardiovascular health, especially in population with baseline metabolic profile of increased risk for metabolic syndrome [98]. The principal mechanisms of action underlying the potential cardioprotective effects of berries include counteracting free radical generation, attenuating inflammatory gene expression, down-regulating foam cell formation, and up-regulating endothelial nitric oxide synthase expression [4, 103]. Bilberry and anthocyanin supplementation have been shown to ameliorate hyperlipidemia in both animals and humans [16, 40].

A majority of the existing literature is based on in vitro and animal studies. Human intervention trials are necessary to confirm the health effects of the berries. Regular consumption of berries and/or berry products may improve plasma lipid profile and reduce the occurrence of metabolic syndrome and CVD which was confirmed by several studies. The randomized controlled clinical trial by *Karlsen* et al. [50] on subjects taking either bilberry juice or water for 4 weeks were directed to assess the effect of bilberry polyphenols supplementation on inflammation. They have shown that bilberry juice could decrease the plasma concentrations of CRP, interleukin (IL)-6 and IL-15. Another randomized controlled trial was by *Basu* et al. [4] on 48 subjects with metabolic syndrome who were taking freeze-dried blueberry beverage (50 g freeze-dried blueberries, approximately 350 g fresh blueberries) or equivalent amounts of fluids (controls, 960 ml water) daily for eight weeks. In this study, blueberry supplementation was shown to decrease systolic and diastolic blood pressures without altering the serum glucose concentration and lipid profile. The study by *Lehtonen* et al. [60] followed a randomized cross-over study design in Finland in which the only difference between the intervention (33–35 days) and wash-out (30–39 days) periods was the bilberry products consumed. There was statistically significant decrease in waist circumference and body weight after bilberry diet. A further randomized, controlled dietary intervention trial study in Finland was by *Kolehmainen* et al. [52] in which participants consumed either a diet rich in bilberries or a control diet. The bilberry group consumed daily an equivalent dose of 400 g fresh bilberries, while the control group maintained their habitual diet. After eight weeks of dietary intervention and four weeks recovery period, no differences were found between the groups in body weight, glucose, or lipid metabolism, but bilberry supplementation tended to decrease serum high-sensitivity CRP, IL-6 and IL-12 concentrations. In a subsequent study involving 122 hypercholesterolemic subjects, supplementation for 24 weeks with anthocyanins (320 mg/day) purified from bilberry and blackcurrant enhanced the HDL-associated protein paraoxonase 1 (PON1) activity, increased the antioxidant effects and enhanced the cholesterol efflux capacity of HDL, and resulted in an increase in HDL cholesterol and decrease in LDL cholesterol concentrations [105]. The study by *Habanova* et al. [27] on women and men taking 150 g of frozen stored bilberries three times a week for six weeks showed that regular intake of bilberries in women was associated with a decrease TG, LDL-C, glucose, and a positive increase in HDL-C. In men, favorable changes were observed in T-C, glucose and HDL-C. *Huang* et al. [38] conducted a meta-analysis with a sequential trial analysis in order to estimate the effect of berry consumption on CVD risk factors and observed that LDL-C was significantly lower for subjects who consumed the berries than for the placebo-treated subjects. The study by de *Mello* et al. [18] was based on eight weeks intervention study on 47 metabolic syndrome patients who were randomized to receive either of bilberry or placebo diet or a control diet. They have shown that fasting serum hippuric acid is increased after consumption of anthocyanin-rich bilberries, and may contribute to the beneficial effect of bilberry consumption. *Arevström* et al. [1] hypothesized that standard medical therapy supplemented with freeze-dried bilberry after acute myocardial infarction (AMI) would have a more beneficial effect on cardiovascular risk markers and exercise capacity than medical therapy alone. The study conducted by *Curtis* et al. [17] showed no favorable effects of the intervention on glucose, insulin resistance index (HOMA-IR), and glycated hemoglobin (HbA1c). In this study, which lasted 24 weeks, a trend towards a dose-related increase in HDL-C was obtained. However, the intervention had no effect on T-C and LDL-C

after the consumption of freeze-dried blueberry (150 g/day or 75 g/day). In addition, TG levels differed significantly in the group treated with 75 g/day of blueberries, compared with the placebo. The study of *Habanova* et al. [26] investigating the effect of berries/apple juice consumption on human lipid profile of healthy volunteers (36 women and 14 men). The consumption of 300 mL juice/day resulted in a significant decrease of T-C and LDL-C levels (only for men group), and significantly increased HDL-C and total antioxidant status. Finally the study of *Chan* et al. [40] showed that bilberry supplementation (1.4 g/day of anthocyanins extract) for 4 weeks had atendency to reduce HbA1c; however, no significant improvement in glycemic control, cardiovascular risk, antioxidant-oxidative stress, or inflammatory status was observed.

To date, there are very few human studies examining the effect of phytonutrients from saskatoon berry on the risk factors of cardiovascular diseases. Saskatoon berry has potential preventative and therapeutic effects on diseases such as diabetes, cancers, inflammatory and cardiovascular diseases, obesity, neurodegenerative pathologies, and muscular degeneration [45, 57, 73, 102]. *Wang* et al. [97] found that a concentrated crude extract of *Amelanchier alnifolia* berries inhibited nitric oxide production in activated macrophages, indicating a potential protective role against chronic inflammation. Results from animal study demonstrated hypoglycemic and hypolipidemic effects of saskatoon berry [102].

CONCLUSION

In the past years, a growing demand for healthy food has been noted in the market. Consumers are primarily interested in food, which is appealing and helps in preventing various diseases and contains high levels of promoted bioactive compounds. Bilberries and saskatoon berry have achieved a superfood status with implications of beneficial health outcomes under a variety of pathological conditions. The polyphenolic composition dominated by anthocyanins are the primary active components for these health claims. Berries have recently received much attention for their health benefits, including antimutagenesis and anticarcinogenic activity for the prevention of various cancers and age-related diseases. This review to demonstrate the beneficial effects of bilberry and saskatoon bery supplementation on risk factors of cardiovascular diseases. Based on these results, regular consumption of fresh berries and/or berry products may improve plasma lipid profile and reduce the occurrence of metabolic syndrome and cardiovascular diseases.

Acknowledgments

This work was supported by the Ministry of Education, Science, Research and Sport of the Slovak Republic project VEGA 1/0159/21 Determination of effects of biologically active substances of small fruit on health of consumers (50%), the Operational Programme Integrated Infrastructure for the project: Long-term strategic research of prevention, intervention and mechanisms of obesity and its comorbidities, IMTS: 313011V344, co-financed by the European Regional Development Fund (50%).

Conflict of interest

The authors declare no conflict of interest.

REFERENCES

1. *Arevström L., Bergh C., Landberg R., Wu H., Rodriguez-Mateos A., Waldenborg M., Magnuson A., Blanc S., Fröbert O.:* Freeze-dried bilberry (Vaccinium myrtillus) dietary supplement improves walking distance and lipids after myocardial infarction: an open-label randomized clinical trial. Nutr Res 2019;62:13-22. doi: 10.1016/j.nutres.2018.11.008.
2. *Bagchi D., Sen C.K., Bagchi M., Atalay M.:* Anti-angiogenic, antioxidant, and anti-carcinogenic properties of a novel anthocyanin-rich berry extract formula. Biochemistry 2004;69(1):75-80, 1 p preceding 75. doi: 10.1023/b:biry.0000016355.
3. *Bakowska-Barczak A.M., Kolodziejczyk P.:* Evaluation of Saskatoon berry (Amelanchier alnifolia Nutt.) cultivars for their polyphenol content, antioxidant properties, and storage stability. J Agric Food Chem 2008;56(21):9933-40. doi: 10.1021/jf801887w. 39.
4. *Basu A., Du M., Leyva M.J., Sanchez K., Betts N.M., Wu M.:* Blueberries decrease cardiovascular risk factors in obese men and women with metabolic syndrome. J Nutr 2010;140 (9):1582-1587.
5. *Battino M., Beekwilder J., Denoyes-Rothan B., Laimer M., McDougall G.J., Mezzetti B.:* Bioactive compounds in berries relevant to human health. Nutr Rev 2009;67 Suppl 1:S145-50. doi: 10.1111/j.1753-4887.2009.00178.x.
6. *Bergh C., Fall K., Udumyan R., Sjöqvist H., Fröbert O., Montgomery S.:* Severe infections and subsequent delayed cardiovascular disease. Eur J Prev Cardiol 2017;24(18):1958-1966. doi: 10.1177/2047487317724009.
7. *Brandhorst S., Longo VD.:* Dietary Restrictions and Nutrition in the Prevention and Treatment of Cardiovascular Disease. Circ Res 2019;124(6):952-965. doi: 10.1161/CIRCRESAHA.118.313352.
8. *Bubalo M. C., Vidović S., Radojčić Redovniković I., Jokić S.:* New perspective in extraction of plant biologically active compounds by green solvents. Food and Bioproducts Processing 2018;108:52-73. doi:10.1016/j.fbp.2018.03.001.

9. *Burdulis D., Sarkinas A., Jasutiené I., Stackevicené E., Nikolajevas L., Janulis V.:* Comparative study of anthocyanin composition, antimicrobial and antioxidant activity in bilberry (Vaccinium myrtillus L.) and blueberry (Vaccinium corymbosum L.) fruits. Acta Pol Pharm 2009;66(4):399-408.
10. *Carbonell-Capella J.M., Buniowska M., Barba F.J., Esteve M.J., Frígola A.:* Analytical Methods for Determining Bioavailability and Bioaccessibility of Bioactive Compounds from Fruits and Vegetables: A Review. Compr Rev Food Sci Food Saf 2014;13(2):155-171. doi: 10.1111/1541-4337.12049.
11. *Cassidy A., Bertoia M., Chiuve S., Flint A., Forman J., Rimm E.B.:* Habitual intake of anthocyanins and flavanones and risk of cardiovascular disease in men. Am J Clin Nutr 2016;104(3):587-94. doi: 10.3945/ajcn.116.133132.
12. *Çelik H.:* The performance of some northern highbush blueberry [Vaccinium corymbosum L.] varietis in North eastern part of Anatolia. Anatolian Journal of Agricultural Sciences 2009;24(3):141-146.
13. *Cocetta G., Karppinen K., Suokas M., Hohtola A., Häggman H., Spinardi A., Mignani I., Jaakola L.:* Ascorbic acid metabolism during bilberry (Vaccinium myrtillus L.) fruit development. J Plant Physiol 2012;169(11):1059-65. doi: 10.1016/j.jplph.2012.03.010.
14. *Colak N., Torun H., Gruz J., Strnad M., Hermosín-Gutiérrez I., Hayirlioglu-Ayaz S., Ayaz F.A.:* Bog bilberry phenolics, antioxidant capacity and nutrient profile. Food Chem 2016;15;201:339-49. doi: 10.1016/j.foodchem.2016.01.062.
15. *Cravotto G., Boffa L., Genzini L., Garella D.:* Phytotherapeutics: an evaluation of the potential of 1000 plants. J Clin Pharm Ther 2010;35(1):11-48. doi: 10.1111/j.1365-2710.2009.01096.x.
16. *Crespo M.C., Visioli F.:* A Brief Review of Blue- and Bilberries' Potential to Curb Cardio-Metabolic Perturbations: Focus on Diabetes. Curr Pharm Des 2017;23(7):983-988. doi: 10.2174/1381612822666161010120523.
17. *Curtis P.J., van der Velpen V., Berends L., Jennings A., Feelisch M., Umpleby A.M., Evans M., Fernandez B.O., Meiss M.S., Minnion M., Potter J., Minihane A.M., Kay C.D., Rimm E.B., Cassidy A.:* Blueberries improve biomarkers of cardiometabolic function in participants with metabolic syndrome-results from a 6-month, double-blind, randomized controlled trial. Am J Clin Nutr 2019;109(6):1535-1545. doi: 10.1093/ajcn/nqy380.
18. *de Mello V.D., Lankinen M.A., Lindström J., Puupponen-Pimiä R., Laaksonen D.E., Pihlajamäki J., Lehtonen M., Uusitupa M., Tuomilehto J., Kolehmainen M., Törrönen R., Hanhineva K.:* Fasting serum hippuric acid is elevated after bilberry (Vaccinium myrtillus) consumption and associates with improvement of fasting glucose levels and insulin secretion in persons at high risk of developing type 2 diabetes. Mol Nutr Food Res 2017;61(9). doi: 10.1002/mnfr.201700019.
19. *Dróżdż P., Šėžienė V., Pyrzynska K.:* Mineral Composition of Wild and Cultivated Blueberries. Biol Trace Elem Res 2018;81(1):173-177. doi: 10.1007/s12011-017-1033-z.
20. *Erlund I., Marniemi J., Hakala P., Alfthan G., Meririnne E., Aro A.:* Consumption of black currants, lingonberries and bilberries increases serum quercetin concentrations. Eur J Clin Nutr 2003;57(1):37-42. doi: 10.1038/sj.ejcn.1601513.
21. *Fang J.:* Nutritional composition of saskatoonberries - a review. Botany 2021; 99(4). doi:10.1139/cjb-2019-0191.
22. *Ference B.A., Ginsberg H.N., Graham I., Ray K.K., Packard C.J., Bruckert E., Hegele R.A., Krauss R.M., Raal F.J., Schunkert H., Watts G.F., Borén J., Fazio S., Horton J.D., Masana L., Nicholls S.J., Nordestgaard B.G., van de Sluis B., Taskinen M.R., Tokgözoglu L., Landmesser U., Laufs U., Wiklund O., Stock J.K., Chapman M.J., Catapano A.L.:* Low-density lipoproteins cause atherosclerotic cardiovascular disease. 1. Evidence from genetic, epidemiologic, and clinical studies. A consensus statement from the European Atherosclerosis Society Consensus Panel. Eur Heart J 2017;21;38(32):2459-2472. doi: 10.1093/eurheartj/ehx144.
23. *Gołba M., Sokół-Łętowska A., Kucharska A.Z.:* Health Properties and Composition of Honeysuckle Berry *Lonicera caerulea* L. An Update on Recent Studies. Molecules 2020;25(3):749. doi: 10.3390/molecules25030749.
24. *Green R.C, Mazza G.:* Relationships Between Anthocyanins, Total Phenolics, Carbohydrates, Acidity and Colour of Saskatoon Berries. Canadian Institute of Food Science and Technology Journal 1986;19(3):107-113. doi:10.1016/S0315-5463[86]71463-6.
25. *Grosso G., Micek A., Godos J., Pajak A., Sciacca S., Galvano F., Giovannucci E.L.:* Dietary Flavonoid and Lignan Intake and Mortality in Prospective Cohort Studies: Systematic Review and Dose-Response Meta-Analysis. Am J Epidemiol 2017;185(12):1304-1316. doi: 10.1093/aje/kww207.
26. *Habanova M., Saraiva J. A., Holovicova M., Moreira S. A., Fidalgo L. G., Haban M., Gazo J., Schwarzova M., Chlebo P., Bronkowska M.:* Effect of berries/apple mixed juice consumption on the positive modulation of human lipid profile. Journal of Functional Foods 2019;60:1756-4646. doi:10.1016/j.jff.2019.103417.
27. *Habanova M., Saraiva J.A., Haban M., Schwarzova M., Chlebo P., Predna L., Gažo J., Wyka J.:* Intake of bilberries (Vaccinium myrtillus L.) reduced risk factors for cardiovascular disease by inducing favorable changes in lipoprotein profiles. Nutr Res 2016;36(12):1415-1422. doi: 10.1016/j.nutres.2016.11.010.
28. *Habtemariam S.:* Bilberries and blueberries as potential modulators of type 2 diabetes and associated diseases. Medicinal Foods as Potential Therapies for Type-2 Diabetes and Associated Diseases, 2019; 135-175. doi:10.1016/B978-0-08-102922-0.00007-9.

29. *Habtemariam S.:* Medicinal Foods as Potential Therapies for Type-2 Diabetes and Associated Diseases. London, Academic Press, 2019.
30. *Halilova H., Ercisli S.:* Several Physico-Chemical Characteristics of Cherry Laurel (*Laurocerasos Officinalis* Roem.) Fruits, Biotechnology & Biotechnological Equipment 2010;24(3):1970-1973, doi:10.2478/V10133-010-0059-6.
31. *Halliwell B.:* Dietary polyphenols: good, bad, or indifferent for your health? Cardiovasc Res 2007;15;73(2):341-7. doi: 10.1016/j.cardiores.2006.10.004.
32. *Hassimotto N. M. A., Genovese M. I., Lajolo, F.M.* Antioxidant Capacity of Brazilian Fruit, Vegetables and Commercially-Frozen Fruit Pulps. J Food Compos Anal 2009; 22:394-396.
33. *Heinonen I.M., Meyer A.S, Frankel E.N.:* Antioxidant Activity of Berry Phenolics on Human Low-Density Lipoprotein and Liposome Oxidation. J Agric Food Chem 1998;46(10):4107-4112. doi:10.1021/jf980181c.
34. *Hellström J., Sinkkonen J., Karonen M., Mattila P.:* Isolation and structure elucidation of procyanidin oligomers from Saskatoon berries (Amelanchier alnifolia). J Agric Food Chem 2007;5(1):157-64. doi: 10.1021/jf062441t.
35. *Hilz H., Bakx E.J., Schols H.A., Voragen A.G.J.:* Cell wall polysaccharides in black currants and bilberries characterisation in berries, juice, and press cake. Carbohydr Polymers 2005; 59(4):477-488. doi:10.1016/j.carbpol.2004.11.002
36. *Hokanson J., E., Austin M. A.:* Plasma triglyceride level is a risk factor for cardiovascular disease independent of high density lipoprotein-cholesterol level: A meta-analysis of population-based prospective studies. J Cardiovasc Risk 1996;3(2), 213-219. doi:10.1097/00043798-199604000-00014.
37. *Hosseinian F.S., Beta T.:* Saskatoon and wild blueberries have higher anthocyanin contents than other Manitoba berries. J Agric Food Chem 2007;55(26):10832-8. doi: 10.1021/jf072529m.
38. *Huang H., Chen G., Liao D., Zhu Y., Xue, X.:* Effects of berries consumption on cardiovascular risk factors: A meta-analysis with trial sequential analysis of randomized controlled trials. Scientific Reports 2016;6(1), 23625. https://doi.org/10.1038/srep23625.
39. *Hughes J., Kee F., O'Flaherty M.:* Modelling coronary heart disease mortality in Northern Ireland between 1987 and 2007: Broader lessons for prevention. Eur J Prev Cardiol 2013;20:310-321. doi:10.1177/2047487312441725.
40. *Chan S.W., Tomlinson B.:* Effects of Bilberry Supplementation on Metabolic and Cardiovascular Disease Risk. Molecules 2020;25(7):1653. doi: 10.3390/molecules25071653.
41. *Chen Z.Y., Jiao R., Ma K.Y.:* Cholesterol-lowering nutraceuticals and functional foods. J Agric Food Chem 2008;56(19):8761-73. doi: 10.1021/jf801566r.
42. *Jaakola L., Määttä-Riihinen K., Kärenlampi S., Hohtola A.:* Activation of flavonoid biosynthesis by solar radiation in bilberry (Vaccinium myrtillus L) leaves. Planta 2004;218(5):721-8. doi: 10.1007/s00425-003-1161-x.
43. *Jacobson T. A., Maki K. C., Orringer C. E., Jones P. H., Kris-Etherton P., Sikand G., La Forge R., Daniels S. R., Wilson D. P., Morris P. B., Wild R. A., Grundy S. M., Daviglus M., Ferdinand K. C., Vijayaraghavan K., Deedwania P. C., Aberg J. A., Liao K. P., McKenney J. M., Ross J. L., Braun L.T., Ito M. K., Bays H. E., Brown W. V., Underberg J. A.:* NLA Expert Panel. National Lipid Association Recommendations for Patient-Centered Management of Dyslipidemia: Part 2. J Clin Lipidol. 2015 Nov-Dec;9(6 Suppl):S1-122.e1. doi: 10.1016/j.jacl.2015.09.002.
44. *Jain K.S., Kathiravan M.K., Somani R.S., Shishoo C.J.:* The biology and chemistry of hyperlipidemia. Bioorg Med Chem 2007;15(14):4674-99. doi: 10.1016/j.bmc.2007.04.031.
45. *Jin A.L., Ozga J.A., Kennedy J.A., Koerner-Smith J.L., Botar G., Reinecke D.M.:* Developmental profile of anthocyanin, flavonol, and proanthocyanidin type, content, and localization in saskatoon fruits (Amelanchier alnifolia Nutt.). J Agric Food Chem 2015;63(5):1601-14. doi: 10.1021/jf504722x.
46. *Juríková T., Balla S., Sochor J., Pohanka M., Mlcek J., Baron M.:* Flavonoid profile of Saskatoon berries (Amelanchier alnifolia Nutt.) and their health promoting effects. Molecules 2013;18(10):12571-86. doi: 10.3390/molecules181012571.
47. *Kalkan Yildirim H.:* Evaluation of colour parameters and antioxidant activities of fruit wines. Int J Food Sci Nutr 2006;57(1-2):47-63. doi: 10.1080/09637480600655993.
48. *Kalt W., MacKinnon S., McDonald J., Vinqvist M., Craft C., Howell A.:* Phenolics of Vaccinium berries and other fruit crops. J Sci Food Agric 2008;88:68-76. doi: 10.1002/jsfa.2991.
49. *Karasawa M.M.G., Mohan C.:* Fruits as Prospective Reserves of bioactive Compounds: A Review. Nat Prod Bioprospect 2018;8(5):335-346. doi: 10.1007/s13659-018-0186-6.
50. *Karlsen A., Paur I., Bøhn S.K., Sakhi A.K., Borge G.I., Serafini M., Erlund I., Laake P., Tonstad S., Blomhoff R.:* Bilberry juice modulates plasma concentration of NF-kappaB related inflammatory markers in subjects at increased risk of CVD. Eur J Nutr 2010;49(6):345-55. doi: 10.1007/s00394-010-0092-0.
51. *Khattab R., Brooks M. S. L., Ghanem A.:* Phenolic Analyses of Haskap Berries (Lonicera caerulea L.): Spectrophotometry Versus High Performance Liquid Chromatography, International Journal of Food Properties 2016;19(8):1708-1725, doi: 10.1080/10942912.2015.1084316.
52. *Kolehmainen M., Mykkänen O., Kirjavainen P.V., Leppänen T., Moilanen E., Adriaens M., Laaksonen D.E., Hallikainen M., Puupponen-Pimiä R., Pulkkinen L., Mykkänen H., Gylling H., Poutanen K., Törrönen R.:* Bilberries reduce low-grade inflammation in individuals with features of metabolic syndrome. Mol Nutr Food Res 2012;56(10):1501-10. doi: 10.1002/mnfr.201200195.

53. *Kong J.M., Chia L.S., Goh N.K., Chia T.F., Brouillard R.:* Analysis and biological activities of anthocyanins. Phytochemistry 2003;64(5):923-33. doi: 10.1016/s0031-9422(03)00438-2.
54. *Konic-Ristic A., Savikin K., Zdunic G., Jankovic T., Juranic Z., Menkovic N.:* Biological activity and chemical composition of different berry juices. Food Chemistry 2011;125(4):1412e1417.
55. *Koskimaki J. J., Hokkanen J., Jaakola L., Suorsa M., Tolonen A., Mattila S.:* Flavonoid biosynthesis and degradation play a role in early defence responses of bilberry [Vaccinium myrtillus] against biotic stress. Eur J Plant Pathol 2009;125:629-640. doi:10.1007/s10658-009-9511-6.
56. *Kowalczyk E., Krzesiński P., Kura M., Szmigiel B., Błaszczyk J.:* Anthocyanins in medicine. Pol J Pharmacol 2003;55(5):699-702.
57. *Lachowicz S., Oszmiański J., Seliga Ł., Pluta S.:* Phytochemical Composition and Antioxidant Capacity of Seven Saskatoon Berry (Amelanchier alnifolia Nutt.) Genotypes Grown in Poland. Molecules 2017;22(5):853. doi: 10.3390/molecules22050853.
58. *Landete J.M.:* Updated knowledge about polyphenols: functions, bioavailability, metabolism, and health. Crit Rev Food Sci Nutr 2012;52(10):936-48. doi: 10.1080/10408398.2010.513779.
59. *Lavola A., Karjalainen R., Julkunen-Tiitto R.:* Bioactive polyphenols in leaves, stems, and berries of Saskatoon (Amelanchier alnifolia Nutt.) cultivars. J Agric Food Chem 2012;60(4):1020-7. doi: 10.1021/jf204056s
60. *Lehtonen H.M., Suomela J.P., Tahvonen R., Yang B., Venojärvi M., Viikari J., Kallio H.:* Different berries and berry fractions have various but slightly positive effects on the associated variables of metabolic diseases on overweight and obese women. Eur J Clin Nutr 2011;65(3):394-401. doi: 10.1038/ejcn.2010.268.
61. *Loza-Mejía M.A., Salazar J.R.:* Sterols and triterpenoids as potential anti-inflammatories: Molecular docking studies for binding to some enzymes involved in inflammatory pathways. J Mol Graph Model. 2015;62:18-25. doi: 10.1016/j.jmgm.2015.08.010.
62. *Mahmoudi M.:* The Pathogenesis of Atherosclerosis. Medicine 2018;46:505-508.
63. *Mazza G.:* Chemical Composition of Saskatoon Berries [Amelanchier alnifolia Nutt.]. Journal of Food Science 2006;47:1730-1731. doi:10.1111/j.1365-2621.1982.tb05022.x.
64. *Meczarska K., Cyboran-Mikolajczyk S., Wloch A., Bonarska-Kujawa D., Oszmianski J., Kleszczynska H.:* Polyphenol content and bioactivity of Saskatoon berry [Amelanchier alnifolia nutt.] leaves and berries. Acta Pol Pharm 2017;74(2):660-669.
65. *Michalska A., Łysiak G.:* Bioactive Compounds of Blueberries: Post-Harvest Factors Influencing the Nutritional Value of Products. Int J Mol Sci 2015;16(8):18642-63. doi: 10.3390/ijms160818642.
66. *Mikulic-Petkovsek M., Schmitzer V., Slatnar A., Stampar F., Veberic R.:* A comparison of fruit quality parameters of wild bilberry (Vaccinium myrtillus L.) growing at different locations. J Sci Food Agric 2015;5;95(4):776-85. doi: 10.1002/jsfa.6897.
67. *Mikulic-Petkovsek M., Schmitzer V., Slatnar A., Stampar F., Veberic R.:* Composition of sugars, organic acids, and total phenolics in 25 wild or cultivated berry species. J Food Sci 2012;77(10):C1064-70. doi: 10.1111/j.1750-3841.2012.02896.x.
68. *Milivojevic J., Slatnar A., Mikulic-Petkovsek M., Stampar F., Nikolic M., Veberic R.:* The influence of early yield on the accumulation of major taste and health-related compounds in black and red currant cultivars (Ribes spp.). J Agric Food Chem 2012;14;60(10):2682-91. doi: 10.1021/jf204627m.
69. *Moeck S.:* Vaccinium. In: *Hansel R., Keller K., Rimpler H., Schneider G.,* editors. Hagers Handbuch der Pharmazeutischen Praxis.Volume 6 (P-Z). Berlin, Springer, 1994.
70. *Moyo M., Aremu A.O., Plačková L., Plíhalová L., Pěnčík A., Novák O., Holub J., Doležal K., Staden J.V.:* Deciphering the growth pattern and phytohormonal content in Saskatoon berry (Amelanchier alnifolia) in response to in vitro cytokinin application. N Biotechnol 2018;42:85-94. doi: 10.1016/j.nbt.2018.02.001.
71. *Nestby R., Percival D., Martinussen I., Opstad N., Rohlo J.:* The European Blueberry [Vaccinium myrtillus L.] and the Potential for Cultivation. A Review. Eur J Plant Sci Biotechnol 2011;5:5-16.
72. *Olas B.:* The multifunctionality of berries toward blood platelets and the role of berry phenolics in cardiovascular disorders. Platelets 2017;28(6):540-549. doi: 10.1080/09537104.2016.1235689.
73. *Ozga J.A., Saeed A., Wismer W., Reinecke D.M.:* Characterization of cyanidin- and quercetin-derived flavonoids and other phenolics in mature saskatoon fruits (Amelanchier alnifolia Nutt.). J Agric Food Chem 2007;55(25):10414-24. doi: 10.1021/jf072949b.
74. *Pappan N., Rehman A.:* Dyslipidemia. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing. 2021.
75. *Paredes-López O., Cervantes-Ceja M.L., Vigna-Pérez M., Hernández-Pérez T.:* Berries: improving human health and healthy aging, and promoting quality life--a review. Plant Foods Hum Nutr 2010;65(3):299-308. doi: 10.1007/s11130-010-0177-1.
76. *Patel S.:* Blueberry as functional food and dietary supplement: the natural way to ensure holistic health. Med J Nutri Metab 2014;7(2):133-143. doi: 10.3233/MNM-140013.
77. *Prakash D., Gupta K. R.* The Antioxidant Phytochemicals of Nutraceutical Importance. The Open Nutraceuticals Journal 2009;2:20-35.
78. *Prior R.L., Cao G., Martin A., Sofic E., McEwen J., Obrien C., Lischner N., Ehlenfeldt M., Kalt W., Krewer G., Mainland C.M.:* Antioxidant Capacity as Influenced by Total Phenolic and Anthocyanin Content, Maturity, and Variety of Vaccinium Species. Journal of Agricultural and Food Chemistry 1998;46(7):2686-2693. doi:10.1021/jf980145d.

79. *Riihinen K., Jaakola L., Kärenlampi S., Hohtola A.:* Organ-specific distribution of phenolic compounds in bilberry (Vaccinium myrtillus) and 'northblue' blueberry (Vaccinium corymbosum x V. angustifolium). Food Chem 2008;110(1):156-60. doi: 10.1016/j.foodchem.2008.01.057.
80. *Rimando A.M., Kalt W., Magee J.B., Dewey J., Ballington J.R.:* Resveratrol, pterostilbene, and piceatannol in vaccinium berries. J Agric Food Chem 2004;52(15):4713-9. doi: 10.1021/jf040095e.
81. *Rogiers S.Y., Knowles, N.R.:* Physical and chemical changes during growth, maturation, and ripening of saskatoon (Amelanchier alnifolia) fruit. Can J Bot 1997;75(8): 1215-1225.
82. *Rop O., Reznicek V., Mlcek J., Jurikova, T., Sochor J., Kizek R., Humpolicek P., Balik J.:* Nutritional values of new Czech cultivars of Saskatoon berries (*Amelanchier alnifolia* Nutt.). *Hortic Sci* 2012;39(3):123-128.
83. *Sabatine M.S., Wiviott S.D., Im K., Murphy S.A., Giugliano R.P.:* Efficacy and Safety of Further Lowering of Low-Density Lipoprotein Cholesterol in Patients Starting With Very Low Levels: A Meta-analysis. JAMA Cardiol 2018;3(9):823-828. doi: 10.1001/jamacardio.2018.2258.
84. *Seeram N.P.:* Berry fruits: compositional elements, biochemical activities, and the impact of their intake on human health, performance, and disease. J Agric Food Chem 2008;13;56(3):627-9. doi: 10.1021/jf071988k.
85. *Smith M. A. I., Marley K. A., Seigler D., Singletary K. W., Meline B.:* Bioactive Properties of Wild Blueberry Fruits. Journal of Food Science 2000;65(2):352-356. doi:10.1111/j.1365-2621.2000.tb16006.x.
86. *Steinberger J., Moorehead C., Katch V., Rocchini A.P.:* Relationship between insulin resistance and abnormal lipid profile in obese adolescents. J Pediatr 1995;126(5 Pt 1):690-5. doi: 10.1016/s0022-3476(95)70394-2.
87. *St-Pierre R., Zatylny A., Tulloch H.:* Evaluation of growth and fruit production characteristics of 15 saskatoon [Amelanchier alnifolia Nutt.] cultivars at maturity. Canadian Journal of Plant Science 2005;85. doi:10.4141/P04-066.
88. *Su Z.:* Anthocyanins and Flavonoids of Vaccinium L. Pharmaceutical Crops 2012;3, 7-37.
89. *Taïwe G.S., Kuete V.:* KUETEENGLISH. Medicinal spicies and vegetables from South Africa, Elsevier, 2017.
90. *Thodberg S., Del Cueto J., Mazzeo R., Pavan S., Lotti C., Dicenta F., Jakobsen Neilson E.H., Møller B.L., Sánchez-Pérez R.:* Elucidation of the Amygdalin Pathway Reveals the Metabolic Basis of Bitter and Sweet Almonds (*Prunus dulcis*). Plant Physiol 2018;178(3):1096-1111. doi: 10.1104/pp.18.00922.
91. *Tran P.H.L., Tran T.T.D.:* Blueberry Supplementation in Neuronal Health and Protective Technologies for Efficient Delivery of Blueberry Anthocyanins. Biomolecules 2021;11(1):102. doi: 10.3390/biom11010102.
92. *Upton R.:* Bilberry fruit Vaccinium myrtillus L. standards of analysis, quality control, and therapeutics. Santa Cruz, CA, American Herbal Pharmacopoeia and Therapeutic Compendium. 2000.
93. *Valentova K., Ultrichova J., Cvak L., Simanek V.:* Cytoprotective effect of a bilberry extract against oxidative damage of rat hepatocytes. Food Chemistry 2006;101:912-917.
94. *Vaneková Z., Vanek M., Škvarenina J., Nagy M.:* The Influence of Local Habitat and Microclimate on the Levels of Secondary Metabolites in Slovak Bilberry (*Vaccinium myrtillus* L.) Fruits. Plants (Basel) 2020;9(4):436. doi: 10.3390/plants9040436.
95. *Veberic R., Jakopic J., Stampar F., Schmitzer V.:* European elderberry [Sambucus nigraL.] rich in sugars, organic acids, anthocyanins and selected polyphenols. Food Chemistry 2009;114:511-515.
96. *Vesna T., Jasna Č.B., Lars G., Sonja D., Gordana Ć.:* Superoxide anion radical scavenging activity of bilberry [*Vaccinium myrtillus* L.]. J Berry Res; 2010;1:13-23.
97. *Wang X., Ouyang Y., Liu J., Zhu M., Zhao G., Bao W., Hu F.B.:* Fruit and vegetable consumption and mortality from all causes, cardiovascular disease, and cancer: systematic review and dose-response meta-analysis of prospective cohort studies. BMJ 2014;349:g4490. doi: 10.1136/bmj.g4490.
98. *Yang B., Kortesniemi M.:* Clinical evidence on potential health benefits of berries. Current Opinion in Food Science 2015;2:36-42. doi:10.1016/j.cofs.2015.01.002.
99. *Yusuf S., Hawken S., Ounpuu S., Dans T., Avezum A., Lanas F., McQueen M., Budaj A., Pais P., Varigos J., Lisheng L.:* INTERHEART Study Investigators. Effect of potentially modifiable risk factors associated with myocardial infarction in 52 countries (the INTERHEART study): case-control study. Lancet 2004;364(9438):937-52. doi: 10.1016/S0140-6736(04)17018-9.
100. *Zafra-Stone S., Yasmin T., Bagchi M., Chatterjee A., Vinson J.A., Bagchi D.:* Berry anthocyanins as novel antioxidants in human health and disease prevention. Mol Nutr Food Res 2007;51(6):675-83. doi: 10.1002/mnfr.200700002.
101. *Zatylny A., Ziehl W., St-Pierre R.:* Physicochemical properties of fruit of 16 saskatoon [Amelanchier alnifolia Nutt.] cultivars. Canadian Journal of Plant Science 2005;85: doi:10.4141/P04-065.
102. *Zhao L., Huang F., Hui A.L., Shen G.X.:* Bioactive Components and Health Benefits of Saskatoon Berry. J Diabetes Res 2020;3901636. doi: 10.1155/2020/3901636.
103. *Zhao R., Xie X., Le K., Li W., Moghadasian M.H., Beta T., Shen G.X.:* Endoplasmic reticulum stress in diabetic mouse or glycated LDL-treated endothelial cells: protective effect of Saskatoon berry powder and cyanidin glycans. J Nutr Biochem 2015;26(11):1248-53. doi: 10.1016/j.jnutbio.2015.05.015.

104. *Zhao, Y.:* Berry fruit. Value-added products for health promotion [Food Science and Technology] [1st ed.]. New York, CRC Press, 2007.
105. *Zhu Y., Huang X., Zhang Y., Wang Y., Liu Y., Sun R., Xia M.:* Anthocyanin supplementation improves HDL-associated paraoxonase 1 activity and enhances cholesterol efflux capacity in subjects with hypercholesterolemia. J Clin Endocrinol Metab 2014;99(2):561-9. doi: 10.1210/jc.2013-2845.
106. *Zoratti L., Klemettilä H., Jaakola L.:* Bilberry [Vaccinium myrtillus L.] Ecotypes. Nutr Compos Fruit Cultiv 2016;83-99.

Received: 16.05.2022
Accepted: 21.06.2022
Published online first: 05.07.2022

Rocz Panstw Zakl Hig 2022;73(3):275-283
http://wydawnictwa.pzh.gov.pl/roczniki_pzh/ https://doi.org/10.32394/rpzh.2022.0219

ORIGINAL ARTICLE

NUTRITIONAL STATUS OF THE ELDERLY IN POLAND

Ewa Rychlik[1], *Agnieszka Woźniak*[1], *Katarzyna Stoś*[1], *Maciej Ołtarzewski*[1]

[1]National Institute of Public Health NIH - National Research Institute,
Department of Nutrition and Nutritive Value of Food, Warsaw, Poland

This study was carried out as part of the following projects:

- Support to National Dietary Surveys in Compliance with the EU Menu methodology (fith support). Lot 2 – "The adults'survey", including subjects from 10 to 74 years old (No OC/EFSA/DATA/2015/03 CT 3);
- Assessment of dietary patterns, nutritional status and selected lifestyle elements of selected population groups in Poland (project of NIPH NIH – NRI FŻ-1/2022).

A financial grant was also awarded by the Polish Ministry of Science and Higher Education to maintain research capacity (No 3876/E-220/S/2018-1).

ABSTRACT

Background. The elderly are at greater risk of underweight and the associated risk of protein and energy malnutrition. On the other hand, the lower energy requirement with an often too high intake from the diet leads to the development of overweight and obesity.

Objective. The aim of the study was to assess the prevalence of underweight, overweight and obesity, including abdominal obesity in Polish elderly.

Material and methods. The study included 300 men and 304 women aged 65 and over from all over the country. The nutritional status was assessed on the basis of anthropometric measurements: body height and weight as well as waist and hip circumferences. Based on BMI (Body Mass Index), the prevalence of underweight (<20.0), overweight (25.0-29.9) and obesity (≥30.0) was assessed. WHR (Waist-to-Hip Ratio) was used to assess abdominal obesity (≥1.0 in men and ≥0.85 in women). Waist circumference was also analysed with regard to increased risk of metabolic complications (≥94 cm in men and ≥80 cm in women).

Results. Underweight was found in 1.3% of men and 4.3% of women. 55.3% of men and 40.1% of women were overweight, 20.3% and 21.7% were obese, respectively. In the case of people with excess body weight, abdominal obesity was observed in 50% of men and 70.1% of women. Waist circumference indicating an increased risk of metabolic complications was found in 44.1% of men and 67.5% of women.

Conclusions. The prevalence of overweight and obesity in Polish elderly was high, especially in men. Overweight and obese people often had abdominal obesity. This type of obesity was more common in women. Elderly people, especially women, often have an increased risk of metabolic complications due to high fat accumulation in the abdomen. It was even found in elderly who were not overweight nor obese. Some elderly, mostly women, were underweight which increased the risk of protein and energy malnutrition.

Key words: *elderly, underweight, overweight and obesity, risk of metabolic complications*

STRESZCZENIE

Wprowadzenie. Osoby starsze są bardziej narażone na występowanie niedoboru masy ciała i związane z tym ryzyko niedożywienia energetyczno-białkowego. Z kolei mniejsze zapotrzebowanie organizmu na energię przy często zbyt wysokim jej spożyciu z diety sprzyja rozwojowi nadwagi i otyłości.

Cel. Celem pracy była ocena częstości występowania niedoboru masy ciała oraz nadwagi i otyłości, w tym otyłości brzusznej, u osób starszych w Polsce.

Materiał i metody. Badaniem objęto 300 mężczyzn i 304 kobiety w wieku 65 lat i więcej z terenu całego kraju. Ocenę stanu odżywienia badanych przeprowadzono na podstawie pomiarów antropometrycznych: wysokości i masy ciała oraz obwodów talii i bioder. Na podstawie wskaźnika BMI oceniono występowanie niedoboru masy ciała (<20,0), nadwagi (25,0-29,9) i otyłości (≥30,0). Wskaźnik WHR służył do oceny otyłości brzusznej (≥1,0 u mężczyzn i ≥0,85 u kobiet). Analizowano również obwód talii związany ze zwiększonym ryzykiem zaburzeń metabolicznych (≥94 cm u mężczyzn i ≥80 cm u kobiet).

Corresponding author: Ewa Rychlik, National Institute of Public Health NIH - National Research Institute, Department of Nutrition and Nutritive Value of Food, Chocimska 24, 00-791 Warsaw, Poland, phone: +48 22 5509821, email: erychlik@pzh.gov.pl

Wyniki. Niedobór masy ciała stwierdzono u 1,3% mężczyzn i 4,3% kobiet. Nadwagą odznaczało się 55,3% mężczyzn i 40,1% kobiet, a otyłością, odpowiednio 20,3% i 21,7%. W przypadku osób z nadmierną masą ciała u 50% mężczyzn i 70,1% kobiet występowała otyłość brzuszna. Obwód talii wskazujący na zwiększone ryzyko powikłań metabolicznych stwierdzono u 44,1% mężczyzn i 67,5% kobiet.
Wnioski. Wśród osób starszych w Polsce, zwłaszcza wśród mężczyzn, często występowała nadwaga lub otyłość. Osoby z nadmierną masą ciała często odznaczały się otyłością brzuszną. Ten typ otyłości częściej występował u kobiet.
U osób starszych, zwłaszcza kobiet, często występowało zwiększone ryzyko powikłań metabolicznych związane z nadmiernym nagromadzeniem tkanki tłuszczowej w okolicy brzucha. Dotyczyło to nawet osób starszych, u których nie występowała nadwaga ani otyłość.
Niektóre osoby starsze, szczególnie kobiety, odznaczały się niedoborem masy ciała, co zwiększało ryzyko niedożywienia energetyczno-białkowego.

Słowa kluczowe: *osoby starsze, niedobór masy ciała, nadwaga i otyłość, ryzyko powikłań metabolicznych*

INTRODUCTION

In many countries, the life expectancy of the population is increasing. According to WHO, life expectancy worldwide has increased by more than 6 years between 2000 and 2019 - from 66.8 years in 2000 to 73.4 years in 2019 [29]. This is due to, inter alia, the improvement of economic and living conditions, earlier diagnosis and better effectiveness of non-communicable diseases treatment.

Due to this situation, the number of elderly people in the society is increasing. This tendency will also continue in the coming years. The same situation is observed in Poland. According to Statistics Poland, in 2020 the share of people aged 60 and more was 25.6% of the country's population [13]. The projection indicates that their share in 2030 will increase to 29%, and in 2050 to 40.4%.

The elderly people are at greater risk of underweight. On the other hand, the lower energy requirement in case of frequent too high energy intake from the diet leads to the development of overweight and obesity [10].

Underweight indicates the risk of energy and protein malnutrition. It may be accompanied by vitamins and minerals deficiencies in the body. Malnutrition occurs when the diet does not supply the body with sufficient quantity and/or quality of essential nutrients [1].

Malnutrition causes functional disturbance of immune system, increasing disability, limitation of mobility, risk of falls, dependence on help from others, and an increased risk of institutionalization [4, 23]. In extreme cases, it may even lead to cachexia [1].

Moreover, poor nutritional status contributes to a worse response to treatment and increases the incidence of complications, which results in prolonged hospitalization and, consequently, an increase in treatment costs [9, 11].

Main causes of overweight and obesity are overconsumption and low physical activity. However, other factors may also contribute to the development of overweight and obesity. These include chronic stress, sleep disturbances, frequent use of electronic devices, and even environmental pollution [2, 21, 24, 31].

The most common consequences of obesity, especially abdominal obesity, include a high risk of metabolic disorders (hyperinsulinemia, incorrect composition of plasma lipids, hyperuricemia and high blood fibrinogen concentration), type 2 diabetes, hypertension and cardiovascular diseases [15, 20].

Excess body weight affects many aspects of life. Obese elderly people report poor quality of life, including impaired physical function, increased body pain, and a lack of vitality [16].

Some studies seem to indicate that being overweight in old age may offer some protective health benefits (obesity paradox) [20]. However, this may be due to the fact that the actual risk is related to visceral obesity, which is not measured by BMI only and may not reflect the protective physiological effect of higher body fat content in later life [6, 7].

The aim of the study was to assess the prevalence of underweight, overweight and obesity, including abdominal obesity in elderly people in Poland. This assessment will allow to identify the risk of health problems related to improper nutritional status and the need to implement preventive measures in Polish elderly.

MATERIALS AND METHODS

This study was a part of the Nationwide Dietary Survey that was carried out on a representative sample of Polish adolescent and adult population (n=2,432) from July 2019 to February 2020. The sample selection for the study was done by the stratified sampling method using the PESEL (in Polish: Powszechny Elektroniczny System Ewidencji Ludności) system taking into account such demographic details as age, gender and place of residence. The study was approved by the Bioethics Committee at the Institute of Food and Nutrition in Warsaw, Poland (opinion dated 4 June 2018). Participation in the study was voluntary. Written informed consent was obtained from each respondent. For the assessment of the prevalence of underweight, overweight and obesity, including abdominal obesity in elderly people in Poland were included 300 men and

304 women aged 65 and over from all over the country in two age group: 65-74 years and 75 years or more.

Anthropometric measurements were carried out in each of the respondents: body height and weight as well as waist and hip circumferences. Anthropometric measurements were made by qualified personnel with the use of certified devices in appropriate conditions. Based on the results of anthropometric measurements, Body Mass Index (BMI) and Waist-to-Hip Ratio (WHR) were calculated. BMI was calculated as weight divided by height squared, WHR – by dividing waist circumference by hip circumference.

The nutritional status was assessed based on the BMI value. The criteria recommended by WHO were adopted, however, the opinion of experts was taken into account that in the elderly, underweight should be assessed at a higher BMI. The same cut-off points were used as in the NU AGE study: for underweight (<20.0 kg/m^2), normal weight (20.0-24.9 kg/m^2), overweight (25.0-29.9 kg/m^2) and obesity (≥30.0 kg/m^2) [19].

The WHR was used to assess abdominal obesity in overweight or obese people (≥1.0 in males and ≥0.85 in females). The risk of metabolic complications in studied subjects was estimated. It was assessed on the basis of the waist circumference as increase (≥94 cm in males and ≥80 cm in females) and substantially increased (≥102 cm in males and ≥88 cm in females). For waist circumference and WHR, cut-off points developed by WHO were used [30].

Statistical analysis

The results were statistically tested using a computer software PQStat 1.8.2. In order to verify whether the distribution was normal, the *Shapiro-Wilk* test was used. The significance of differences was assessed using *Student's* t-test for normal distribution of data or the *Mann-Whitney* U test for nonparametric data and the chi-square for qualitative data. Relationships between the sex and other parameters were examined using the *Pearson* correlation (parametric data) or *Spearman* correlation (nonparametric data). For all analyses, the significance level α=0.05 was assumed.

RESULTS

The mean age of men and women was similar (74.0±5.8 and 74.1±6.1 years, respectively). A significant relationship was observed between sex and body weight (r=-0.4541, p<0.0001), height (rs=-0.7214, p<0.0001), waist circumference

Table 1. Anthropometric parameters of men and women

Parameter	Men (n=300)			Women (n=304)			M vs W
	X±SD	Median	min-max	X±SD	median	min-max	p*
Age (years)	74±5.8	74.5	65-95	74.1±6.1	74.5	65-96	0.9191
Weight (kg)	82.9±11.5	84	54-120	71.1±11.8	70	45-105	<0.0001
Height (cm)	174.5±5.9	175	155-189	163.3±5.5	164	150-179	0.0001
BMI (kg/m^2)	27.2±3.6	26.9	16.7-38.9	26.6±4.2	26.2	16.1-41	0.0239
Waist circumference (cm)	92.8±13.4	90	59-135	85.5±14.4	85.7	42-138	0.0001
Hip circumference (cm)	97.6±12.3	96	68-148	98.5±15.4	98	45-159	0.188
WHR**	0.95±0.08	0.94	0.72-1.29	0.87±0.09	0.87	0.58-1.21	0.0001

X±SD – mean ± standard deviation, min-max – minimum – maximum, M vs W – men versus women
* *Student's* t-test or *Mann-Whitney* U test, statistically significant difference - p<0.05
** respondents with overweight or obesity

Table 2. Assessment of anthropometric parameters of men and women

Parameter	Interpretation	Men		Women		M vs W
		n	%	n	%	p*
BMI	Underweight	4	1.3	13	4.3	0.0004
	Normal weight	69	23.0	103	33.9	
	Overweight	166	55.3	122	40.1	
	Obesity	61	20.3	66	21.7	
WHR**	Without abdominal obesity	111	50	55	29.9	<0.0001
	Abdominal obesity	111	50	129	70.1	
Risk of metabolic complications	Low	165	55.9	95	32.5	<0.0001
	Increased	58	19.7	68	23.3	
	Substantially increased	72	24.4	129	44.2	

M vs W – men versus women; *chi-square test, statistically significant difference - p<0.05,
** respondents with overweight or obesity

(rs=-0.2479, p<0.0001) as well as BMI (-0.0919, p=0.0238) and WHR (-0.4379, p<0.0001). The mean values of these parameters were lower in women (Table 1).

Most of the men and women were overweight or obese (75.6% of men and 61.8% of women). Only a few percent of respondents were underweight (1.3% of men and 4.3% of women). A significant relationship was observed between sex and BMI status (rs=-0.0981, p=0.0158). The prevalence of overweight was greater in men than in women (55.3% vs 40.1%) and more women than men had normal body weight (33.9% vs 23.0%) (Table 2).

A significant relationship was also observed between sex and the prevalence of abdominal obesity or the risk of metabolic complications (rs=0.2036, p<0.0001; rs=0.2461, p<0.0001, respectively). Among respondents with excess body weight, the abdominal obesity was much more common in women than men (70.1% vs 50.0%). In a greater percentage of women than men the risk of increased or substantially increased metabolic complications was found (23.3% vs 19.7%, 44.2% vs 24.4%).

Men and women aged ≥75 years had lower height and weight than men and women aged 65-74 years. In women aged ≥75 years lower hip circumference was also observed than in younger group of women (Table 3). In both age groups higher values of anthropometric parameters such as body weight, height, waist circumference and WHR were observed in men compered to women.

In both men and women, the prevalence of underweight, overweight and obesity did not differ significantly between the age groups of 65-74 years and ≥75 years. The differences between the prevalence of abdominal obesity in men aged 65-74 years and 75 years or more were also not significant. Among women, this type of obesity was much more common in subjects aged ≥75. The differences between the percentage of males and females at risk of metabolic complications in two analyzed age groups were not significant (Table 4). In both age groups the prevalence of overweight was greater in men than in women while more women than men had a normal body weight. The percentage of men and women with abdominal obesity in the age group of 65-74 years did not differ but in the age group of ≥75 years the percentage of women with this type of obesity was much higher than men (81.1% vs 47.1%, p<0.0001). In both age groups more women than men at the risk of substantially increased metabolic complications was observed. Furthermore, in the age group of ≥75 years the percentage of women at increased metabolic complications was higher than men.

Increased or substantially increased risk of metabolic complications occurred mainly among overweight and obese people: in 54.7% of males

Table 3. Anthropometric parameters of men and women in the age groups of 65-74 and ≥75 years

Parameter	Men (n=300)					Women (n=304)					M vs W 65-74 years	M vs W ≥75 years
	65-74 years (n=150)		≥75 years (n=150)		65-74 years vs ≥75 years	65-74 years (n=152)		≥75 years (n=152)		65-74 years vs ≥75 years		
	X±SD median	min-max	X±SD median	min-max	p*	X±SD median	min-max	X±SD median	min-max	p*	p*	p*
Weight (kg)	84.4±11.2 85	55-120	81.4±11.6 80	54-120	0.0075	72.8±11.8 71	45-105	69.3±11.6 69	45-96	0.0113	<0.0001	<0.0001
Height (cm)	175.4±6.2 176	159-189	173.5±5.4 174.5	155-184	0.0093	164.3±5 164	152-179	162.4±5.7 162	150-179	0.0017	0.0001	0.0001
BMI (kg/m^2)	27.5±3.5 27.1	18.6-37.9	27±3.6 26.7	16.7-38.9	0.196	26.9±4.15 26.3	17.8-41	26.3±4.2 26.1	16.1-38	0.1651	0.1082	0.0937
Waist (cm)	93.6±13 93	61-135	92.1±13.9 90	59-135	0.2121	86.1±12.7 87.5	56-120	84.9±15.8 84.5	42-138	0.4539	<0.0001	0.0001
Hip (cm)	98.6±12.4 98	73-144	96.7±12.1 95	68-148	0.1845	100.3±14.1 99	59-140	96.7±16.5 97	45-159	0.0453	0.2759	0.6937
WHR**	0.95±0.08 0.95	0.72-1.13	0.95±0.08 0.94	0.72-1.29	0.8044	0.86±0.09 0.86	0.64-1.21	0.88±0.08 0.89	0.6-1.15	0.129	<0.0001	0.0001

X±SD – mean ± standard deviation, min-max – minimum – maximum, M vs W – men versus women, * *Student's* t-test or *Mann-Whitney* U test, statistically significant difference - p<0.05, ** respondents with overweight or obesity

Table 4. Assessment of anthropometric parameters of men and women in the age groups of 65-74 and ≥75 years

Parameter	Interpretation	Men					Women					M vs W 65-74 years	M vs W ≥75 years
		65-74 years		≥75 years		65-74 years vs ≥75 years	65-74 years		≥75 years		65-74 vs ≥75 years		
		n	%	n	%	p*	n	%	n	%	p*	p*	p*
BMI	Underweight	2	1.3	2	1.3	0.6735	5	3.3	8	5.3	0.7271	0.023	0.0262
	Normal weight	30	20	39	26		49	32.2	54	35.5			
	Overweight	86	57.3	80	53.3		63	41.4	59	38.8			
	Obesity	32	21.3	29	19.3		35	23.1	31	20.4			
WHR**	Without abdominal obesity	54	47.8	57	52.9	0.5021	38	40.4	17	18.9	0.0014	0.2886	<0.0001
	Abdominal obesity	59	52.2	52	47.1		56	59.6	73	81.1			
Risk of metabolic complications	Low	75	51.7	90	60	0.2954	46	31.5	49	33.5	0.0736	0.0001	<0.0001
	Increased	33	22.8	25	16.7		27	18.5	41	28.1			
	Substantially increased	37	25.5	35	23.3		73	50	56	38.4			

M vs W – men versus women, *chi-square test, statistically significant difference - $p<0.05$, ** respondents with overweight or obesity

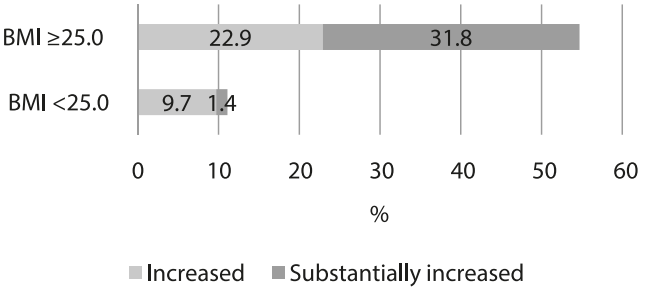

Figure 1. Increased or substantially increase risk of metabolic complications in males according to BMI

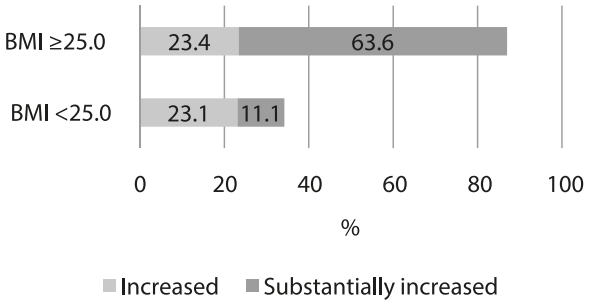

Figure 2. Increased or substantially increased risk of metabolic complications in females according to BMI

(Figure 1) and 87% of females (Figure 2). However, some subjects, especially women, with BMI below 25.0 kg/m^2 also have increased (9.7% of males and 23.1% of females) or substantially increased (respectively 1.4% and 11.1%) risk of metabolic complications.

DISCUSSION

The analysis of anthropometric measurements of the studied subjects showed that the mean and median BMI in men and women exceeded 25.0 kg/m^2 – a value indicating the presence of excess body weight. The mean and median WHR values in men were less than 1.0, indicating abdominal obesity. In women, these values exceeded 0.85, the cut-off point for abdominal obesity. The mean and median waist circumference of men exceeded 92 cm, and women – 80 cm, values indicating an increased risk of metabolic complications.

A representative PolSenior 2 study conducted in 2018-2019 [8] showed that the average BMI was 28.5 in men and 29.3 kg/m^2 in women aged ≥60 years. Waist circumference was respectively: 103.6 and 97.4 cm. Similar trends were noted earlier in a nationwide representative survey carried out in 2000 [27]. Among subjects aged 60 and more, the average BMI was 26.6 in men and 28.4 kg/m^2 in women, average WHR – 0.94 and 0.85 respectively and average waist circumference – 94.7 and 92.3 cm. High average values of BMI (28.3 in males and 28.8 kg/m^2 in females) and waist

circumference (103.7 and 96.3 cm respectively) were also noted among the rural elderly from the Oleśnica district, in the Lower Silesia Voivodship (southern region of Poland) [32].

The comparison of data on the nutritional status of older people is difficult due to different criteria for this group used by some authors. WHO has not established recommendations regarding the use of different BMI values in older than younger people [28]. Nevertheless, some authors use higher BMI values as cut-off points for underweight, overweight, and obesity in the elderly [3, 14].

Different criteria relate primarily to the classification of underweight. In our study, we adopted BMI <20.0 kg/m^2, similar to the NU AGE study [19], assuming that when used at older age, BMI <18.5 kg/m^2 may not allow for a proper assessment of the problem.

Some studied subjects were underweight, although the percentage was not high (1.3% of males and 4.3% of females). Underweight occurred mainly in women.

Among those surveyed in 2000, underweight was also rare: in 1.8% of men and 1.1% of women, although it was classified at BMI <18.5 kg/m^2 according to WHO interpretation [27].

Some authors believe that higher BMI values should be used as a cut-off point for underweight. In the study in Drawsko Pomorskie (Poland) no subject was underweight according to WHO interpretation of BMI, while 17% were underweight according to Queensland Government (QG) interpretation (<23.0 kg/m^2) [5].

An analysis of data on the nutritional status of all adults participating in the Nationwide Dietary Survey in Poland showed the prevalence of overweight and obesity increased with age [26]. However, the data presented in this manuscript showed that the changes after the age of 75 were not significant.

Excess body weight in studied elderly aged ≥65 years was very common (75.6% of males and 61.8% of females).

In the WOBASZ II study conducted in 2013/14, excess body weight was found in 78.3% of men and 82.6% of women aged 65-74 and respectively in 69.1% and 77.4% of subjects aged ≥75 years [25]. In the PolSenior2 study obesity in people aged 60 and more was found in 31.3% of men and 38.7% of women, while overweight – in 42.4% and 35.1%, respectively. Excess body weight was most common at the age of 65-69, and its prevalence clearly decreased after the age of 85 [8].

High prevalence of overweight and obesity in Poland was also noted in previous years. Survey carried out in 2000 showed that overweight was present in 46.1% of men and 35.5% of women over 60 years of age, obesity in 19.3% and 37.2%, respectively [27].

Changes in obesity with age in Poland are similar to those in Europe, the incidence of obesity increases with age, peaking around 60 years. Subsequently, body weight does not change much and begins to decline in older age [20].

The high prevalence of overweight and obesity in the elderly is also indicated by studies of selected groups from Polish towns The analysis of nutritional status of the elderly from Drawsko Pomorskie indicated that 29% of examined subjects (males and females) were overweight and 42% were obese [5]. Among the residents of nursing homes and a day-care center in Nysa aged ≥60 years overweight was found in 40% of men and 37.3% of women, and obesity in 30% and 39.2%, respectively [33]. Among patients of the health and rehabilitation centre in Mielno-Uniescie 50% of males and 13% of females were overweight while 50% of males and 79.9% of females had obesity [12]. In studies of elderly people carried out in Lublin 24.7% of men and 43.9% of women were obese [22].

The authors of above-mentioned studies used the same criteria to assess the prevalence of overweight and obesity as in this manuscript.

Abdominal obesity was common among overweight and obese subjects (50.0% of males and 70.1% of females). It was found more often in women. Moreover, in women after the age of 75, the percentage of subjects with abdominal obesity increased significantly.

Among the subjects studied in 2000, the incidence of abdominal obesity was also analysed, but slightly different criteria were adopted (WHR ≥0.95 in males and ≥0.80 in females). Abdominal obesity has been found in 44.6% of overweight or obese men aged 60 or over. Women with excess body weight had it much more often – 84.4% [17].

Accumulation of fatty tissue around the abdomen indicates an increased risk of developing metabolic complications. It is determined by the waist circumference which gives a better prediction of visceral and even total fat and of disease risks than waist to hip ratio [15].

An increased or substantially increased risk of metabolic complications was found in a large group of respondents (44.1% of males and 67.5% of females). Among women, this risk was most often substantially increased. It should be emphasized that an increased or even substantially increased risk of metabolic complications also occurred in subjects whose BMI did not indicate excess body weight.

The frequent occurrence of an increased risk of metabolic complications related to the accumulation of visceral adipose tissue among the elderly was confirmed by the WOBASZ II study from 2013-2014. Increased risk has been found in 23.5% and 25.4% of males aged 65-74 and ≥75 years and in 17.3% and 12.3% of females respectively. There was a very high

percentage of the elderly with a substantially increased risk: 53.7% and 44.4% of men aged 65-74 and ≥75 years and in 74.5% and 75.9% of women respectively [25].

In a 2000 study, waist circumference indicating a substantially increased risk of metabolic complications was also found in a large proportion of the elderly population (≥60 years): 24.9% of men and 63.1% of women [17].

Regional studies also indicate high risk of metabolic complications. In studies conducted in Lublin nearly 86% of men of 93% of women had too large waist circumference [22].

In the above-mentioned studies, as in the studies presented in this manuscript, the risk of developing metabolic disorders was found more often in women than in men.

Our research confirmed that obesity is a serious problem in the elderly. Decreased physical activity and reduced energy expenditure promote the accumulation of adipose tissue [16]. On the other hand, muscle mass decreases, so BMI may not fully reflect the increase in body fat. Also, lower height resulting from vertebral compression and kyphosis affects the relationship between BMI and adipose tissue [22]. Waist circumference is a better indication of obesity. Therefore, some subjects were found to have too large waist circumference, even though their BMI did not indicate overweight nor obesity.

Underweight among the respondents was much less frequent than excess body weight, but this problem cannot be underestimated. Underweight can lead to malnutrition, which is associated with many serious health problems, especially in old age.

In the elderly, in order to assess the occurrence of malnutrition, it is advisable to conduct detailed assessment, based not only on BMI values. The PolSenior2 study showed that over a quarter of Polish residents aged 60 or more had a poor nutritional status (PNS). The prevalence of risk of malnutrition was estimated at 25.3%, while malnutrition at 2.8% based on the Mini Nutritional Assessment – Short Form. PNS was most often observed in the oldest age group, more often in females than in males [18].

CONCLUSIONS

Overweight and obesity were very common health disorders in Polish elderly. Overweight was more often found in men, but the prevalence of obesity in both populations was similar. Abdominal obesity was common in people with excess body weight. It was more common in women, especially after the age of 75.

Elderly people, especially women, were often at increased or substantially increased risk of metabolic complications due to excessive accumulation of adipose tissue in the abdomen. It applied not only to overweight and obese persons, but also to some people without excess body weight. Some elderly people, especially women, might be at risk of protein and energy malnutrition related to underweight.

Nutritional status of the elderly should be systematically monitored and therapeutic interventions applied in the event of any irregularities. It is very important to implement health programs to prevent overweight and obesity as well as underweight and possible malnutrition of the elderly. Effective early prevention can significantly improve their quality of life.

Acknowledgements

This work was supported by the European Food Safety Authority (No OC/EFSA/DATA/2015/03 CT 3) and the Polish Ministry of Science and Higher Education (No 3876/E-220/S/2018-1). This study was also performed under the project of the National Institute of Public Health NIH – National Research Institute, Poland (FŻ-1/2022).

Conflict of interest

The authors declare no conflict of interest.

REFERENCES

1. *Agarwal E., Miller M., Yaxley A., Isenring E.:* Malnutrition in the elderly: a narrative review. Maturitas 2013;76(4):296-302. doi: 10.1016/j.maturitas.2013.07.013.
2. *An R., Ji M., Yan H., Guan C.:* Impact of ambient air pollution on obesity: a systematic review. Int J Obes (Lond) 2018;42(6):1112-1126. doi: 10.1038/s41366-018-0089-y.
3. *Babiarczyk B., Turbiarz A.:* Body Mass Index in elderly people - do the reference ranges matter? Prog Health Sci 2012;1(2):58-67.
4. *Besora-Moreno M., Llauradó E., Tarro L., Solà, R.:* Social and Economic Factors and Malnutrition or the Risk of Malnutrition in the Elderly: A Systematic Review and Meta-Analysis of Observational Studies. Nutrients 2020;12(3):737. doi: 10.3390/nu12030737.
5. *Bogacka A., Heberlej A., Usarek A., Okoniewska J.:* Diet and nutritional status of elderly people depending on their place of residence. Rocz Panstw Zakl Hig 2019;70(2):185-193. doi: 10.32394/rpzh.2019.0069.
6. *Bosello O., Vanzo A.:* Obesity paradox and aging. Eat Weight Disord 2021;26(1):27-35. doi: 10.1007/s40519-019-00815-4.
7. *Bowman K., Atkins J.L., Delgado J., Kos K., Kuchel G.A., Ble A., Ferrucci L., Melzer D.:* Central adiposity and the overweight risk paradox in aging: follow-up of 130,473 UK Biobank participants. Am J Clin Nutr 2017;106(1):130-135. doi: 10.3945/ajcn.116.147157.

8. *Brzeziński M., Puzianowska-Kuźnicka M., Błeszyńska E., Kujawska-Donecka H., Bandosz P., Zdrojewski T.:* Nadwaga i otyłość [Overweight and obesity]. In. *Błędowski P., Grodzicki T., Mossakowska M., Zdrojewski T.* (eds.). PolSenior2. Badanie poszczególnych obszarów stanu zdrowia osób starszych, w tym jakości życia związanej ze zdrowiem [PolSenior2. Study of specific areas of the health status of elderly people, including health-related quality of life]. Gdańsk, Gdański Uniwersytet Medyczny, 2021, 433-447. ISBN 978-83-67147-00-2 (in Polish).
9. *Cuervo M., García A., Ansorena D., Sánchez-Villegas A., Martínez-González M., Astiasarán I., Martínez J.:* Nutritional assessment interpretation on 22,007 Spanish community-dwelling elders through the Mini Nutritional Assessment test. Public Health Nutr 2009;12(1):82-90. doi: 10.1017/S136898000800195X.
10. *de Morais C., Oliveira B., Afonso C., Lumbers M., Raats M., de Almeida M.D.:* Nutritional risk of European elderly. Eur J Clin Nutr 2013;67(11):1215-9. doi: 10.1038/ejcn.2013.175.
11. *Dent E., Hoogendijk E.O., Visvanathan R., Wright O.R.L.:* Malnutrition Screening and Assessment in Hospitalised Older People: a Review. J Nutr Health Aging 2019;23(5):431-441. doi: 10.1007/s12603-019-1176-z.
12. *Dymkowska-Malesa M., Swora-Cwynar E., Karczewski J., Grzymisławska M., Marcinkowska E., Grzymisławski M.:* Stan odżywienia i skład ciała osób starszych jako przesłanki do stosowania żywienia dietetycznego [Nutrition status and body composition of elderly patients as indications for dietary management]. Forum Zaburzeń Metabolicznych 2017;8(1):29–35 (in Polish).
13. Główny Urząd Statystyczny, Urząd Statystyczny w Białymstoku. Sytuacja osób starszych w Polsce w 2020 roku [The situation of elderly people in Poland in 2020]. Warszawa, Białystok, 2021 (in Polish).
14. *Grzegorzewska A., Wołejko K., Kowalkowska A.,Kowalczyk G., Jaroch A.:* Proper BMI ranges for the elderly in the context of morbidity, mortality and functional status. Gerontol Pol 2016;24(2):114-118.
15. *Han T.S., Sattar N., Lean M.:* ABC of obesity. Assessment of obesity and its clinical implications. BMJ 2006;333(7570):695-8. doi: 10.1136/bmj.333.7570.695.
16. *Han T.S., Wu F.C., Lean M.E.:* Obesity and weight management in the elderly: a focus on men. Best Pract Res Clin Endocrinol Metab 2013;27(4):509-25. doi: 10.1016/j.beem.2013.04.012.
17. *Jarosz M., Szponar L., Rychlik E., Respondek W., Ołtarzewski M.G., Dzieniszewski J., Wardak J.:* Nadwaga, otyłość, niedożywienie Polaków [Overweight, obesity, malnutrition of Poles]. In: *Jarosz M.* (ed.). Otyłość, żywienie, aktywność fizyczna, zdrowie Polaków [Obesity, nutrition, physical activity, health of Poles]. Warszawa, Instytut Żywności i Żywienia, 2006, 45-114. ISBN 83-86060-67-0 (in Polish).
18. *Krzymińska-Siemaszko R., Deskur-Śmielecka E., Kaluźniak-Szymanowska A., Kaczmarek B., Kujawska-Danecka H., Klich-Rączka A., Mossakowska M., Małgorzewicz S., Dworak L.B., Kostka T., Chudek J., Wieczorowska-Tobis K.:* Socioeconomic Risk Factors of Poor Nutritional Status in Polish Elderly Population: The Results of P olSenior2 Study. Nutrients 2021;13(12):4388. doi: 10.3390/nu13124388.
19. *Marseglia A., Xu W., Fratiglioni L., Fabbri C., Berendsen A.A.M., Bialecka-Debek A., Jennings A., Gillings R., Meunier N., Caumon E., Fairweather-Tait S., Pietruszka B., De Groot L.C.P.G.M., Santoro A., Franceschi C.:* Effect of the NU-AGE Diet on Cognitive Functioning in Older Adults: A Randomized Controlled Trial. Front Physiol 2018;9:349. doi: 10.3389/fphys.2018.00349. eCollection 2018.
20. *Mathus-Vliegen E.M., Basdevant A., Finer N., Hainer V., Hauner H., Micic D., Maislos M., Roman G., Schutz Y., Tsigos C., Toplak H., Yumuk V., Zahorska-Markiewicz B.:* Prevalence, pathophysiology, health consequences and treatment options of obesity in the elderly: a guideline. Obes Facts 2012;5(3):460-83. doi: 10.1159/000341193.
21. *Miller A.L., Lumeng J.C., LeBourgeois M.K.:* Sleep patterns and obesity in childhood. Curr Opin Endocrinol Diabetes Obes 2015;22(1):41-7. doi: 10.1097/MED.0000000000000125.
22. *Mulawka P., Dereziński T., Jaroszyński A.J., Wolf J.:* Rozpowszechnienie otyłości u osób w podeszłym wieku [Prevalence of obesity in elderly]. Forum Medycyny Rodzinnej 2015;9(2):88–90 (in Polish).
23. *Phillips M.B., Foley A.L., Barnard R., Isenring, E.A., Miller M.D.:* Nutritional screening in community-dwelling older adults: a systematic literature review. Asia Pac J Clin Nutr 2010;19(3):440-9.
24. *Sinha R., Jastreboff A.M.:* Stress as a common risk factor for obesity and addiction. Biol Psychiatry 2013;73(9):827-35. doi: 10.1016/j.biopsych.2013.01.032.
25. *Stepaniak U., Micek A., Waśkiewicz A., Bielecki W., Drygas W., Janion M., Kozakiewicz K., Niklas A., Puch-Walczak A., Pająk A.:* Prevalence of general and abdominal obesity and overweight among adults in Poland. Results of the WOBASZ II study (2013-2014) and comparison with the WOBASZ study (2003-2005). Pol Arch Med Wewn 2016;126(9):662-671. doi: 10.20452/pamw.3499.
26. *Stoś K., Rychlik E., Woźniak A., Ołtarzewski M., Jankowski M., Gujski M., Juszczyk G.:* Prevalence and Sociodemographic Factors Associated with Overweight and Obesity among Adults in Poland: A 2019/2020 Nationwide Cross-Sectional Survey. Int J Environ Res Public Health 2022;19(3):1502. doi: 10.3390/ijerph19031502.
27. *Szponar L., Sekuła W., Rychlik E., Oltarzewski M., Figurska K.:* Badania indywidualnego spożycia żywności i stanu odżywienia w gospodarstwach domowych [Household food consumption and anthropometric survey]. Prace IŻŻ 101. Warszawa, Instytut Żywności i Żywienia, 2003. ISBN 83-86060-60-3 (in Polish).
28. WHO Consultation on Obesity & World Health Organization. Obesity: preventing and managing the global epidemic: report of a WHO consultation. WHO Technical Report Series 894. Geneva, 2000. ISBN 9241208945.

29. World Health Organization. GHE: Life expectancy and healthy life expectancy. Available: https://www.who.int/data/gho/data/themes/mortality-and-global-health-estimates/ghe-life-expectancy-and-healthy-life-expectancy (accessed: 05.07.2022).
30. World Health Organization. Waist circumference and waist-hip ratio: report of a WHO expert consultation, Geneva, 8-11 December 2008. Geneva, 2011. ISBN: 9789241501491.
31. *Wright S.M., Aronne L.J.* Causes of obesity. Abdom Imaging 2012;37(5):730-2. doi: 10.1007/s00261-012-9862-x.
32. *Wyka J., Biernat J., Mikołajczak J., Piotrowska E.:* Assessment of dietary intake and nutritional status (MNA) in Polish free-living elderly people from rural environments. Arch Gerontol Geriatr 2012;54(1):44-49. doi: 10.1016/j.archger.2011.02.001.
33. *Zołoteńka-Synowiec M.E., Malczyk E., Całyniuk B., Grzesik I., Hajuga M., Oknińska E.:* Ocena stanu odżywienia i składu ciała osób starszych – pensjonariuszy domów pomocy społecznej i Dziennego Domu Pobytu w Nysie [Assessment of nutritional status and body composition of elderly people living in nursing homes and a day-care center in Nysa, Poland]. Piel Zdr Publ 2018;8(4):245–251 (in Polish).

Received: 15.07.2022
Accepted: 09.08.2022
Published on line first: 12.08.2022

Rocz Panstw Zakl Hig 2022;73(3):285-291
http://wydawnictwa.pzh.gov.pl/roczniki_pzh/ https://doi.org/10.32394/rpzh.2022.0225

ORIGINAL ARTICLE

SUPPLY OF ENERGY AND SELECTED NUTRIENTS IN MEALS CONSUMED BY MOROCCAN STUDENTS AT HOME AND ON A UNIVERSITY CAMPUS

Maria Elarbaoui[1], *Ali Jafri*[2], *Houria Makhlouki*[1], *Basma Ellahi*[3], *Abdelfettah Derouiche*[1]

[1]Laboratory of Biology and Health, Research Unit: Human Nutrition, URAC 34, Faculty of Sciences Ben Msik, Hassan II University of Casablanca, Casablanca, Morocco
[2]Mohammed VI University of Health Sciences, UM6SS, Casablanca, Morocco
[3]Faculty of Health and Social Care, Chester University, Chester, United Kingdom

ABSTRACT

Background. Student life is often accompanied by changes in eating behavior. Adopting a balanced and varied diet and healthy eating habits can promote the health, growth and intellectual development of young people at different stages of life. According to the WHO, a healthy diet helps protect against all forms of malnutrition, as well as against non-communicable diseases. The nutritional and energy intake must follow nutritional norms, for example energy intake must be adapted to expenditure. To avoid excessive weight gain, fat should not exceed 30% of total energy intake

Objective. The goal of this study was to compare energy consumption, macronutrients and selected minerals in food rations consumed by students at university campus and at home.

Materials and Method. The subjects were chosen at random from among volunteer students from Hassan II University in Casablanca. A sample of 130 students (54 women and 76 men) aged 18 to 25, participated in this study. Anthropometric measurements were performed to assess general characteristics, and records of one-day food intakes at university and at the parental home were performed by 24-hour food diary, and as well as conducting face-to-face. Variables were expressed as mean ± standard deviation (SD). The Kolmogorov–Smirnov test was used to check the normality of data.

Results. In accordance with the body mass index classifications, 69.5% of male university students and 77.7% of female were in normal weight categories. The overweight classes were 25.1% and 5.6%, respectively, for men and women. Assessment of the energy and nutritional intakes of university and home meals shows that students consume more calories, protein, carbohydrates, sodium, potassium, and iodine at home than at university, but these contributions remain insufficient in relation to the RDAs. Students consume more fat, especially saturated fatty acids, at university than at home. More than half of students exceed the recommended daily recommendations for saturated fatty acids.

Conclusion. These results indicate that a university students' diet is influenced by their behaviors, attitudes, and knowledge. Hence the importance of nutrition education, based on what foods to consume rather than what foods to avoid, a societal issue that requires a multidisciplinary, multisectoral and culturally appropriate approach.

Key words: *nutrients, intakes, university students, anthropometric measurements, energy consumption*

INTRODUCTION

Good physical activity and proper nutrition are essential for a healthy lifestyle, affecting not only health but also physical and mental development [1]. In a Greek study, it was reported that students who lived away from home developed more unfavorable eating habits than students who lived at home [2]. Bad eating habits have also been observed among Turkish university students [3]. Both of these studies reinforce my previous point that it is not young people who pay less attention to healthier diets/foods, but environmental factors, such as (in this case) being away from home. In Morocco, the nutrition of young adults is not yet qualified, because there are not yet studies to elucidate this aspect.

Consuming a balanced and healthy diet throughout the life-course helps to prevent malnutrition in all its forms as well as a range of noncommunicable diseases (NCDs). However, People are now consuming more foods high in energy, fats, free sugars and salt/sodium, and many people do not eat enough fruit, vegetables and other dietary fibre such as whole grains.

Corresponding author: Maria Elarbaoui, Laboratory of Biology and Health, Research Unit: Human Nutrition, URAC 34, Faculty of Sciences Ben Msik, Hassan II University of Casablanca, Morocco, Avenue Cdt Driss El Harti, B.P 7955, Sidi Othmane, Casablanca /20660, Morocco, e-mail: elarbaonuilamaria@gmail.com or maria.elarbaoui-etu@etu.univh2c.ma

In addition, the exact make-up of a diversified, balanced and healthy diet will vary depending on individual characteristics (e.g. age, gender, lifestyle and degree of physical activity), cultural context, locally available foods and dietary customs [4].

In an American study, it was reported that Americans are also heavily influenced by many factors. Some examples of these influences that contribute to an individual's food choices include individual factors, such as knowledge, personal taste preference, mood, hunger level, health status, special diet requirements, ethnicity...or also environmental factors such as weather etc. Alternatively, advertisements also influence food choices. Restaurants and markets often take advantage of this. On the other hand, the indirect factors outside of one's control may also affect food choices [5].

Morocco has made limited progress towards meeting targets for diet-related non-communicable diseases (NCDs) [6]. This last is located on the southwest coast of the Mediterranean Sea. This country is undergoing a demographic and epidemiological transition. Over during last decades, Morocco has experienced a nutritional transition. The causes of this nutritional transition are not well understood. But, maybe it's about socioeconomic or demographic factors that are related to dietary habits in Morocco [7]. Besides, the country has shown no progress towards the obesity target, with around 32.2% of adult women (aged 18 and over) and 19.4% of adult men living with obesity. The prevalence of obesity in Morocco is higher than the regional average of 18.4% for women and 7.8% for men. At the same time, diabetes is estimated to affect 13.4% of adult women and 14.0% of adult men.' It is therefore not a problem of "young people", but a problem of society in general [6].

The current study's aim was to determine students' nutritional status using anthropometric measurements, as well as compare and analyze their energy and nutritional intakes (protein, carbohydrates, fat, potassium, sodium and iodine) of meals consumed by students at the university and at home, with the standard reference values of energy and individual nutrients of university students.

MATERIAL AND METHODS

Design Study

A cross-sectional study was carried out between December 2017 and March 2018 to collect data. The study was conducted with 130 volunteer students aged 18 to 25 years (76 men and 54 women), enrolled at the Hassan II University of Casablanca, Morocco. Most of the students were from Casablanca. Based on the parameters applied in this research study, the methodology we are using is the convenience and snowballing sampling. The assessment of their daily food intake on campus and in the parental home was carried out by 24-hour food diary. The composition of all food intakes has been photographed. The conversion of the meals into nutritional data was carried out using the food composition table (Ciqual) and the intakes per 100 g of macro- and micronutrients were calculated by the EuroFIR method.

Study participants

The study concerned adults aged 18 to 25 years from the Ben Msik Faculty of Sciences, Hassan II University in Casablanca. Students were healthy adults aged between 18 and 25 years with no history of renal disease.

Inclusion criteria

Subjects aged between 18 and 25 years old.

Exclusion criteria

(1) Students under 18 and over 25 years; (2) Students refusing to sign the informed consent; (3) Students who brought back their 24 hours food records incomplete; (4) Students who are not enrolled at the university or have chronic diseases (illnesses that could affect the results – heart or kidney failure, diabetes, etc); (5) Students being taking antidiuretic drugs the week before the study.

Data collection

The evaluation of the energy and nutritional intakes of student meals taken at university and at home was carried out on a collection of intakes over defined days (one day on campus and one day at home) of 103 students by food diary.

The participants are led to indicate the types and times of consumption of food and drinks with an estimate of the quantities consumed; taking pictures of their meals (at each meal, the participant weighs the food and gives a very detailed description of the dish eaten by photographing it at the beginning). The collection of these data allows the determination of consumption profiles and also to evaluate the different nutritional intakes. In the event that the participants did not photograph the food or meals before consuming them, the quantification is left to the discretion of the investigator.

24 hours food diary

Food recording food journals is considered the gold standard because it provides accurate information on food intake. In this survey, students were asked to write down in the food diary the details of their 24-hour food and drink consumption and the pictures of the catches. The recording is made in real time at the time of food intake.

The Ciqual database

Data conversion is done through a French ANSES - Ciqual food composition table [8]. Database allows the conversion of food into nutrients per 100 g of food which makes it possible to estimate the nutritional and energy intake of the nutrients consumed during the day and therefore the nutritional quality of student meals. The conversion of the data of the nutrients obtained in 100 g by Ciqual into the actual weight consumed is done by means of the EuroFIR calculation method [9]. EuroFIR provides the nutrient content of foods using the calculation methods used to determine the nutrient values of multicomponent foods.

Anthropometric measurements

Anthropometric measurements were taken at the university, according to the World Health Organization (WHO) recommendations, and collected by qualified researchers according to WHO standards [10] Weight was measured by the Omron HBF-511T-E body composition monitor in participants wearing light clothing and barefoot [11]. The height was measured using a stadiometer graduated in centimeters (Seca 213). Body mass index (BMI) was calculated by dividing weight by the square of height (kg / m2), (BMI <18.5 – underweight; 18.5<BMI<25 - normal weight; 25<BMI<30 - overweight; BMI> 30 - obese) [12].

Statistical analysis

SPSS Statistics software (IBM SPSS Statistics 25.0) was used for all statistical analyse [13].Variables were expressed as mean ± standard deviation (SD). The *Kolmogorov–Smirnov* test was used to check the normality of data. Since the variables are normally distributed, parametric tests were therefore performed. To compare the differences between the groups, the Student test was conducted. The significance level was established as 5% (<0.05).

RESULTS

The macronutrient and micronutrient intake of Moroccan students was estimated in this pilot study. Results are presented as mean ± standard deviation.

Characteristics of the participants

In our study, men represent 41.6% (54/130) and women 58.5% (76/130). For all recruited students, the average age of participants was 21.57 ± 0.14 years. For all parameters, statistical analysis revealed no significant difference between men and women (Table 1).

The means of energy supply from university and home meals (Table 2) show that energy intakes from home meals are significantly higher ($p<0.05$). Analysis of average protein intakes from university and home

Table 1. General characteristics of the studied population by sex

	Men		Women		Total	
	n	Mean ± SD	n	Mean ± SD	n	Mean ± SD
Age (years)	76	21.76 ± 0.27	54	21.41 ± 0.13	130	21.57 ± 0.14
Height (cm)	76	176.32 ± 0.95	54	161.71 ± 0.71	130	168.66 ± 0.93
Weight (kg)	76	69.33 ± 1.58	54	60.74 ± 1.92	130	64.83 ± 1.32
BMI (Body Mass Index) (kg/m2)	76	22.32 ± 0.42	54	23.15 ± 0.67	130	22.76 ± 0.40
WC (waist circumference), cm	76	80.82 ± 1.14	54	73.59 ± 1.41	130	76.88 ± 0.99
WHR(waist-hip ratio)	76	0.82 ± 0.01	54	0.73 ± 0.01	130	0.77 ± 0.01

SD - standard deviation

Table 2. Average energy supply from meals consume at university and at home for both sexes

	Average energy supply from meals consumed at university in kcal /d	Average energy supply from meals consumed at home in kcal /d	p value
Total (n=130)	1576 ± 44.09	1834 ± 52.62	0.0456*
Women (n=54) Mean ± SD	1604 ± 59.27	1803 ± 67.61	0.0283*
Men (n=76) Mean ± SD	1556 ± 62.80	1855 ± 76.41	0.0030**

SD – standard deviation; The significance level was established as 5% ($p<0.05$).
*Statistically significant differences defined as $p<0.05$.

Table 3. Average nutrients and minerals supply from meals consume at university and at home

Nutrients, minerals	Meals consumed at university (n=130) Mean ±SD	Meals consumed at home (n=130) Mean ±SD	*p*-value
Protein (%)	14.08 ± 0.39	16.26 ± 0.92	0.030*
Carbohydrate (%)	48.69 ± 1.11	50.5 ± 1.21	0.270
Lipids (%)	34.18 ± 0.90	30.21 ± 0.84	0.001**
FA (Fatty acids)	44.88 ± 2.01	48.16 ± 1.93	0.240
FAT(Trans fatty acids)	17.92 ± 0.98	19.19 ± 0.91	0.340
MUFA(Monounsaturated fatty acids), (g)	18.61 ± 0.99	19.58 ± 0.90	0.470
PUFA (Polyunsaturated fatty acids), (g)	8.35 ± 0.44	9.385 ± 0.56	0 .150
Lipid index of meals	0.744 ± 0.04	0.762 ± 0.04	0.780
Potassium (mg)	1289 ± 64.32	1905 ± 76.44	<0.0001****
Sodium chloride (g)	4.896 ± 0.20	6.925 ± 0.26	<0.0001****
Iodine (µg)	67.97 ± 5.53	90.94 ± 5.04	0.002**

SD - standard deviation. Variables are normally distributed (*Kolmogorov–Smirnov* test), p values by t- *Student's* test for medians. *Statistically significant differences defined as p<0.05.

meals shows that the difference between the intake of home meals (16.26%) and university meals (14.08%) is significant p<0.05. On the other hand, the difference between the carbohydrate intake at university and at home is not significant (p>0.05). The mean lipid intakes shown in Table 3 indicate that the lipid intakes from university meals are higher than those taken at home, 34.18% and 30.21% respectively.

Analysis of potassium and sodium chloride intakes in meals taken at home and at the university shown in Table 3 indicates that the difference between meals taken at university and at home is highly significant p<0.0001. With regard to average intakes of iodine the difference between meals taken at the University and at home is significant p<0.05.

DISCUSSION

In this survey, 130 university students participated in our study to evaluate the one-day energy and nutritional intake of their food on campus and at home. Data analysis allows us to assess the quantity and quality of food consumed by participants, as well as their nutritional habits.

An assessment of the energy intake from university and home meals for 130 students shows that the energy intake from home students is higher than the energy intake at university (1834 52.62 kcal/d vs 1576 44.09 kcal/d). However, these data show that the energy intake is lower than the recommended values of 2000 – 2700 kcal/day. For moderate physical activity [14]. Analysis of these energy intakes shows that 61% of students consume more energy at home, while 27% consume more energy at university than at home, while 12% of participants have no fluctuations in their diet.

These results are different from those of a study of university students' food consumption and out-of-home eating habits. A total of 289 students aged 18–24 years old from three major Albanian universities were polled for this study. Despite the fact that carbohydrate, sugar, and protein content are all high at home (AH) and saturated fats, amounts appear higher outside of the home (OH). There are no significant differences between foods consumed AH and foods consumed OH [15]

The nutritional intake of 130 participants shows that protein intake at home 16.26± 0.92% is high compared to protein intake at university 14.08± 0.39%, indicating that students consume more protein at home than university. Analysis of the results of protein intake shows that 41% of students during a day at home have a protein intake that is higher than the reference values (16.26 ± 0.92), and 37% of students during a day at university have a protein intake (14.08 ± 0.39), these values fall within the reference values of 12 to 15% according to the WHO.

Similarly, studies have in Albania shown that the Dietary composition of AH intake was richer in proteins, while OH intake was richer in saturated fats[15]. These results are not similar to the results of Energy and nutrient intake and food patterns among Turkish university students. This survey was conducted on a sample of 400 students (167 female and 233 male) aged between 19 and 24 years five universities in Ankara. The percentages of energy supplied from proteins at university were found to be (12.9% in males, and 13.2% in females) [3].

The carbohydrate intake of students from meals taken at university is 48.69 ± 1.11% and 50.5 ± 1.218% at home, this carbohydrate intake is close to the reference values which correspond to 50% - 60% according to the World Health Organization (WHO). These results indicate that students' carbohydrate needs are met, and that there is no significant difference between carbohydrate intakes from college and home meals.

The evaluation of our lipid intake from meals taken at university and at home indicates that students' home lipid intakes fall within the reference values which are 25 to 30% [16], which is contrary to university. In addition, the lipid intakes of 67% of students during a day at university and 47% of students during a day at home is over 30%, which almost exceeds the recommended daily intakes (25 -30%), this shows that half of students consume more fat.

These results are similar to the results found in a survey by the university of Valencia in Spain (n = 918) which found that according to the percentage of lipid 35.05% of students have values above the Spanish nutritional objectives (<35%) [17].

The evaluation of the fatty acids (FA) intake in meals taken at university and at home shows that approximately 45% to 48% of the total energy is supplied by fatty acids and that the FA intake of meals taken at home is higher than the intake of meals taken at university (48.16 ± 1.93; 44.88 ± 2.01 respectively), which is higher than the reference value which represents 30% according to the WHO [18].

Intakes of saturated fatty acids from meals taken at university are 17.92 ± 0.99 % and 19.19 ± 0.92% for meals taken at home. 88% of daytime students at university and 89% of daytime students at home have an AGS intake greater than 8%, which falls within the reference values of 5 - 10% according to the WHO. This shows that more than half of the students favor the consumption of saturated fatty acids.

Regarding the lipid index, there is no significant difference between meals taken at university and meals taken at home 0.74 ± 0.05 and 0.76 ± 0.04 respectively, which is higher than the reference value which is <0.4.

The assessment of sodium chloride intake in students indicates that students consume more salt at home than at university. Their sodium chloride intake at home is higher than 5g/d, which exceeds the recommended intake according to the WHO [18] . 40% of college students and 59% of home students exceed recommended salt intake. Comparing college meals to those eaten at home shows that students consume more salt at home than at college.

In the same vein, the University of São Paulo conducted a study to determine the sodium chloride concentration in the 24-hour meals of 19 students (9 women and 10 men) and they found that in their diets, 85 % of men exceeds 6 g of salt per day [19].

Our sodium results higher than the recommendations could be explained by the change in eating behavior. In fact, Morocco has recorded a nutritional transition, resulting in changes in eating habits and lifestyle changes, especially in larger cities like the region of Casablanca. And as a result, processed foods and fast-food restaurants have increased dramatically [20].

Analysis of potassium intakes in meals taken at home and at university show that students consume during a day at home (1905 ± 76.44 kcal /d) and at university (1289 ± 64.32 kcal /d), which is lower than the recommended daily allowance which corresponds to 3500 mg (WHO, 2015) and that only 2% of meals at university and 5% at home cover the needs in potassium.

Cook and Col's studies has shown that people who consume more sodium or less potassium have a higher risk of hypertension and high blood pressure [21]. Although high blood pressure is linked to an increased risk of cardiovascular disease, the connection between sodium or potassium intake and cardiovascular disease incidence or mortality is not always clear. Some studies have indicated that the combination of sodium and potassium intakes is a more significant risk factor for hypertension and CVD than each factor alone [22]. A survey of the estimated usual sodium and potassium intake of 12,267 adults in the United States who were tracked for an average of 14.8 years found a strong monotonic relationship between increased sodium-to-potassium ratio and risk of death from cardiovascular disease and ischemic heart disease [20] .

Analysis of the iodine intake of meals taken at university and at home shows that students during a day at university consume 67.97 ± 5.535 µg/d and during a day at home consume 90.94 ± 5.044 µg/d, which indicates that the students consume iodine in a small amount, which is far from the recommended iodine intakes (150 µg/d). 89% of students for a day at university and 86% of students for a day at home have an iodine intake of less than 150 µg/d. Our results are similar to the results of a nutritional intake assessment survey of 805 University of Leipzig employees and students, which found that nutritional iodine deficiency is 32% to 46% [23].

Since it is the most common cause of preventable mental illness worldwide, iodine deficiency remains a significant global challenge to health and development [24] . Hence a lot of effort has been spent into fighting iodine deficiency disorders [25] .

The assessment of energy and nutritional intakes from university and home meals shows a deficiency in student energy intakes from both, university and parental home meals compared to recommendations of 2,000 - 2,700 kcal/day [26]. Differences in nutritional, protein and fat intakes between university and home

meals. Indeed, energy and protein intakes are higher in home meals and lipids are higher in those taken at university. For micronutrient intakes, the results indicate increased potassium deficiency exceeding 50% of daily recommendations. A home sodium intake exceeding 40% of recommendations and an iodine intake deficiency of 89% and 86% respectively in food intakes on campus and at home.

Eating habits were examined among a sample of University of Alberta female students who had all completed at least one nutrition course, to determine whether the female students had similar eating habits to those recommended in the Canada's Food Guide (CFG) or the Traditional Mediterranean Diet Pyramid (PRMT). None of the students consumed the minimum number of servings of legumes, seeds, nuts, olive oil.

The majority of participants did not follow the minimum recommendations, such is our case. The results of this study suggest that nutrition education alone may be insufficient to ensure optimal eating habits among female university students.

Limitations

Our study has a few limitations. First, the study was carried out only on students of the University Hassan II, of the city of Casablanca, even if it is the largest city of Morocco, but it is not sufficiently large and representative sample of the population. The main was a 24-hour food diary, which was semi-quantitative or quantitative. This method was created to provide information about food consumption, frequency of consumption, and servings; however, it is unreliable for measuring total diet, total energy intake, or total nutrient intake. Among the limits also the quality of our sampling. Further research will be needed with better methodology. Besides, the 24-hour Dietary Recall (24HR). A single administration of a 24HR is unable to account for day-to-day variation, two or more non-consecutive recalls are required to estimate usual dietary intake distributions. Multiple administrations are also recommended when 24HRs are used to examine diet and health or other variables.

This study was also limited by contextual technicalities, such as the lack of national food composition tables and little to no research in this area. Due to similarities in dieting and nutritional habits, the Ciqual nutritional composition table managed by ANSES (the French Agency for Food, Environmental and Occupational Health Safety) was used [8].

CONCLUSIONS

This pilot cross-sectional study shows that students have eating habits that do not meet their needs for macronutrients and micronutrients, which will have an impact on their health. All forms of malnutrition are risk factors for non-communicable diseases such as diabetes, heart disease, stroke, and cancer.

Acknowledgments

The authors express their gratitude to the researchers and students who decided to take part in this pilot study.

Statement of authorship:

Abdelfettah Derouiche, Maria Elarbaoui, Ali Jafri, Houria Makhlouki and Basma Ellahi contributed to preparation and revision of the manuscript for publication. Ali Jafri and Maria Elarbaoui performed the statistical analysis. The final manuscript has been read and approved by all the authors.

Conflict of interest

No conflicts of interest declared by Authors.

Funding sources

This study was funded by the British Council.

Ethical approval

In this study, all participants signed a statement of consent.

REFERENCES

1. *Drewnowski A., Evans W.J.*: Nutrition, Physical Activity, and Quality of Life in Older Adults: Summary. J Gerontol: Series A, 2001;56(suppl_2):89-94, doi: 10.1093/gerona/56.suppl_2.89.
2. *Papadaki A., Hondros G. , Scott J.A., Kapsokefalou M.*: Eating habits of university students living at, or away from home in Greece, Appetite, 2007;49(1):169-176, doi: 10.1016/j.appet.2007.01.008.
3. *Neslişah, R., Emine, A.Y.*: Energy and nutrient intake and food patterns among Turkish university students. Nutr Res Practice, 2011;5(2):117-123. doi: 10.4162/nrp.2011.5.2.117.15.
4. Healthy diet. https://www.who.int/news-room/fact-sheets/detail/healthy-diet (consulté le 29 avril 2022).
5. Fof_lesson_7_final.pdf. Lesson 7 – Understanding Influences on Food Choices. Available at: https://cns.ucdavis.edu/sites/g/files/dgvnsk416/files/inline-files/fof_lesson_7_final.pdf (Accessed: 29 April 2022).
6. Rapport mondial sur la nutrition [Global Nutrition Raport]. 2021 (Accessed at: 29 January 2022).
7. *El Rhazi K, Nejjari C, Romaguera D, Feart C, Obtel M, Zidouh A, Bekkali R, Gateau PB.*: Adherence to a Mediterranean diet in Morocco and its correlates: cross-sectional analysis of a sample of the adult Moroccan population. BMC Public Health. 2012 May11;12:345. doi: 10.1186/1471-2458-12-345.
8. ANSES. Ciqual Table de composition nutritionnelle des aliments. https://ciqual.anses.fr/ (consulté le 30 avril 2021).

9. *Church S.M*: EuroFIR Synthesis report No 7: Food composition explained. Nutrition Bulletin, 2009;34(3):250-272, doi: 10.1111/j.1467-3010.2009.01775.x.
10. de *Onis M*., *Habicht J.P*.: Anthropometric reference data for international use: recommendations from a World Health Organization Expert Committee. Am. J. Clin. Nutr. 1996;64:650–658. https://doi.org/10.1093/ajcn/64.4.650
11. *Jafri A, Jabari M, Dahhak M, Saile R, Derouiche A*.: Obesity and its related factors among women from popular neighborhoods in Casablanca, Morocco. Ethn Dis. 2013;23(3):369-73.
12. *Han, S.S., Kim, K.W., Kim, K.-I., Na, K.Y., Chae, D.-W., Kim, S., Chin, H.J*.: Lean Mass Index: A Better Predictor of Mortality than Body Mass Index in Elderly Asians. J Am Geriatr Soc. 2010;58:312-317. https://doi.org/10.1111/j.1532-5415.2009.02672.x.
13. *Ibm C.R*.: IBM SPSS Statistics for Windows, Version Q3 25.0. Armonk, NY: IBM Corporation, 2017.
14. *Kotseva K, De Backer G, De Bacquer D, Rydén L, Hoes A, Grobbee D, Maggioni A, Marques-Vidal P, Jennings C, Abreu A, Aguiar C, Badariene J, Bruthans J, Castro Conde A, Cifkova R, Crowley J, Davletov K, Deckers J, De Smedt D, De Sutter J, Dilic M, Dolzhenko M, Dzerve V, Erglis A, Fras Z, Gaita D, Gotcheva N, Heuschmann P, Hasan-Ali H, Jankowski P, Lalic N, Lehto S, Lovic D, Mancas S, Mellbin L, Milicic D, Mirrakhimov E, Oganov R, Pogosova N, Reiner Z, Stöerk S, Tokgözoğlu L, Tsioufis C, Vulic D, Wood D;* EUROASPIRE Investigators: Lifestyle and impact on cardiovascular risk factor control in coronary patients across 27 countries: Results from the European Society of Cardiology ESC-EORP EUROASPIRE V registry. Eur J Prev Cardiol. 2019May;26(8):824-835. doi: 10.1177/2047487318825350
15. *Llanaj E., Ádány R., Lachat C., D'Haese M*.: Examining food intake and eating out of home patterns among university students, PLOS ONE, 2018;13(10):e0197874, doi: 10.1371/journal.pone.0197874.
16. *Bachmann, P., Marti-Massoud, C., Blanc-Vincent, M. P., Desport, J. C., Colomb, V., Dieu, L., & Senesse, P*.: Summary version of the standards, options and recommendations for palliative or terminal nutrition in adults with progressive cancer. British J. Cancer, 2001;89(1), S107-S110.
17. *Soriano J.M., Rico H., Moltó J.C., Mañes J*.: Effect of introduction of HACCP on the microbiological quality of some restaurant meals. Food Control 2002;13(4):253-261, doi: 10.1016/S0956-7135(02)00023-3.
18. WHO. World Health Statistics 2015. World Health Organization, 2015.
19. *McLean, R. M., Farmer, V. L., Nettleton, A., Cameron, C. M., Cook, N. R., Campbell, N. R.,* & TRUE Consortium (International Consortium for Quality Research on Dietary Sodium/Salt). Assessment of dietary sodium intake using a food frequency questionnaire and 24-hour urinary sodium excretion: a systematic literature review. The Journal of Clinical Hypertension, 2017;19(12), 1214-1230. », 2017.
20. *Yang, Q., Liu, T., Kuklina, E. V., Flanders, W. D., Hong, Y., Gillespie, C., Khoury, M. J.*et al.: Sodium and potassium intake and mortality among US adults: prospective data from the Third National Health and Nutrition Examination Survey. Arch Intern Med, 2011;171(13):1183-1191, doi: 10.1001/archinternmed.2011.257.
21. *Ridker P. M., Buring, J. E., Rifai, N., Cook, N. R*.: Development and validation of improved algorithms for the assessment of global cardiovascular risk in women: the Reynolds Risk Score. Jama, 2007;297(6):611-619.
22. *Umesawa, M., Iso, H., Date, C., Yamamoto, A., Toyoshima, H., Watanabe, Y., Inaba, Y.* & JACC Study Group: Relations between dietary sodium and potassium intakes and mortality from cardiovascular disease: the Japan Collaborative Cohort Study for Evaluation of Cancer Risks. Am J Clin Nutr, 2008;88(1):195-202.
23. *Brauer VF, Brauer WH, Führer D, Paschke R*.: Iodine nutrition, nodular thyroid disease, and urinary iodine excretion in a German university study population. Thyroid. 2005 Apr;15(4):364-70. doi: 10.1089/thy.2005.15.364.
24. *Andersson, M., Karumbunathan, V., Zimmermann, M.B.:* Global iodine status in 2011 and trends over the past decade. J. Nutr, 2012;142(4):744-750.
25. *Jafri A, Elarbaoui M., Elkardi Y., Makhlouki H., Ellahi B., Derouiche A*.: Assessment of sodium and iodine intake among university students in Casablanca, Morocco. Nutrition Clinique et Métabolisme, 2021;35(3), 222-225. https://doi.org/10.1016/j.nupar.2020.11.003
26. World Health Organization. WHO_TRS_724_(chp1-chp6).pdf. Consulté le: 29 avril 2022. [On line]. Available at: https://apps.who.int/iris/bitstream/handle/10665/39527/WHO_TRS_724_(chp1-chp6).pdf;jsessionid=C2BDD6EEF37A79FCE8E8EA7EA6E8E277?sequence=1

Received: 11.03.2022
Accepted: 22.07.2022

Rocz Panstw Zakl Hig 2022;73(3):293-301
http://wydawnictwa.pzh.gov.pl/roczniki_pzh/ https://doi.org/10.32394/rpzh.2022.0221

ORIGINAL ARTICLE

DIETARY DIVERSITY SCORE AND THE INCIDENCE OF CHRONIC KIDNEY DISEASE IN AN AGRICULTURAL MOROCCAN ADULTS POPULATION

Rachida Moustakim[1], Mohamed Mziwira[2], Mohammed El Ayachi[1], Rekia Belahsen[1]

[1]Laboratory of Biotechnology, Biochemistry & Nutrition. Training and Research Unit on Nutrition and Food Sciences. Chouaib Doukkali University, Faculty of Sciences, El Jadida, Morocco
[2] Laboratory of Bio-Geosciences and Materials Engineering, Higher Normal School, Hassan II University, Casablanca, Morocco

ABSTRACT

Background. Healthy diet plays an important role in the management of chronic kidney disease (CKD) and in the prevention of related comorbidities. Dietary diversity score (DDS) is well recognized as an indicator for assessing diet quality and food security. However, its association with CKD has not been investigated.

Objective. The aim of this study was to estimate the prevalence of CKD and to evaluate its association with DDS among a Moroccan adults from Sidi Bennour province.

Materials and methods. A cross sectional study was conducted among 210 individuals. General information among others was collected. Weight, height and waist circumference were measured and body mass index (BMI) was calculated. Blood samples were collected and the serum creatinine was determined. Subsequent glomerular filtration rate (eGFR) was estimated by the modification of diet in renal disease (MDRD) formula and the chronic kidney disease was defined by an eGFR<60 ml/min/1.73m². Dietary intake was assessed using a 24-hours dietary recall, and DDS was computed according to the FAO guidelines.

Results. The participants mean age was 54.18±13.45 years, with a sex ratio of 0.38 and 4.4% as the prevalence of chronic kidney disease. The dietary diversity score was lower than 3 (lowest DDS) in 14.4% of the subjects, between 4 and 5 (medium DDS) in 72.5% and higher than 6 (high DDS) in 13.1% of the subjects. Subjects with higher DDS consistently have a higher level of eGFR compared to those with lower DDS while the DDS was not associated with the incidence of CKD in the present study.

Conclusion. Even if no statistically significant association was found between CKD and dietary diversity, there is a relationship of higher eGFR levels among the study participants with higher dietary diversity.

Key words: *chronic kidney disease, diet, dietary diversity score, food security, 24-h dietary recall*

INTRODUCTION

Chronic kidney disease (CKD) is recognized as a serious public health problem around the world and it is associated with high cardiovascular morbidity, mortality and low quality of life [1, 2, 3]. Several metabolic and cardiovascular disease (CVD) factors including obesity, diabetes, hypertension, and metabolic syndrome are in continuous increase worldwide including in Morocco [4, 5, 6]. In addition to these CKD risk, diet is thought to play a major role in the development of these diseases [7].

Dietary diversity is a best indicator of healthy diet [8, 9]. Dietary diversity score (DDS) is an easy and key index used to assess overall dietary quality and reflects the consumption of various foods between and within each food group [10]. The result of some studies among women of different age groups have displayed that higher dietary diversity score is related to increased nutrient adequacy of the diet [9].

Previous studies have also demonstrated the association between dietary diversity score and chronic diseases, as well as its correlation with prolonged longevity and improved health status [11]. According data from several investigations, an inverse relationship of dietary diversity score with metabolic syndrome [11], cardiovascular diseases [12], cancer [13], high blood pressure [14] and anxiety [15] has been reported.

Corresponding author: Rekia Belahsen, Laboratory of Biotechnology, Biochemistry and Nutrition, Training and Research Unit on Nutrition and Food Sciences, Department of Biology, Faculty of Sciences, Chouaib Doukkali University, El Jadida 24 000, Morocco, Phone: +212 664971616, e-mail: rbelahsen@yahoo.com or b.rekia@gmail.com

Furthermore, DDS is positively correlated with the intake of macronutrients and micronutrients [15].

The dietary diversity is a qualitative measure of food consumption, it also reflects household access to a variety of foods, and is also a proxy of nutrient adequacy of the individuals diet [6, 16, 17].

Ideally, the most adopted approach to measure dietary diversity is the qualitative recall over the previous 24-hours, a reference period, of all the foods consumed by the study sample [18, 19, 20]. The analysis of dietary diversity data is done by the measurement of DDS which is obtained by calculating the total number of food groups consumed over the previous 24-hours by the individuals. The total number of food groups consumed reflects, thus the degree of dietary diversity [9, 21].

Evidence suggests that diet play an important role in chronic kidney disease [22]. Dietary diversity score is well recognized as an indicator for assessing diet quality and food and nutrition security status [9, 18, 23]. In the present study, 9 food groups were selected for the calculation of the DDS in accordance with the classification proposed by the FAO [9]. The DDS therefore varied between 0 and 9. In fact, the higher dietary score indicated more diversification of the diet, and hence reflecting the nutritional quality of the food ration and therefore of the diet adopted [18]. To the best of our knowledge, no study has examined the relationship between dietary diversity score and chronic kidney disease.

The purpose of this study was therefore to determine the association between chronic kidney disease and dietary diversity score in an agricultural Moroccan adult's population attend the health centers.

MATERIALS AND METHODS

Sample

The current study was carried out between January and December 2017 on a sample of 210 subjects aged 18 years and over, living in an agricultural province, Sidi Bennour and randomly selected from primary health care. The study was supported by the Moroccan Ministry of Higher Education and Research and the Ministry of Health of Morocco. Only people aged 18 years old and older with normal mental health were included. Pregnant women, patients with paralysis and person with antecedent of kidney disease were excluded from this investigation.

Data collection

Information is mainly gathered using questionnaire to collect data on sociodemographic and socioeconomic status (age, sex, marital status, area of residence, profession, monthly income and education level), personal and family health history (hypertension, diabetes and kidney disease) and lifestyle (smoking, alcohol consumption, physical activity) and dietary habits. Blood pressure and anthropometric parameters (weight, height, waist and hip circumferences) were likewise carefully measured. All anthropometric and clinical measurements were performed by the same well trained nurse in order to reduce subjective errors.

Anthropometric measurement

The weight was measured in light clothing and without shoes to the nearest 0.1 kg on a mechanical scale, and height was recorded to the nearest of 0.1 cm with a stadiometer while the subjects were in a standing position, not wearing shoes and with shoulders in normal position. Body Mass Index (BMI) was calculated by dividing weight (kg) by the square of height (m^2). According to the World Health Organization (WHO) criteria, normal weight was defined as $18 \leq BMI \leq 24.9$ kg/m², overweight as $25 \leq BMI < 30$ kg/m² and overall obesity was defined as BMI>30 kg/m². Waist circumference (WC) in (cm) was measured at midway between the lowest rib and the iliac crest and the hip circumference (HC) at the level of the greater trochanter using a flexible tape and expressed in (cm) and the waist to hip ratio (WHR) was calculated as WC divided by HC. WC is a marker for central obesity and WHR for body fat distribution. According to the NCEP-ATP III reference values, WC larger than 88 cm for females and 102 cm for males was considered to be high.

Laboratory measurements

Blood samples were collected by venipuncture after an overnight fast of at least 12 hr and all analyses were made on the day of blood collection. Serum creatinine was measured according to the standard colorimetric *Jaffe-Kinetic* reaction method.

Chronic kidney disease

Estimated glomerular filtration rate (eGFR) was calculated using the modification of diet in renal disease (MDRD) formula as follows [24, 25, 26]:

$$eGFR = 186 \times (\text{serum creatinine})^{-1.154} \times (\text{age})^{-0.203} \times (0.742 \text{ if female}) \times (1.210 \text{ if African- American})$$

Subjects were classified based on their eGFR levels by the national kidney foundation guidelines, when eGFR ≥60 ml/min/1.73 m² as without CKD and when eGFR <60 ml/min/1.73 m² as with CKD.

Dietary diversity score (DDS)

A 24-hours dietary recall questionnaire was completed for a subsample of 160 participants in a face-to-face interview. According to the FAO guidelines, dietary diversity questionnaire was used to determine the dietary diversity score of each participant [9,18]. In accordance to the structure of this guideline, all

the food items were categorized into 9 food groups, including: (1) cereals and white roots, (2) green leafy vegetables, (3) other vegetable and fruits, (4) vitamin A-rich vegetables and fruits, (5) organ meat, (6) meat, fish and sea food, (7) eggs, (8) nut, seeds and legumes, (9) milk and dairy products. The DDS was calculated using a minimum consumption of at least half serving of one food item from each of the mentioned food groups. The score of dietary diversity was the total number of all consumed food groups. The range of dietary diversity score was from 0 to 9. Dietary diversity score was classified into three groups; (1) low (≤3 food groups), (2) medium (between 4 and 5 food groups) and (3) high (≥6 food groups) [9,18].

Ethical consideration

The authorities were previously informed by the delegation of the Ministry of Health about the realization of the study, its objectives and terms. Also, the procedures and objectives of the study were clearly explained to the participants who provided written informed consent.

Statistical analyses

All calculations were performed using the SPSS statistics program version 24.0. Continuous variables were expressed by mean ± SD and categorical variables were reported by frequency and proportions. *Student's* t-test was used to compare differences in means and Chi-square test was used to compare differences in proportions. Analysis of variance (ANOVA) was used to determine the relationship between DDS categories and quantitative variables. In all statistical tests, p-value less than 0.05 was considered statistically significant.

RESULTS

Dietary diversity score

The mean age of the participants studied was 54.18 ±13.45 years. The prevalence of CKD (eGFR<60ml/min/1.73 m^2) was 4.4%. In our study, only a sub group of 160 individuals were able to complete their dietary questionnaire, the mean DDS was 4.49 ± 0.92 food groups, the dietary diversity value was between 2 and 7 and the median was 5 (Figure 1).

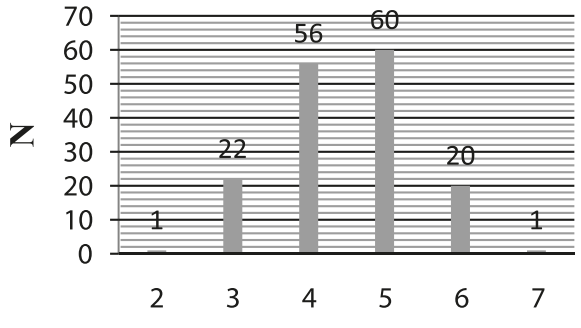

Figure 1. Individual dietary diversity score

Table 1 shows the distribution of the mean DDS according to the basic characteristics of the participants. Socio-economic status and education level were found to be associated with DDS ($p<0.05$). However, the analysis of dietary diversity according to gender and area of residence seems not to have any impact on the dietary diversity in this study population. On the other hand, for the age groups, a slight decrease in the mean value of the DDS was noted, going from 5.20 ± 0.63 for the youngest age category of (18-29) years old to 4.28 ± 0.75 for the category aged 70 years old and over, this difference is however not statistically significant. The same observation was made for the anthropometric status. Individuals with normal BMI have a higher mean of DDS than those with abnormal BMI. Regarding the incidence of CKD, it is noted that while not statistically significant, subjects without CKD have a relatively higher DDS value compared to those with CKD.

The respondents' dietary diversity score ranged from 2 to 7; 14.4% had low dietary diversity score, 72.5% had medium DDS and 13.1% had high DDS.

The table 2 gathered the results concerning the distribution of the participants according to their characteristics and dietary diversity scores categories. Generally, the majority of the study population (72.5%) had a medium DDS. The bivariate analysis is used to compare the proportions of these characteristics according to three categories qualified as low, medium or high DDS. With regard to age, the analysis shows that the youngest age group (18-29) was characterized by high DDS (30%) followed by the (30-44) age group (16.1%). However, no significant difference was reported regardless the age group. Concerning gender characteristics, it is noted that the proportion of high DDS is greater among men than women with a DDS mean nearly similar in both genders. The comparison by area of residence show that a medium DDS in both areas with a relatively higher DDS mean in urban areas (4.69 ± 0.89) than in rural ones (4.41 ± 0.92). This difference is not statistically significant.

The comparison of dietary diversity according to the SES showed that participants with a low SES had a low DDS with a clearly significant difference. Similarly the same observation is noted for education level, revealing a low DDS in participants with a low level of education.

With regard to the anthropometrical status, the comparison of dietary diversity revealed that the majority of subjects with normal weight, overweight and obese have a medium DDS whereas 22% of subjects with normal weight have a high DDS reflecting a diverse diet in this weight category. However, there was no significant difference between these groups.

Based on CKD incidence, the results show that the eGFR increases with the food diversity score,

Table 1. Comparison of DDS according to the study participants' characteristics

Characteristics	n (%)	DDS (mean ±SD)	P- value
Gender			
Men	45 (28.1)	4.69 ± 0.90	0.095
Women	115(71.9)	4.42 ± 0.92	
Age groups (years)			
18 – 29	10 (6.3)	5.20 ± 0.63	
30 – 44	31 (19.4)	4.48 ± 1.02	0.060
45 – 59	55 (34.4)	4.35 ± 0.94	
60 – 69	46 (28.8)	4.61 ± 0.88	
≥ 70	18 (11.3)	4.28 ± 0.75	
Area of residence			
Urban	49 (30.6)	4.69 ± 0.89	0.069
Rural	111 (69.4)	4.41 ± 0.92	
SES			
Low	95 (59.4)	4.25 ± 0.91	**0.000**
Medium	47 (29.4)	5.02 ± 0.76	
High	18 (11.3)	4.39 ± 0.85	
Education level			
Unable to read/write	116 (72.5)	4.38 ± 0.90	
Koranic	8 (5.0)	4.38 ± 0.91	**0.007**
Primary school	16 (10.0)	4.63 ± 0.80	
Secondary school	13 (8.1)	4.85 ± 1.06	
University	7 (4.4)	5.53 ± 0.53	
BMI categories			
Under weight	3 (1.9)	3.67 ± 1.15	
Normal weight	41 (25.6)	4.73 ± 0.89	0.113
Overweight	55 (34.4)	4.38 ± 0.95	
Obesity	61 (38.1)	4.48 ± 0.88	
Chronic kidney disease			
With CKD	7 (4.5)	4.29 ± 0.75	0.601
Without CKD	148 (95.5)	4.47 ± 0.92	

DDS: dietary diversity score; SD: standard deviation; SES: socio economic status;
BMI: body mass index; CKD: chronic kidney disease

Table 2. Association between the characteristics of study participants and the dietary diversity score

Characteristics	Low DDS 23 (14.4%)	DDS medium 116 (72.5%)	High DDS 21 (13.1%)	P-value
Gender, n (%)				
Men	5 (11.1)	32 (72.1)	8 (17.8)	0.469
Women	18 (15.7)	84 (73.0)	13 (11.3)	
Age, years, (mean±ET)	52.65±11.25	54.01±14.40	49.90±15.19	0.461
Ages groups, n (%)				
18 – 29	0 (0.0)	7 (70.0)	3 (30.0)	
30 – 44	5 (16.1)	21 (67.8)	5 (16.1)	0.466
45 – 59	11 (20.0)	39 (70.9)	5 (9.1)	
60 – 69	5 (10.9)	34 (73.9)	7 (15.2)	
≥ 70	2 (11.1)	15 (83.3)	1 (5.6)	
Area of residence, n (%)				
Urban	4 (8.2)	37 (75.5)	8 (16.3)	0.281
Rural	19 (17.1)	79 (71.2)	13 (11.7)	
SES, n (%)				
Low	20 (21.1)	67 (70.5)	8 (8.4)	**0.009**
Medium	1 (2.1)	35 (74.5)	11 (23.4)	
High	2 (11.1)	14 (77.8)	2 (11.1)	

Level of education, n (%)				
Unable to read/write	20 (17.2)	85 (73.3)	11 (9.5)	**0.006**
Koranic	1 (12.5)	6 (75.0)	1 (12.5)	
Primary school	0 (0.0)	15 (93.8)	1 (6.3)	
Secondary school	2 (15.4)	7 (53.8)	4 (30.8)	
University	0 (0.0)	3 (42.9)	4 (57.1)	
BMI categories, n (%)				
Underweight	2 (66.7)	1 (33.3)	0 (0.0)	0.059
Normal weight	3 (7.3)	29 (70.7)	9 (22.0)	
Overweight	10 (18.2)	39 (70.9)	6 (10.9)	
Obesity	8 (13.1)	47 (77.0)	6 (9.8)	
CKD, n (%)				
With CKD	1 (14.3)	6 (85.7)	0 (0.0)	0.587
Without CKD	22 (14.9)	107 (72.3)	19 (12.8)	
eGFR, ml/min/1.73m², mean± SD	88.83±18.98	91.72±21.57	96.84±25.76	0.487

DDS: dietary diversity score; BMI: body mass index; CKD: chronic kidney disease; SES: socioeconomic status; eGFR: estimated glomerular filtration rate.

which proves that people with normal kidney function have a more diverse diet. However, no significant difference was noted for this criterion ($p>0.05$). Also the participants with CKD do not achieve high DDS compared to those without CKD (12.8%).

Food consumption

The assessment of dietary diversity in the study population was also carried out by the consumption of the different food groups. Indeed, as shown in the (Figure 2), there is a predominance of the consumption of the cereals group as almost 100% of the individuals had consumed cereal products, 98.8% consumed vegetables and fruits, 88.1% meat and fish, 69.4% vegetables and fruits rich in vitamin A and 47.5% consumed milk and dairy products. The least consumed food groups were: eggs consumed by 21.9%, legumes by 21.3% and lastly green leafy vegetables and organ meat were consumed in a proportion of 1.3% each.

The distribution of food consumption between the three DDS categories is shown in Table 3. The intakes of cereal group were not different between the groups of dietary diversity score (100 %). About the same results are found for the fruits and vegetables group and the meat and fish group. However, the intake of the other groups, such as organ meats, eggs, nuts, seeds and legumes and milk and dairy products, were higher in subjects with higher DDS compared to the group with low DDS.

Table 4 shows the distribution of food consumption between the two groups of CKD status. Thus, the intake of all food groups seems to be independent of

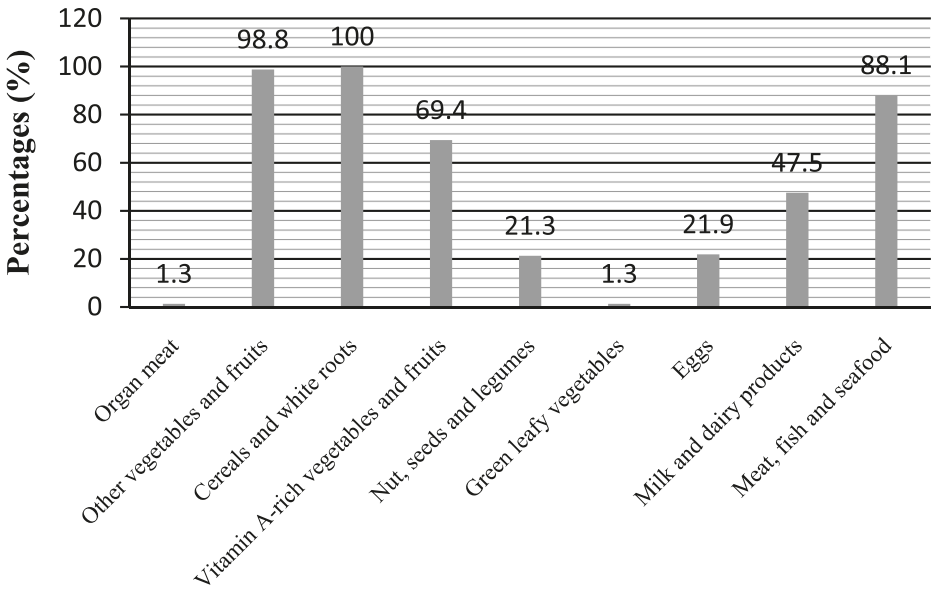

Figure 2. Food groups consumed by the study participants during the previous 24 hours

Table 3. Food groups consumption across the DDS categories in the study population

	Low DDS 23 (14.4%)	Medium DDS 116 (72.5%)	High DDS 21 (13.1%)	P-value
DDS (mean±SD)	2.96±0.20	4.52±0.50	6.05±0.21	0.000
Food groups, n (%)				
Cereals and white roots	23 (100)	116 (100)	21 (100)	1.00
Green leafy vegetables	0 (0.0)	2 (1.7)	0 (0.0)	0.681
Vitamin A-rich vegetables & fruits	2 (8.7)	91 (78.4)	18 (85.7)	0.000
Other vegetable and fruits	21 (91.3)	116 (100)	21 (100)	0.002
Organ meat	0 (0.0)	0 (0.0)	2 (9.65)	0.001
Meat, fish and sea food	12 (52.2)	108 (93.1)	21 (100)	0.000
Eggs	3 (13.0)	17 (14.7)	15 (71.4)	0.000
Nut, seeds and legumes	1 (4.3)	18 (15.5)	15 (71.4)	0.000
Milk and dairy products	6 (26.1)	56 (48.3)	14 (66.7)	0.025

DDS: Dietary diversity score; SD: standard deviation

Table 4. Food consumption across CKD status in the study population

	With CKD, n=7 (4.4%)	Without CKD, n=148 (95.6%)	p-value
DDS (mean±SD)	4.29±0.75	4.47±0.92	0.601
Food groups, n (%)			
ereals and white roots	7 (100)	148 (100)	1.00
Green leafy vegetables	0 (0.0)	2 (1.4)	0.757
Vitamin A-rich vegetables & fruits	5 (71.4)	101 (68.2)	0.859
Other vegetable and fruits	7 (100)	146 (98.6)	0.757
Organ meat	0 (0.0)	2 (1.4)	0.757
Meat, fish and sea food	7 (100)	129 (87.2)	0.312
Eggs	2 (28.6)	31 (20.9)	0.630
Nut, seeds and legumes	1 (14.3)	33 (22.3)	0.617
Milk and dairy products	1 (14.3)	70 (47.3)	0.087

CKD: chronic kidney disease; DDS: dietary diversity score; SD: standard deviation

this characteristic. DDS values are slightly similar between the two groups (p=0.601). Cereal products, fruits and vegetables, meats and fish are considerably consumed regardless the status of CKD. However, no significant difference was recorded in the consumption of the food groups according to this trait (p> 0.05).

DISCUSSION

This study is the first to explore the relationship between dietary diversity assessed by dietary diversity score (DDS) and the incidence of CKD. The present data revealed a consistent association of higher estimated glomerular filtration rate (eGFR) levels among the study participants with higher compared to those with lower dietary diversity. However no association was found between CKD and dietary diversity score.

Dietary diversity has already been pointed out as one of the best indicators of a healthy diet [8]. The latter is also found to be inversely associated with chronic diseases, including CKD risk factors such as diabetes and hypertension. Diet is indeed considered to be a modifiable factor involved in CKD that could contribute to impacting a major clinical and public health problem as well as preventing or delaying the renal function decline [7, 22].

The present data reports also the effects of the many factors examined in this study on DDS, especially education level and socio economic status. Thus, lower socioeconomic status and lower education level were associated with lower dietary diversity score. Similar finding was highlighted by other studies [23, 27, 28, 29]. However, gender, sex and anthropometric status of the participants were neither associated with their dietary diversity scores nor with the consumption of same food groups. This association being controversial

in the literature, this result is in accordance with previous studies [30] but not with others [23].

The food quality assessment in the present study was performed using both the dietary diversity score and the consumption of different food groups. The calculation of the DDS and the list of foods used were established according to FAO recommendations [9, 31]. The seasonality factor was also taken into account since the survey took place over periods which covered approximately the 4 seasons of the year.

The diet of the majority of this population was moderately diversified, characterized by a DDS between 4 and 5 in a proportion of 72.5% with a mean DDS as 4.49±0.92. The maximum DDS of 9 points was never reached in this study population. We have The DDS was categorized according to the FAO recommendations, in 3 classes representing as low, medium, or high the dietary diversity [9, 27]. The food groups found to be the most consumed by the participants are: cereals and white roots, vegetables and fruits, meat, fish and seafood, vitamin A-rich vegetables and fruits, milk and dairy products; the least consumed were legumes, eggs, organ meats, and green leafy vegetables. The high consumption of the above foods can be explained by the abundance of these products in the weekly market especially in this agricultural region. Moreover, the consumption of cereals products also remains very important regardless of the factor studied. These are staple foods that characterize and dominate the food consumption pattern of the Moroccan population [32]. According a study revealed a great dietary diversity among 263 students adolescents of 12 to 16 years old, from Kenitra, another country agricultural province, reporting a DDS average of 10.2, out of 12 food groups that was slightly higher in rural than in urban area [32, 33]. At the African continent level, a study carried out among a representative sample of 691 mothers of young children living in a disadvantaged rural area of Burkina Faso has reported a mean DDS of 5.1±1.7 food groups. High diversity was mainly characterized by higher consumption of meat, legumes, fats and sugar. The DDS was also positively and mostly correlated with socio-economic characteristics of the participants [27].

With regards to the incidence of CKD, the present survey does not show any influence of the disease by food consumption. Indeed, the majority of subjects with CKD consumed more meat, fish and sea food compared to those without CKD. The latter had a relatively high consumption of legumes. Although, this difference was not statistically significant; this finding can probably highlight the value of plant proteins as a protective factor over those of animal products in the context of chronic kidney disease, This result is in agreement with the study of Miller et al. indicating that, the absence of legumes from the daily diet was significantly associated with hypertension [14]. Likewise, despite, the non-significant difference between the two groups, the mean DDS was slightly higher in the group without apparent CKD than in the group with CKD.

Limitations

This study has few limitations. Firstly, the sample size was relatively small. Secondly, like all other cross-sectional studies, we could not determine the causal-effect relationship between CKD and dietary diversity. Thirdly, the 24-hours recall relies on subject recall which may be prone to memory bias from the participants. Despite these limitations, this seems to be the first study to evaluate the link between CKD and dietary diversity.

CONCLUSION

In conclusion, even if no statistically significant association was found between CKD and dietary diversity, there is a relationship of higher eGFR levels among the study participants with higher dietary diversity. Further researches are also needed to confirm this finding. The study data suggest that further efforts should be made to increase the dietary diversity following the recommendations of dietary guidelines, in the aim to potentially prevent or delay the probability of having the chronic kidney disease and its related risk factors.

Acknowledgements

The authors wish to thank the medical delegation of Sidi Bennour Province, Ministry of Health of Morocco and the medical and biomedical team for their cooperation. Furthermore, special thanks are extended to the staff of all health care centers in the region of Sidi Bennour for allowing us to collect data. The Survey was supported by the Moroccan Ministry of Higher Education and Research.

Author Contributions

Moustakim R. and Belahsen R. were responsible for designing the review protocol, writing the protocol and report. Moustakim R. and Mziwira M. were responsible for collecting the data.

Moustakim R. and Belahsen R. contributed to analyzing the data and interpreting results.

Moustakim R. wrote the first draft of the article and then all co-authors contributed to finalize the manuscript.

Funding

No funding was received for this article.

Competing interests
No conflicting financial interests exist.

REFERENCES

1. *Jha V, Garcia-Garcia G, Iseki K, Li Z, Naicker S, Plattner B, et al*. Chronic kidney disease: global dimension and perspectives. The Lancet. juill 2013;382(9888):260-72.
2. *Moustakim R, El Ayachi M, Mziwira M, Belahsen R.:* Undiagnosed chronic kidney disease and its associated risk factors in an agricultural Moroccan adult's population. Néphrologie Thérapeutique. mai 2020;16(3):147-52. DOI: 10.1016/j.nephro.2019.12.003
3. *Moustakim R., Mziwira M., El Ayachi M., Belahsen R.*: Assessment of nutritional status, dietary intake and adherence to dietary recommendations in hemodialysis patients. GSC Adv Res Rev. 30 mai 2020;3(2):009-19.
4. *Belahsen R., Bermudez O.I., Mohamed M., Fatima F., Newby P.K., Tucker L.K.:* Obesity and related metabolic disorders are prevalent in Moroccan women of childbearing age. Int J Diabetes Metab. 2005;13(3):159-66.
5. *Grundy SM.*: Metabolic Syndrome Pandemic. Arterioscler Thromb Vasc Biol. avr 2008;28(4):629-36.
6. *Mziwira M, El Ayachi M, Lairon D, Belahsen R.* Mediterranean Diet and Metabolic Syndrome in Adult Moroccan Women. J Res Obes. 20 févr 2015;1-18.
7. *Anderson CAM, Nguyen HA, Rifkin DE.:* Nutrition Interventions in Chronic Kidney Disease. Med Clin North Am. nov 2016;100(6):1265-83.
8. *Hatlùy A, Torheim L, Oshaug A.* Food variety - a good indicator of nutritional adequacy of the diet? A case study from an urban area in Mali, West Africa. Eur J Clin Nutr. 1998;52(12):8.
9. *Kennedy PG, Ballard T.* Guide pour mesurer la diversité alimentaire au niveau du ménage et de l'individu. 2013;56.
10. *Azadbakht L, Esmaillzadeh A.* Dietary diversity score is related to obesity and abdominal adiposity among Iranian female youth. Public Health Nutr. janv 2011;14(1):62-9.
11. *Azadbakht L, Mirmiran P, Azizi F.* Dietary diversity score is favorably associated with the metabolic syndrome in Tehranian adults. Int J Obes. nov 2005;29(11):1361-7.
12. *McCullough ML, Feskanich D, Stampfer MJ, Giovannucci EL, Rimm EB, Hu FB, et al.* Diet quality and major chronic disease risk in men and women: moving toward improved dietary guidance. Am J Clin Nutr. 1 déc 2002;76(6):1261-71.
13. *Fernandez E, Negri E, La Vecchia C, Franceschi S.* Diet Diversity and Colorectal Cancer. Prev Med. juill 2000;31(1):11-4.
14. *Miller WL, Crabtree BF, Evans DK.* Exploratory Study of the Relationship between Hypertension and Diet Diversity among Saba Islanders. Public Health Rep. 1992;107(4):426-32.
15. *Poorrezaeian M, Siassi F, Qorbani M, Karimi J, Koohdani F, Asayesh H, et al.* Association of dietary diversity score with anxiety in women. Psychiatry Res. déc 2015;230(2):622-7.
16. *Hoddinott J, Yohannes Y.* Dietary Diversity as a Food Security Indicator. Int Food Policy Res Inst. 2002;(136):94.
17. Sablan Dgu.: Dietary diversity and nutritional status of selected muslim adults (aged 19 to 59 years) in barangay Batong Malake, Los Baños, Laguna. 2018 2017;54.
18. Kennedy G, Ballard T, Dop M-C. Guidelines for measuring household and individual dietary diversity. Rome: FAO; 2011.
19. *Ikizler TA, Burrowes JD, Byham-Gray LD, Campbell KL, Carrero J-J, Chan W, et al.* KDOQI Clinical Practice Guideline for Nutrition in CKD: 2020 Update. Am J Kidney Dis. sept 2020;76(3):S1-107.
20. *Dahl H, Warz S, Welland NL, Arnesen I, Marti H, Dierkes J.:* Factors associated with nutritional risk in patients receiving haemodialysis assessed by Nutritional Risk Screening 2002 (NRS2002). J Ren Care. 11 mai 2021;jorc.12374.
21. *Kennedy GL, Pedro MR, Seghieri C, Nantel G, Brouwer I.* Dietary Diversity Score Is a Useful Indicator of Micronutrient Intake in Non-Breast-Feeding Filipino Children. J Nutr. 1 févr 2007;137(2):472-7.
22. *Snelson M, Clarke R, Coughlan M.* Stirring the Pot: Can Dietary Modification Alleviate the Burden of CKD? Nutrients. 11 mars 2017;9(3):265.
23. *Karoune R, Mekhancha DE, Benlatreche C, Badaoui B, Nezzal L, Dahel-Mekhancha CC.* Évaluation de la qualité de l'alimentation d'adolescents algériens par le score d'adéquation aux recommandations nutritionnelles du PNNS (France). Nutr Clin Métabolisme. mai 2017;31(2):125-33.
24. *Vinhas J, Gardete-Correia L, Boavida JM, Raposo JF, Mesquita A, Fona MC, et al.* Prevalence of Chronic Kidney Disease and Associated Risk Factors, and Risk of End-Stage Renal Disease: Data from the PREVADIAB Study. Nephron Clin Pract. 2011;119(1):c35-40.
25. *Levey AS, Coresh J.* Chronic kidney disease. The Lancet. janv 2012;379(9811):165-80.
26. *Farhadnejad H, Asghari G, Mirmiran P, Yuzbashian E, Azizi F.* Micronutrient Intakes and Incidence of Chronic Kidney Disease in Adults: Tehran Lipid and Glucose Study. Nutrients. 20 avr 2016;8(4):217.
27. *Savy M, Martin-Prével Y, Sawadogo P, Kameli Y, Delpeuch F.* Use of variety/diversity scores for diet quality measurement: relation with nutritional status of women in a rural area in Burkina Faso. Eur J Clin Nutr. mai 2005;59(5):703-16.
28. *Madrigal JM, Cedillo-Couvert E, Ricardo AC, Appel LJ, Anderson CAM, Deo R, et al.* Neighborhood Food Outlet Access and Dietary Intake among Adults with Chronic Kidney Disease: Results from the Chronic Renal Insufficiency Cohort Study. J Acad Nutr Diet. juill 2020;120(7):1151-1162.e3.
29. *Akter R, Sugino H, Akhter N, Brown CL, Thilsted SH, Yagi N.* Micronutrient Adequacy in the Diet of Reproductive-Aged Adolescent Girls and Adult Women in Rural Bangladesh. Nutrients. 23 janv 2021;13(2):337.
30. *Savy M, Martin-Prével Y, Danel P, Traissac P, Dabiré H, Delpeuch F.* Are dietary diversity scores related to the socio-economic and anthropometric status of

women living in an urban area in Burkina Faso? Public Health Nutr. 2007;11(2):132-41.
31. FAO. Report on use of the Household Food Insecurity Access Scale and Household Dietary Diversity Score in two survey rounds in Manica and Sofala Provinces, Mozambique, 2006-2007. FAO food security project GCP/MOZ/079/BEL.
32. FAO. Profil nutritionnel de pays royaume du maroc. Popul Fr Ed. 2011;5(4):764.
33. *Aboussaleh, Y., Farsi, M., El Hioui, M., Ahami, A.:* Transition nutritionnelle au Maroc: Coexistence de l'anémie et de l'obésité chez les femmes au Nord Ouest marocain. Antropo, 2009. 19, 67-74. www.didac.ehu.es/antropo

Received: 14.02.2022
Accepted: 15.07.2022
Published online first: 02.09.2022

Rocz Panstw Zakl Hig 2022;73(3):303-313
http://wydawnictwa.pzh.gov.pl/roczniki_pzh/ https://doi.org/10.32394/rpzh.2022.0222

ORIGINAL ARTICLE

ASSESSMENT OF NUTRITIONAL STATUS, DIETARY INTAKE AND ADHERENCE TO DIETARY RECOMMENDATIONS IN TYPE 1 DIABETIC CHILDREN AND ADOLESCENTS

Sanaa El–Jamal[1], Houda Elfane[1], Hamid Chamlal[1], Khadija Sahel[1], Imane Barakat[1], Mohamed Mziwira[1,2], Aziz Fassouane[1,3], and Rekia Belahsen[1]

[1]Laboratory of Biotechnology, Biochemistry and Nutrition, Training and Research Unit on Nutrition and Food Sciences, Department of Biology, Faculty of Sciences, Chouaib Doukkali University, El Jadida 24 000, Morocco
[2]Higher Normal School, Hassan II University, Casablanca, Morocco
[3]Ibn Zohr University, Agadir, Morocco

ABSTRACT

Background. Currently, T1D is one of the most common chronic diseases in children and adolescents. The International Diabetes Federation (IDF) estimates that more than 1.1 million children and adolescents are living with (T1D). A few studies have evaluated the relationship between dietary intake and glycemic control (GC) in people with T1D, and in particular, children and adolescents.

Objective. The aim of this study was to evaluate the relationship between anthropometric characteristics, lipid profile, inflammation, dietary intake and GC in comparison with international guidelines.

Materials and methods. The study included a sample of 240 children, aged 15 years old or less with T1D. A structured questionnaire was used to collect information on the socio-demographic status, disease characteristics, and diet of the participants. Weight, height, and WC were measured and WHtR and BMI were calculated. Biochemical measurements were determined. Dietary intake was assessed using three 24-hour recalls.

Results. Saturated fat intake was five times higher than recommended. Only 8.3% of participants reached the recommended level of fiber. Overweight, obesity, TC, TG, HDL and CRP were significantly higher in children with poor GC to those with good GC. In addition, participants with poor GC had significantly low intakes of calories, carbohydrates, fiber, MUFAs, and PUFAs and high intakes of fat and SFAs. The use of Bivariate correlation analyses showed that calorie, protein, fat and fiber intake were positively correlated with weight, height, WC, and GO, whereas carbohydrate intake was negatively associated with these parameters. On the contrary, CO showed a negative correlation with calorie, protein, fat and fiber intake and a positive correlation with carbohydrate intake.

Conclusions. The results revealed that the dietary quality was poor and adherence to dietary recommendations was low with insufficient fiber intake and excess SFA. These results suggest that GC can be improved by a healthy, balanced diet by increasing fiber intake and limiting SFA intake.

Key words: *type 1 diabetes; overweight, obesity, lipid profile, dietary intake, glycemic control*

Abbreviations:

T1D: Type 1 diabetes; **GC**: glycemic control; **IDF**: International Diabetes Federation; **CVD**: cardiovascular diseases; **SFA**: Saturated Fatty Acids; **WHO**: World Health Organization; **HbA_1c**: Glycated hemoglobin; **WC**: Waist circumference; **WHtR**: Waist-to-Height ratio; **BMI:** Body Mass Index; **GO**: general obesity; **CO**: central obesity; **SD**: standard deviations; **FBG**: Fasting blood glucose; **PPG**: Postprandial blood glucose; **TC:** Total cholesterol; **LDL**: Low density lipoproteins; **HDL**: high density lipoproteins; **TG:** triglycerides; **CRP**: C-reactive protein; **TEI**: Total Energy Intake; **PUFA**: Polyunsaturated fatty acids; **MUFA**: Monounsaturated fatty acids; **ADA**: American Diabetes Association; **ISPAD**: International Society for Paediatrics and Adolescent Diabetes.

Corresponding author: Rekia Belahsen, Laboratory of Biotechnology, Biochemistry and Nutrition, Training and Research Unit on Nutrition and Food Sciences, Department of Biology, Faculty of Sciences, Chouaib Doukkali University, El Jadida 24 000, Morocco, Phone: +212664971616, e-mail: rbelahsen@yahoo.com or b.rekia@gmail.com

INTRODUCTION

Type 1 diabetes (T1D) is an autoimmune disease characterized by the destruction of beta cells in the islets of Langerhans of the pancreas that are responsible for insulin production [1]. Currently, T1D is one of the most common chronic diseases in children and adolescents [2]. The International Diabetes Federation (IDF) estimates that more than 1.1 million children and adolescents are living with T1D [1].

Young people with T1D have an increased risk of cardiovascular disease (CVD) [3]. Nutrition therapy is one of the cornerstones of the management of T1D. The main objectives of this therapy are the maintenance of stable blood glucose levels, with a reduction in complications, and the frequency of hypoglycemic and hyperglycemic episodes [4, 5].

In addition, the diet for children and adolescents with T1D is similar to that of the general population [6]. However, studies conducted to assess their dietary intake have shown that they do not meet dietary guidelines and their diets are less healthy than those of children without diabetes [7]. Indeed, research has shown that poor glycemic control (GC) was associated with low carbohydrate and high fat intake, particularly saturated fatty acids (SFAs) [8]; higher added sugar intake with insufficient fiber, fruits, vegetables and whole grains [9, 10]. However, results are conflicting; some studies have shown that better GC was associated with low carbohydrate intake [11]. Other studies have shown no association [12].

On the other hand, diabetes is often accompanied by a high prevalence of overweight/obesity and altered lipid profile [13]. Unfortunately, rates of overweight and obesity are steadily increasing, not only among the healthy population, but also among adolescents with T1D [14]. Furthermore, excessive weight is associated with an increased risk of CVD [15]. In Morocco, as in other developing countries, the prevalence of childhood obesity has increased in alarming proportions. Thus, according to the World Health Organization (WHO), Morocco is ranked among the countries with a prevalence of overweight/obesity of 10 to 14.9%, alongside Algeria and Tunisia [16]. This increase can be explained in part by changes in dietary habits in the general Moroccan population, whose consumption of high-calorie foods and beverages is increasing in association with an increase in sedentary behaviour [17].

It is also well known that inflammation is more claimed in people with T1D [18]. Unfortunately, few studies have evaluated the relationship between dietary intake and GC in people with T1D, and in particular, children and adolescents. Moreover, to our knowledge, there are no studies in a Moroccan population that have evaluated the same topic. Therefore, the objective of this study was to evaluate the relationship between anthropometric characteristics, lipid profile, inflammation, dietary intake and GC in Moroccan children and adolescents with T1D in comparison with international guidelines.

MATERIALS AND METHODS

Study population

This is a prospective descriptive study conducted at the level of the pediatric unit of the Mohamed V Provincial Hospital of El Jadida over a period from January 2018 to December 2020. The target population was 240 diabetic children, aged 15 years or less, with T1D for 12 months to avoid the remission period due to the residual secretion of endogenous insulin during recent diabetes.

A structured questionnaire was used and completed with the patients or their parents to collect data on the socio-demographic and socio-economic characteristics, family history, the disease characteristics (duration of diabetes, fasting and postprandial blood glucose), measurement of the HbA_1c level on the same day, diabetes management (number of daily insulin injections and frequency blood glucose self-monitoring per day), lipid profile and anthropometric measurements were also measured (weight, height, WC, sum of skinfolds, WHtR and BMI).

The interview was conducted with the parents (or the participant's guardian) when the child's age was less than 11 years and with the child him/herself when the child's age was 11 years or older. Treating physicians and medical records were also used as sources of data.

Socio-demographic and socio-economic characteristics

Data collected on participants' socioeconomic status (SES) and sociodemographic status are collected through structured interviews included, age, sex, area of residence, parental education and household income.

Anthropometric measurements

These parameters were measured on participants in the pediatrics unit on the day of the interview according to the World Health Organization (WHO) standards [19]. Weight was measured in kilogram to the nearest 100 g, on children lightly dressed and without shoes, on a mechanical scale. The height was measured in the participants to the nearest 0.1 centimeter using a wall scale with heels joined, legs straight, arms dangling and shoulders relaxed.

Waist circumference (WC) was measured on respondents standing with feet 2.5 cm apart, legs

straight, arms dangling and shoulders relaxed, the measuring tape was placed uncompressed at midway between the iliac crest and the last rib, at the end of expiration. The Waist-to-Height Ratio (WHtR) was calculated and the WHtR cut-off of 0.5 is used to define abdominal obesity for both boys and girls [20].

The amount and distribution of body fat were assessed by measuring the thickness of the subcutaneous adipose tissue with a Lange Skinfold Calliper (Cambridge Scientific Industries, Inc. Cambridge, Maryland). The Skinfold thickness was measured on the left side of the body at four sites: biceps and triceps (limb), sub scapular and supra-iliac (trunk). The sum of the four skinfold thickness measures were considered as an indicator of total subcutaneous fat.

Finally, the body mass index (BMI), a measure that estimates the fat mass of individuals, was calculated by dividing the weight in kg by the square of the height expressed in meters (kg/m2): BMI = Weight (kg)/Height^2 (m^2). The references established by WHO in 2007 are used to calculate Z Score values for BMI for age using WHO software, AnthroPlus (Version 1.0.4, 2010), to assess the growth of children and adolescents worldwide [21]. Children under five years old are considered underweight when Z score < −2 standard deviations (SD), overweight when a Z score > +2 SD and obese if Z score > +3 SD [22]. For the children aged 5 to 19 years, they were classified into 3 categories: underweight when Z Score < −2 SD, overweight if Z Score > +1 SD and obese if Z Score > +2 SD [23, 24].

HbA_1c

Metabolic control was assessed by HbA_1c levels. The level of this parameter was measured by boronate affinity chromatography, with the same assay kits (A_1C EZ 2.0; Bio-Hermes). According to ISPAD recommendations, HbA_1c level is optimal if <7.5%; suboptimal if $7.5\% \leq HbA_1c \leq 9.0\%$; and high risk when $HbA_1c > 9.0\%$ [25]. The patients were divided into two groups: a poor GC group if $HbA_1c > 9.0\%$; and a group with good GC when $HbA_1c \leq 9.0\%$.

Biochemical measurements

Venous blood samples were collected by venepuncture after a minimum of 10 hours of overnight fasting, except for postprandial blood glucose, which was collected 2 hours after eating. All analyses were performed on the day of blood collection using a Dirui automated system (CS-1200 Package; Dirui). Blood tests included fasting blood glucose (FBG), postprandial blood glucose (PPG), triglycerides (TG), total cholesterol (TC), low density lipoproteins (LDL), high density lipoproteins (HDL), and C-reactive protein (CRP).

FBG and PPG were measured using a glucose oxidase enzymatic method. TG and TC used an enzymatic method with glycerol phosphate oxidase and cholesterol esterase and cholesterol oxidase respectively. HDL cholesterol was measured, by the direct method, and LDL cholesterol was calculated using the Friedewald formula [LDL = TC− (HDL+TG/5)]. CRP was quantified in serum by turbidimetry and nephelometry.

On the basis of the biochemical data, the occurrence of lipid disorders was assessed in each patient. Abnormal values were identified with high TC ≥ 200 mg/dl, high LDL cholesterol with concentrations ≥ 100mg/dl, high TG with concentrations ≥ 150 mg/dl and low HDL cholesterol with concentrations ≤ 40mg/dl [26]. Serum concentrations of > 5 mg/l CRP were considered to indicate acute inflammation [27].

Dietary intake assessment

Dietary intake data are collected by the three 24-hour dietary recalls technique to list all foods ingested and participant's macronutrient and micronutrient intake (including two weekdays and one weekend day). Each respondent is asked to describe precisely everything they consumed (drunk and eaten) in the previous 24 hours, from rising the night before until the same time on the day of the survey. The respondent was also asked to quantify the foods described, with her own measures (household measures), using an iconographic manual [28]. Dietary intakes were converted to estimate energy and their composition in nutritional intakes using the BILNUT software (SCDA NUTRISOFT-BILNUT, version 2.01). The values obtained were then compared to the reference dietary intakes.

A semi-quantitative food frequency questionnaire (FFQ) was also completed in the participants to this study to transcribe their food consumption habits. The food frequency is composed of two parts: a closed list of foods and a section where consumption frequencies can be indicated (for example, several times a day, 3 to 4 times a week, 1 to 2 times a week, 1 to 2 times a month...) and a section with more detailed questions about the size the portion consumed and its composition.

Dietary intakes were compared to the International Society for Paediatric and Adolescent Diabetes (ISPAD) guidelines for fat intake (30-35% of total energy intake (TEI)) with saturated fatty acids (SFA) (<10% of TEI), polyunsaturated fatty acid (PUFA) intake (<10% of TEI), monounsaturated fatty acid (MUFA) intake (>10% of TEI, up to 20%), protein intake (15% to 20% of TEI) and carbohydrate intake (45-50% of TEI). The adequate fiber intake of children aged 1 year or more is 14 g/1000 kcal/day, while for

children aged > 2 years, the fiber intake is equal to: the child's age in years + 5 [4].

Statistical analysis

Data analysis was performed using SPSS for Windows (Statistical Package for the Social Sciences) software version 23.0. A descriptive analysis was conducted to describe the characteristics of the participants in this study, namely socio-demographic variables and anthropometric and biological measures. T test and *Chi*-square tests are applied for comparison of means ± SD and proportions with percentages of continuous and categorical variables, respectively. P values below 0.05 are considered statistically significant for all tests. In addition, Pearson correlations between macronutrients and anthropometric and biochemical variables were performed.

Ethical aspects

The questionnaire used in this study was validated by a scientific committee of the Chouaib Doukkali University of El Jadida and data collection began after obtaining an authorization from the Regional Health Directorate of the Casablanca-Settat region in Morocco. For each child, free and informed written consent was obtained from the parents or guardians before starting the survey. The procedures and objectives of the study were also clearly explained to the participants. The confidentiality and anonymity of the information collected were also respected.

RESULTS

Socio-demographic, anthropometric and biological characteristics by HbA_1c level

The results in Table 1 show that the proportions of diabetic children aged 11 to 15 years, patients living in rural areas, patients with mothers never attended, and children of low-income parents were significantly higher in T1D children with poor GC compared to those with good GC. In addition, there was a significant association between GC and disease duration. GC was associated with family history of diabetes, number of insulin injections, and self-monitoring of blood glucose ($P \leq 0.001$).

The table data show that BMI, sum of skinfolds, FBG, HbA_1C, TC, TG, HDL and CRP were also significantly higher in diabetic children with poor GC than those with good GC. In addition, overweight and obesity were significantly higher in children with poor than good GC ($P \leq 0.001$).

Association of dietary intake with GC

Dietary intake, including food group intake, is presented in Table 2. Compared with children with T1D of good GC, participants with poor GC had low calorie, carbohydrate, MUFA, and PUFA intakes, slightly high protein intakes, and higher fat and SFA intakes. All of these differences were significant.

Poorly balanced T1D patients also had lower fiber, Calcium, Zinc, Iron and Vit C intake and higher Sodium, Potassium and Phosphorus intake than those with good GC.

On the other hand, children with T1D with good GC had significantly higher intakes of cereals, vegetables/legumes and fish and lower intakes of potatoes, meat/poultry, and oils/fats.

Dietary intakes of macro- and micro-nutrients compared to dietary recommendations

Table 3 shows the mean dietary intake and the number (%) of participants adhering to recommended intakes for all nutrients assessed.

The mean energy intake was 1448.04±484.12 kcal/day. According to ISPAD guidelines for nutritional management, the contribution to TE of the mean carbohydrate intake represented 52% (higher than recommended), that of protein intake is approximately 14% (slightly lower than recommended). The mean fat intake was about 34% of TEI (in line with recommendations) and saturated fat intake averaged 48% of TEI which is significantly higher than recommended. The mean ± SD intake of fiber was 11.78±7.09 g/d with only 8.3% of the participants reaching the recommended level.

On the other hand, about three quarters of the respondents had inadequate Sodium and Phosphorus intake. However, Vit E and Potassium intake was very low in the T1D children (4.2% and 0.8% respectively) and about one quarter of T1D children had met the recommended intakes of Magnesium, Zinc, Vit B1, Vit C and Folate.

Correlations between macronutrients and anthropometry and biochemical variables in children T1D

The use of bivariate correlation analyses showed that calorie, protein, fat, and fiber intakes were positively correlated with weight, height, WC, sum of skinfolds and GO (BMI). In contrast, carbohydrate intake was negatively associated with these parameters (Table 4). On the other hand, CO (WHtR) showed a significantly negative correlation with calorie, protein, fat and fiber intake and a positive correlation with carbohydrate intake.

On the other hand, HbA1c and LDL were significantly and positively associated with fat and SFA intake and negatively associated with carbohydrate, MUFA, PUFA and fiber intake. Finally, HDL was positively associated with carbohydrate and PUFA intake and negatively with SFA intake.

Table 1. Socio-demographic, anthropometric, and biological characteristics by HbA_1c level

Variables (n=127)		Good HbA_1c (n=113)	Poor HbA_1c (n=240)	Total	P-value
Socio-demographic characteristics					
Sex	Male	64(55.7%)	51(44.3%)	115(100%)	0.415
	Female	63(50.4%)	62(49.6%)	125(100%)	
Age category	<= 4 years	32(56.1%)	25(43.9%)	57(100%)	≤ 0.001
	5-10 years	57(68.7%)	26(31.3%)	83(100%)	
	11-15 years	38(38%)	62(62%)	100(100%)	
Area of residence	Urban	84(58.7%)	59(41.3%)	143(100%)	0.028
	Rural	43(44.3%)	54(55.7%)	97(100%)	
Education attainment of the father	Never attended	76(47.2%)	85(52.8%)	161(100%)	0.087
	Primary school	11(57.9%	8(42.1%)	19(100%)	
	College school	14(66.7%)	7(33.3%)	21(100%)	
	Secondary school	7(53.8%)	6(46.2%)	13(100%)	
	University	19(73.1%)	7(26.9%)	26(100%)	
Education attainment of the mother	Never attended	81(46.0%)	95(54.0%)	176(100%)	0.005
	Primary school	23(65.7%)	12(34.3%)	35(100%)	
	College school	9(69.2%)	4(30.8%)	13(100%)	
	Secondary school	8(88.9%)	1(11.1%)	9(100%)	
	University	6(85.7%)	1(14.3%)	7(100%)	
Household income	Low	98(47.3%)	109(52.7%)	207(100%)	≤ 0.001
	Medium	22(84.6%)	4(15.4%)	26(100%)	
	High	7(100%)	0(0%)	7(100%)	
Characteristics of the disease					
Duration of diabetes (years)		2.66±1.80	3.89±2.78	3.24±2.39	≤ 0.001
History of diabetes	No previous history	102(82.9)	21(17.1)	123(100%)	≤ 0.001
	History of diabetes	25(21.4)	92(78.6)	117(100%)	
Number of injections / day	2 times/day	111(57.2%)	83(42.8%)	194(100%)	≤ 0.001
	3 times/day	11(26.8%)	30(73.2%)	41(100%)	
	4 times/day	5(100%)	0(0%)	5(100%)	
Self-monitoring of blood glucose	< 4times/day	98(46.9%)	111(53.1%)	209(100%)	≤ 0.001
	≥ 4 times/day	29(93.5%)	2(6.5%)	31(100%)	
Anthropometric characteristics					
Weight (kg)		25.56±12.29	33.76±16.20	29.41±14.81	≤ 0.001
Height (m)		1.21±0.21	1.31±0.23	1.25±0.23	0.143
WC (cm)		54.29±6.11	57.82±6.74	55.95±6.64	0.356
BMI (kg/m2)		18.29±4.32	20.9±5.59	19.51±5.12	≤ 0.001
WHtR (cm)		0.45±0.054	0.44±0.055	0.45±0.05	0.852
Sum of skinfolds (mm)		30.66±6.17	33.44±8.01	31.97±7.22	0.001
BMI Categories	Normal weight	98(59.4%)	67(40.6%)	165(100%)	≤ 0.001
	Overweight	8(21.1%)	30(78.9%)	38(100%)	
	Obese	2(33.3%)	4(66.7%)	6(100%)	
	Underweight	19(61.3%)	12(38.7%)	31(100%)	
Categories WHtR	No abdominal obesity	96(52.2%)	88(47.8%)	184(100%)	0.676
	Abdominal obesity	31(55.4%)	25(44.6%)	56(100%)	
Biological characteristics					
FBG (mg/dl)		2.6±0.08	2.94±0.06	2.76±0.05	0.033
PPG (mg/dl)		3.37±0.09	3.78±0.07	3.57±0.06	0.109

HbA_1c (%)		7.85±0.07	11.6±0.17	9.62±0.15	≤ 0.001
TC (mg/dl)		1.52±0.021	1.7±0.020	1.61±0.015	0.024
HDL (mg/dl)		0.65±0.01	0.47±0.01	0.56±0.01	0.001
LDL (mg/dl)		0.63±0.02	0.98±0.02	0.80±0.02	0.637
TG (mg/dl)		1.15±0.02	1.23±0.01	1.19±0.01	0.003
CRP (mg/l)		2.98±0.22	4.78±0.36	3.83±0.21	0.013

Abbreviations: Data are presented as mean ± standard deviation (SD) or number (%).
BMI: Body Mass Index; WHtR: Waist-to-Height ratio; FBG: fasting blood glucose; PPG: postprandial blood glucose; HbA_1c: Glycated hemoglobin; TC: Total cholesterol; HDL: High density lipoprotein cholesterol; LDL: Low density lipoprotein cholesterol; TG, Triglycerides; CRP: C-reactive protein.
The differences between socio-demographic, anthropometric and biological characteristics according to the level of HbA_1c were compared by the t test for continuous variables and by Chi2 test for categorical variables. The mean difference is significant at the 0.05 level.

Table 2. Association of dietary intake with GC

	Good HbA_1c (n=127)	Poor HbA_1c (n=113)	Total (n=240)	P-value
Calories and macronutrient intake				
Calories (kcal/d)	1480.41±530.31	1411.66±425.71	1448.04±484.12	0.012
Carbohydrates (%AET)	54.69±4.66	49.21±6.92	52.11±6.44	0.001
Protein (% AET)	14.09±2.19	14.14±2.94	14.11±2.57	0.01
Lipids (% AET)	31.21±4.08	36.66±5.68	33.78±5.59	0.007
AGS(%)	43.81±11.01	53.41±8.00	48.33±10.81	≤ 0.001
MUFA(%)	42.54±7.7	37.78±6.05	40.3±7.35	0.006
PUFA(%)	13.63±8.68	8.78±5.00	11.35±7.57	≤ 0.001
Micronutrient intake				
Fibers (g/d)	12.91±8.56	10.52±4.67	11.78±7.09	≤ 0.001
Cholesterol (mg/d)	105.01±85.46	89.02±78.68	97.48±82.56	0.368
Sodium (mg/d)	2364.23±1309.54	2450.44±1194.46	2404.82±1254.81	0.002
Magnesium (mg/d)	201.96±102.51	194.11±111.79	198.26±106.83	0.173
Calcium (mg/d)	714.8±386.06	445.09±286.88	587.81±367.89	≤ 0.001
Potassium (mg/d)	1576.63±676.08	1734.15±1016.38	1650.8±855.15	0.036
Zinc (mg/d)	5.58±3.07	4.65±2.11	5.14±2.7	≤ 0.001
Iron (mg/d)	6.31±2.35	5.81±2.72	6.08±2.54	0.028
Phosphorus (mg/d)	888.68±419.75	1044.62±463.11	962.1±446.63	0.049
Vit E (mg/d)	2.41±1.97	1.94±1.52	2.19±1.78	0.075
Vit B1 (mg/d)	0.54±0.22	0.52±0.2	0.53±0.21	0.885
Vit C (mg/d)	29.8±19.52	23.72±16.12	26.94±18.22	0.005
Folate (µg/d)	173.19±84.28	185.27±94.25	178.88±89.13	0.171
Food group intake (g/d)				
Cereals	416.74±186.59	336.19±116.76	378.81±162.38	≤ 0.001
Potatoes	59.46±28.66	73.63±39.77	66.13±34.99	≤ 0.001
Vegetables	167.58±65.19	123.69±45.86	146.92±60.89	0.003
Legumes	7.318±3.92	7.019±4.71	7.17±4.30	0.033
Fruits	109.17±37.9	83.33±39.79	97±40.82	0.626
Meat/poultry	36.86±14.99	49.06±24.43	42.60±20.87	0.028
Fish	22.01±14.78	17.40±9.05	13.90±8.76	≤ 0.001
Eggs	12.71±6.10	15.23±10.89	19.84±12.60	0.579
Dairy products	266.82±103.28	199.88±110.60	235.30±111.70	0.632
Oils/fats	16.42±8.41	21.25±11.84	18.7±10.43	≤ 0.001

Abbreviations: TEI: Total Energy Intake; SFA: saturated fatty acids; MUFA: monounsaturated fatty acids; PUFA: polyunsaturated fatty acids.

Table 3. Daily macronutrient and micronutrient intake and proportion of participants meeting recommended targets

Nutrients	Average daily intake	n(%) meeting goals of ISPAD[a] or RDA[b]
Energy, calories	1448.04±484.12	-
Carbohydrates, % TE[a]	52.11±6.44	62(25.8%)
Proteins, % TE[a]	14.11±2.57	89(37.1%)
Lipids, % TE[a]	33.78±5.59	84(35%)
AGS, % TE[a]	48.33±10.81	0(0%)
Fibers (g/d) [a]	11.78±7.09	20(8.3%)
Sodium (mg/d) [b]	2404.82±1254.81	72(30%)
Magnesium (mg/d) [b]	198.26±106.83	55(22.9%)
Calcium (mg/d) [b]	587.81±367.89	27(11.3%)
Potassium (mg/d) [b]	1650.8±855.15	2(0.8%)
Zinc (mg/d) [b]	5.14±2.7	54(22.5%)
Iron (mg/d) [b]	6.08±2.54	43(17.9%)
Phosphorus (mg/d) [b]	962.1±446.63	66(27.5%)
Vit E (mg/d) [b]	2.19±1.78	10(4.2%)
Vit B1 (mg/d) [b]	0.53±0.21	56(23.3%)
Vit C (mg/d) [b]	26.94±18.22	55(22.9%)
Folate (µg/d) [b]	178.88±89.13	51(21.3%)

Abbreviations: Data are presented as mean ± standard deviation (SD). % TE: Percentage of total energy. ISPAD[a]: International Society for Pediatric and Adolescent Diabetes; RDA[b]: Recommended Dietary Allowance. Food and Nutrition Board. Institute of Medicine. National Academies. 2010.

[a] Target values recommended by ISPAD: **Carbohydrate**: 45-50% of TEI; **protein**: 15-20% of TEI; **Total Fat**:30-35% of TEI; Saturated Fat <10% of TEI; **Fiber:** 1 year or greater: 14 g/1000 kcal; > 2 years old: age (years) + 5.

[b] Target values recommended by RDA: [b] **Sodium**: Children 1-3y: 1g; Children 4-8y: 1.2g; Male or female 9-15y: 1.5g; [b] **Magnesium**: Children 1-3y: 80mg; Children 4-8y: 130mg; Male or female 9-13y: 240mg; Male 14-15y: 410mg; Female 14-15y: 360; [b] **Calcium**: Children 1-3y: 700mg; Children 4-8y: 1000mg; Male or female 9-15y: 1300mg; [b] **Potassium**: Children 1-3y: 3g; Children 4-8y: 3.8g; Male or female 9-13y: 4.5g; Male or female 14-15y: 4.7g; [b] **Zinc**: Children 1-3y: 3mg; Children 4-8y: 5mg; Male or female 9-13y: 8mg; Male 14-15y: 11mg; Female 14-15y: 9mg;[b] **Iron:** Children 1-3y: 7mg; Children 4-8y: 10mg; Male or female 9-13y: 8mg; Male 14-15y: 11mg; Female 14-15y: 15mg;[b]**Phosphorus:** Children 1-3y: 460mg; Children 4-8y: 500mg; Male or female 9-15y: 1250mg; [b] **Vit E**:Children 1-3y: 6; Children 4-8y: 7; Male or female 9-13y: 11; Male or female 14-15y: 15; [b] **Vit B1**:Children 1-3y: 0.5mg; Children 4-8y: 0.6mg; Male or female 9-13y: 0.9mg; Male 14-15y 1.2mg; Female 14-15y: 1mg; [b]**Vit C**: Children 1-3y: 15mg; Children 4-8y: 25mg; Male or female 9-13y: 45mg; Male 14-15y: 75mg; Female 14-15y: 65mg; [b] **Folates**: Children 1-3y: 150 µg; children 4-8y: 200 µg; Male or female 9-13y: 300 µg; Male or female 14-15y: 400 µg.

Table 4. Correlations between macronutrients and anthropometry, and biochemical variables in children T1D

	Calories (kcal/d)	Carbohydrates (%TEI)	Protein (%TEI)	Lipids (%TEI)	SFA (%)	MUFA (%)	PUFA (%)	Fibers (g/d)
Anthropometric characteristics								
Weight (kg)	0.491**	-0.406**	0.235**	0.363**	0.035	-0.008	-0.043	0.309**
Height(m)	0.533**	-0.377**	0.258**	0.318**	-0.013	0.026	-0.008	0.303**
WC (cm)	0.445**	-0.361**	0.246**	0.303**	0.044	0.023	-0.085	0.285**
BMI (kg/m^2)	0.492**	-0.372**	0.193**	0.344**	0.011	0.002	-0.018	0.335**
WHtR (cm)	-0.436**	0.241**	-0.152*	-0.210**	0.091	-0.036	-0.094	-0.238**
Sum of skinfolds	0.386**	-0.304**	0.179**	0.269**	0.034	0.039	-0.087	0.302**
Biological characteristics								
FBG (mg/dl)	0.023	-0.03	-0.078	0.069	0.021	-0.047	0.014	-0.056
PPG (mg/dl)	0.042	-0.048	-0.075	0.089	0.038	-0.075	0.018	-0.098
HbA_1c (%)	0.003	-0.375**	0.012	0.427**	0.415**	-0.323**	-0.279**	-0.258**
TC (mg/dl)	-0.084	-0.124	-0.023	0.156*	0.123	-0.117	-0.061	-0.142*

HDL (mg/dl)	0.082	0.150*	-0.108	-0.123	-0.340**	0.218**	0.273**	0.093
LDL (mg/dl)	-0.113	-0.174**	0.054	0.177**	0.289**	-0.209**	-0.208**	-0.159*
TG (mg/dl)	-0.036	-0.128*	-0.028	0.163*	0.157*	-0.145*	-0.084	-0.086
CRP	-0.021	-0.083	-0.011	0.114	0.09	-0.158*	0.025	-0.038

Abbreviations: BMI: Body Mass Index; WHtR: Waist-to-Height ratio; FBG: fasting blood glucose; PPG: Postprandial glycaemia. HbA_1c: Glycated hemoglobin; TC: Total cholesterol; HDL: High density lipoprotein cholesterol; LDL: Low density lipoprotein; TG, Triglycerides; CRP: C-reactive protein; ** significance level: <0.01; * significance level: <0.05.

DISCUSSION

The results of the present study show that the average dietary intake of Moroccan children with T1D was far from dietary recommendations. In this current study, daily intake and dietary quality were assessed in patients with T1DM at the Paediatric service to examine whether they adhered to the international dietary recommendations. The study revealed that SFA intake was almost five times the recommended maximum and fiber intake was inadequate in most young people. In addition, the present data also indicate an association of socio-demographic, anthropometric, biological characteristics, and dietary intake with GC in children and adolescents with T1D.

To our knowledge, few studies have focused on the assessment of nutritional status in children and adolescents with T1D. The prevalence of obesity is increasing in subjects with T1D [29]. Indeed, several factors associated with T1D may favor overweight, among them difficulty in managing GC and an unhealthy diet rich in animal fats [10]. In addition, the introduction of insulin treatment is associated with excess body weight in patients with T1D [7]. The results of the present study showed that the majority of children with T1D (68.8%) had normal nutritional status. However, about 16% of the participants were overweight and 2.5% were obese. This may be explained by the third phase of the nutritional transition that Morocco is going through, in addition to globalization, accompanied by considerable changes towards unhealthy lifestyles, including low physical activity and the adoption of Western eating habits with increasing consumption of fast food, rich in fat and sugar [17]. Previous data have also shown that overweight and GO are associated with poor GC. An association between HbA_1c and high fat accumulation in the abdominal region has been reported in the literature [30]. In contradiction with these studies, however, no significant difference was between CO and GC in the present study. On the other hand, a significant difference between TC, HDL, TG and HbA_1c was in the sample of children studied. Previous studies have also reported changes in lipid profile in patients with poor GC and long disease duration [31].

It is therefore clear that a healthy diet is essential for better GC and for the management of obesity and dyslipidemia, which contribute to CVD risk in diabetic subjects [32]. The children with T1DM in this study had a dietary intake far from the nutritional recommendations, particularly regarding to SFA and fiber intakes. These results agree with several studies that have reported poor adherence to dietary recommendations in children and adolescents with T1DM [33]. Furthermore, this poor adherence was associated with poor GC and thus risk of complications and CVD [34].

The results of this study agree with previous studies that indicated that high dietary carbohydrate intake was associated with low HbA_1c [35]. In contrast, another study showed that increased carbohydrate intake increased HbA_1c levels in young patients with T1D [36]. Other macronutrients that should be considered in the dietary recommendations include fat, protein, and fiber intake [37]. In addition, the American Heart Association has advocated moderate fat intake while reducing SFAs and replacing them with MUFAs and PUFAs [38]. In agreement with previous studies [39], the data reported in the present study show an association of fat and SFA with HbA_1c levels. However, this association is controversial in the literature. Some of studies have found no association between fat intake and GC [40], while on the contrary; other studies have reported that high fat intakes are associated with poor GC [41]. These different results could be explained by the replacement of carbohydrates by fats [42]. Protein intake was also associated with HbA_1c in the sample of participants in this study. In contrast, studies have found that low protein intake was associated with better GC [35].

The beneficial role of fiber intake in weight management, CVD prevention and digestive health is reported in the literature [43]. Mixed results regarding this role have been reported. Indeed, an association between low fiber intake and poor GC has been found by studies and confirmed by the present study [8]. In contrast, other studies have found no association between fiber and HbA_1c [41].

Furthermore, in this study, children, and adolescents with T1D of poor GC consumed fewer cereals and vegetables and more fat than youth with better GC. Our results are consistent with other studies that have found that adolescents with T1D with better optimal GC had low consumption of added sugars and high

consumption of fiber, fruits, and vegetables compared to those with less optimal GC [9].

Strengths and limitations of the study

This study has strengths and limitations. To our knowledge, this is the first study to assess daily dietary intake in Morocco in children and adolescents with T1D in relation to international recommendations. The strengths of the study include the use of three 24-hour recalls assessing dietary intake, which is one of the best methods for collecting dietary data, and the use of the food frequency questionnaire to more accurately determine the amount of food portions ingested.

Nevertheless, our study has some limitations that need to be considered. Firstly, the majority of participants are treated with two insulin injections and, therefore, the results may not be generalizable to adolescents with T1D on other types of diets. Secondly, it should be mentioned that the estimation of food intake is often misreported, and especially underreported, by children and adolescents with T1D. Thirdly, the number of subjects who participated in the study should be larger. This limitation is, however, offset by the accurate clinical measures that were collected. Finally, the study population may not be representative of the Moroccan population of children with diabetes. It would be wise to extend this study to a larger representative sample to generalize the results obtained.

CONCLUSION

In conclusion, the results of the present study indicate that the quality of the diet of children with T1D was poor and adherence to dietary recommendations was low with insufficient fiber intake and excess of SFA. Considering our results, we suggest continued nutrition education for children and parents by focusing on a healthy, balanced diet and limiting high-fat foods and increasing consumption of fiber-rich foods such as fruits and vegetables to optimize growth, maintain normal weight, reduce CVD risk, and improve GC in young diabetics.

Acknowledgements

The authors would like to thank the study participants (children, parents, or guardians of the participant), the Provincial Delegation of the Province of El Jadida, the Regional Health Directorate of Casablanca-Settat, and the medical and paramedical team for their cooperation. We would also like to thank the director of the Provincial Hospital Center and the staff of the biological analysis laboratory of this hospital for their cooperation. Special thanks are due to the staff of the pediatric unit of this hospital for allowing us to collect data. The study was supported by the Moroccan Ministry of Higher Education and Research.

Funding sources

This research has not received any specific grants from funding agencies in the public, commercial, or not-for-profit sectors.

Conflicts of interest

The authors declare that they have no competing interests.

REFERENCES

1. *International Diabetes Federation (IDF):* Diabetes in the young: a global perspective. In: IDF Diabetes Atlas. Ninth Edition. Brussels: International Diabetes Federation 2019. Available from: www.idf.org.
2. *Simmons K.M:* Type 1 Diabetes: A Predictable Disease. World J. Diabetes 2015; 6:380–390.
3. *Benjamin EJ, Blaha MJ, Chiuve SE, Cushman M, Das SR, DeFerranti SD, et al.* Heart Disease and Stroke Statistics Update: A Report from the American Heart Association. Circulation 2017;135:e146–e603.
4. *DiMeglio L.A., Acerini C.L., Codner E., Craig M.E., Hofer S.E., Pillay K., and al:* ISPAD Clinical Practice Consensus Guidelines: GC Targets and Glucose Monitoring for Children, Adolescents, and Young Adults with Diabetes.
5. Pediatric Diabetes 2018;19:105–114.
6. *American Diabetes Association*: Children and Adolescents: Standards of Medical Care in Diabetes—2021. Diabetes Care 2021.44 (Suppl. 1), S180–S199.
7. *Smart C., Aslander-van Vliet E., Waldron S:* Nutritional management in children and adolescents with diabetes. Pediatr Diabetes 2009; 10(suppl 12):100-117.
8. *Rovner AJ, Nansel TR:* Are children with type 1 diabetes consuming a healthy diet?: a review of the current evidence and strategies for dietary change. Diabetes Educ 2009;35:97–107.
9. *Katz ML, Mehta S, Nansel T, Quinn H, Lipsky LM, Laffel LM:* Associations of nutrient intake with GC in youth with T1D: differences by insulin regimen. Diabetes Technol Ther 2014;16(8):512–518.
10. *Overby NC, Margeirsdottir HD, Brunborg C, Andersen LF, Dahl-Jorgensen K:* The influence of dietary intake and meal pattern on blood glucose control in children and adolescents using intensive insulin treatment. Diabetology 2007;50:2044–51.
11. *Mayer-Davis E, Nichols M, Liese A et al :* Dietary intake among youth with diabetes: the SEARCH for Diabetes in Youth Study. J. Am. Diet 2006; Assoc 106(5), 689–697.
12. *Meissner T, Wolf J, Kersting M, Frohlich-Reiterer E, Flechtner-Mors M, Salgin B, and al:* Carbohydrate intake in relation to BMI, HbA1c and lipid profile in children and adolescents with type 1 diabetes. Clin Nutr 2014;33(1):75–8.

13. *Michaliszyn SF, Shaibi GQ, Quinn L, Fritschi C, Faulkner MS*: Physical fitness, dietary intake, and metabolic control in adolescents with type 1 diabetes. Pediatric Diabetes 2009;10:389–94.
14. *American Diabetes Association*: Nutrition recommendations and interventions for diabetes. A position statement of the American Diabetes Association. Diabetes Care 2006:29:2140–2157.
15. *Minges, KE; Whittemore, R.; Grey, M:* Overweight and obesity in youth with type 1 diabetes. Ann. Rev. Nurs. Res 2013;31:47–69.
16. *Van Vliet M., Van der Heyden J.C., Diamant M., et al:* Overweight is highly prevalent in children with type 1 diabetes and associates with cardiometabolic risk. J Pediatr 2010;156 (6):923-929.
17. *World Health Organization*: World diabetes report 2016. Available from:
18. https://apps.who.int/iris/bitstream/handle/10665/254648/9789242565256-eng.pdf.
19. *Belahsen R.* Nutrition transition and food sustainability. Proc Nutr Soc 2014;73:385–388.
20. *Snell-Bergeon J.K., West N.A., Mayer-Davis E.J., Liese A.D., Marcovina S.M., D'Agostino R.B., and al:* Inflammatory Markers Are Increased in Youth with Type 1 Diabetes: The SEARCH Case-Control Study. J. Clin. Endocrinol. Metab 2010;95:2868–2876.
21. *Purnell J.Q:* Definitions, Classification, and Epidemiology of Obesity 2000. [Updated 2018 Apr 12]. In: *Feingold KR, Anawalt B, Boyce A*, and al., editors. Endotext [Internet]. South Dartmouth (MA): MDText.com, Inc. Available from:https://www.ncbi.nlm.nih.gov/books/NBK279167.
22. *McCarthy H.D., Ashwell M:* A study of central fatness using waist-to-height ratios in UK children and adolescents over two decades supports the simple message—"keep your waist circumference to less than half your height". Int J Obes 2006; (London) 30:988–992.
23. WHO *Anthro* (version 1.0.4, January 2011) and macros [Internet] 2011: [cited 2012 May 5]. Available from: http://www.who.int/childgrowth/software/en/.
24. WHO. Child Growth Standards: Length/height-for-age, weight-for-age, weight-for-length, weight-for-height and body mass index-for-age. Methods and development 2006. Available from: https://www.who.int/childgrowth/standards/Technical_report.pdf?ua=1.
25. World Health Organization*:* WHO Child Growth Standards. Growth reference data for 5– 19 years. 2007. Available from: http://www.who.int/growthref/en/.
26. *Butte N.F., Garza C., de Onis M*: Evaluation of the feasibility of international growth standards for school-aged children and adolescents. J Nutr 2007;137:153–157.
27. *Rewers M., Pihoker C., Donaghue K* : Assessment and monitoring of GC in children and adolescents with diabetes. Pediatr Diabetes 2007;8:408–418.
28. *Donaghue K.C., Wadwa R.P., Dimeglio L.A:* ISPAD Clinical Practice Consensus Guidelines. Microvascular and macrovascular complications in children and adolescents. Pediatr Diabetes. 2014;15(Supplement 20):257–269.
29. *Thurnham D.I., Mccabe L.D., Haldar S., Wieringa F.T., Northrop-clewes C.A., Mccabe G.P:* Adjusting plasma ferritin concentrations to remove the effects of subclinical inflammation in the assessment of iron deficiency: 2018; (August):546–555.
30. *Elmoumni K:* Typical foods and preparations of the Moroccan population, Tool for estimating food consumption, Center for Information and Research on Food Intolerances and Hygiene (CIRIHA), 2008.
31. *DuBose S.N., Hermann J.M., Tamborlane W.V:* Obesity in youth with T1D in Germany, Austria, and the United States. J Pediatr 2015; 167(3):627–632.
32. *Valerio G., Iafusco D., Zucchini S., Maffeis C:* Study-Group on Diabetes of Italian Society of Pediatric Endocrinology and Diabetology [ISPED]. Abdominal adiposity and cardiovascular risk factors in adolescents with T1D. Diabetes Res Clin Pract 2012;97(1):99–104.
33. *Wysocka-Mincewicz M, Kołodziejczyk H, Wierzbicka E, et al*: Overweight, obesity and lipid abnormalities in adolescents with type 1 diabetes. Pediatr Endocrinol Diabetes Metab 2015;21(2):70–81.
34. *Evert A.B., Dennison M., Gardner C.D., Garvey W.T., Lau K.H.K., MacLeod J., Mitri J., et al*: Nutrition Therapy for Adults With Diabetes or Prediabetes: A Consensus Report. Diabetes Care 2019; 42:731–754.
35. *Mackey E.R., Rose M., Tully C., Monaghan M., Hamburger S., Herrera N., and al:* The Current State of Parent Feeding Behavior, Child Eating Behavior, and Nutrition Intake in Young Children with Type 1 Diabetes. Pediatric Diabetes 2020;21:841–845.
36. *Mackey E.R., O'Brecht L., Holmes CS, Jacobs M, Streisand R:* Teens with Type 1 Diabetes: How Does Their Nutrition Measure Up? J. Diabetes Res 2018 Sept.6;2018:5094569. Doi : 10.1155/2018/5094569. eCollection 2018.
37. *Nansel T.R, Lipsky L.M, Liu A:* Greater diet quality is associated with more optimal GC in a longitudinal study of youth with type 1 diabetes. Am J Clin Nutr 2016;104:81–87.
38. *Lamichhane A.P., Crandell J.L., Jaacks L.M., Couch S.C., Lawrence J.M., Mayer-Davis E.J:* Longitudinal associations of nutritional factors with glycated hemoglobin in youth with type 1 diabetes: the SEARCH Nutrition Ancillary Study. Am J Clin Nutr 2015;101:1278–1285.
39. *Smart C.E., Annan F., Higgins L.A., Jelleryd E., Lopez M., Acerini C.L*: ISPAD Clinical Practice Consensus Guidelines: Nutritional Management in Children and Adolescents with Diabetes. Pediatric Diabetes 2018;19:136–154.
40. *Sacks F., Lichtenstein A., Wu JHY et al*: Dietary Fats and Cardiovascular Disease: A Presidential Advisory from the American Heart Association 2017. Traffic.13 (3), e1–e23.
41. *Mehta SN, Volkening LK, Quinn N, Laffel LM:* Intensively managed young children with type 1 diabetes consume high-fat, low-fiber diets similar to age-matched controls. Nutr. Res 2014;34:428–435.
42. *Lamichhane A.P., Crandell J.L., Jacks L.M., Couch S.C., Lawrence J.M., Mayer-Davis E.J:* Longitudinal Associations of Nutritional Factors with Glycated

Hemoglobin in Youth with Type 1 Diabetes: The SEARCH Nutrition Ancillary Study. Am. J. Clin. Nutr 2015;101:1278–1285.
43. *Delahanty L.M., Nathan D.M., Lachin J.M., Hu F.B., Cleary P.A., Ziegler G.K., and al:* Association of diet with glycated hemoglobin during intensive treatment of type 1 diabetes in the Diabetes Control and Complications Trial. Am J Clin Nutr 2009;89:518–524.
44. *Soedamah-Muthu S.S., Chaturvedi N., Fuller J.H., Toeller MGroup EPCS:* Do European people with type 1 diabetes consume a high atherogenic diet? 7-year follow-up of the EURODIAB Prospective Complications Study. Eur J Nutr 2013;52:1701–1710.
45. *Dahl W., Stewart M:* Position of the Academy of Nutrition and Dietetics: Health Implications of Dietary Fiber. J. Acad. Nutr. Diet 2015;115(11):1861–1870.

Received: 27.03.2022
Accepted: 06.07.2022
Published online first: 02.09.2022

Rocz Panstw Zakl Hig 2022;73(3):315-323
http://wydawnictwa.pzh.gov.pl/roczniki_pzh/ https://doi.org/10.32394/rpzh.2022.0217

ORIGINAL ARTICLE

AN EVALUATION OF THE KNOWLEDGE ON SPECIFIC NUTRITIONAL NEEDS AND FACTORS AFFECTING PREGNANCY OUTCOME IN WOMEN OF REPRODUCTIVE AGE

Julianna Kostecka[1], Monika Bojanowska[2], Joanna Kostecka-Jarecka[3], Katarzyna Kolasa[2], Małgorzata Kostecka[2]

[1]Faculty of Medicine, Medical University of Lublin, Chodźki 19, 20-093 Lublin, Poland
[2]University of Life Science, Faculty of Food Science and Biotechnology, Department of Chemistry, Akademicka 15, 20-950 Lublin, Poland
[3]Independent Public Healthcare Center in Łęczna, Krasnystawska 52, 21-010 Łęczna, Poland

ABSTRACT

Background. Pregnancy is one of the few periods in a woman's life when extra weight is not perceived as a negative factor, but has positive connotations with healthy fetal development, which runs against the modern cult of a perfect, slim and healthy body. Most research studies focus on excessive rather than insufficient weight gain in successive trimesters of pregnancy.

Objective. The aim of this study was to evaluate women's knowledge about the influence of diet on pregnancy outcome and to assess changes in body weight and eating behaviors during pregnancy.

Materials and methods. The study consisted of an online survey. A total of 325 correctly and completely filled in questionnaires were considered. The respondents were divided into two groups: women without children (44.92%) and pregnant women and mothers (55.08%). The respondents' knowledge about eating behaviors during pregnancy was compared between the two groups. The responses given by mothers were used to evaluate weight gains during pregnancy and the nutritional status of pregnant women.

Results. Changes in body weight during pregnancy were regarded as acceptable and pregnancy weight gain was considered a normal process by 92% of the respondents. Pregnant women had greater knowledge about the need for increased caloric intake in successive trimesters ($p=0.0012$). The respondents' knowledge about maternal health and healthy fetal development was assessed with the use of 10 true or false questions. The average score was 6.3±1.8 points, and no significant differences were found between mothers/pregnant women and women without children (6.6±1.3 vs 6.2±1.7, $p>0.05$). In the present study, 67% of the respondents were of the opinion that they followed a healthy diet, 14% claimed that they did not eat right, but were not motivated to make any changes.

Conclusions. The respondents were aware that weight gain during pregnancy is a normal physiological process, but 1/3 of the respondents did not feel comfortable with the observed changes. The respondents did not have sufficient knowledge about the influence of maternal weight on fetal development. The use of diuretics and laxatives by pregnant women without medical consultation is a worrying phenomenon. These results indicate that women should have better access to knowledge about the impact of healthy nutrition on pregnancy outcome.

Key words: *eating disorders, pregnancy, pregorexia, malnutrition, body weight*

STRESZCZENIE

Wprowadzenie. Ciąża to jeden z nielicznych okresów w życiu kobiety, w których nadwaga nie jest postrzegana jako czynnik negatywny, ale kojarzy się pozytywnie ze zdrowym rozwojem płodu, co sprzeczne jest ze współczesnym kultem idealnego, szczupłego i zdrowego ciała. Większość badań naukowych skupia się na nadmiernym, a nie niedostatecznym przybieraniu na wadze w kolejnych trymestrach ciąży.

Cel. Celem pracy była ocena wiedzy kobiet na temat wpływu diety na przebieg ciąży oraz ocena zmian masy ciała i zachowań żywieniowych w okresie ciąży.

Materiał i metody. Badanie składało się z ankiety internetowej, uwzględniono 325 poprawnie wypełnionych ankiet. Respondenci zostali podzieleni na dwie grupy: kobiety bezdzietne (44,92%) oraz kobiety w ciąży i matki (55,08%). Porównano wiedzę respondentek na temat zachowań żywieniowych w czasie ciąży między obiema grupami. Odpowiedzi udzielone przez matki zostały wykorzystane do oceny przyrostów masy ciała w czasie ciąży oraz stanu odżywienia kobiet w ciąży.

Corresponding author: Małgorzata Kostecka, University of Life Science, Faculty of Food Science and Biotechnology, Department of Chemistry, Akademicka 15, 20-950, Lublin, Poland, phone number: +48 81 445 68 46; e-mail: kostecka.malgorzatam@gmail.com

Wyniki: Zmiany masy ciała w czasie ciąży respondentki uznały za dopuszczalne, a przyrost masy ciała w ciąży zaakceptowało 92% badanych. Kobiety w ciąży miały większą wiedzę na temat potrzeby zwiększenia spożycia kalorii w kolejnych trymestrach (p=0,0012). Wiedza respondentek na temat zdrowia matki i zdrowego rozwoju płodu została oceniona za pomocą 10 pytań. Średni wynik wyniósł 6,3±1,8 punktu i nie stwierdzono istotnych różnic między matkami/kobietami w ciąży a kobietami nieposiadającymi dzieci (6,6±1,3 vs 6,2±1,7, p>0,05). Wśród kobiet w ciąży aż 67% respondentek było zdania, że stosują zdrową dietę, 14% twierdziło, że nie odżywia się prawidłowo, ale nie ma motywacji do wprowadzania zmian.
Wnioski. Respondentki miały świadomość, że przyrost masy ciała w czasie ciąży jest normalnym procesem fizjologicznym, jednak 1/3 badanych nie czuła się komfortowo z obserwowanymi zmianami. Kobiety nie posiadały wystarczającej wiedzy na temat wpływu masy matki na rozwój płodu. Stosowanie leków moczopędnych i przeczyszczających przez kobiety w ciąży bez konsultacji lekarskiej jest niepokojącym zjawiskiem. Istnieje potrzeba zwiększenia dostępności szkoleń z zakresu roli i prawidłowego żywienia kobiet w ciąży.

Słowa kluczowe: *zaburzenia odżywiania, ciąża, pregoreksja, niedożywienie, masa ciała*

INTRODUCTION

Eating disorders are a serious and complex health and social problem that pose a particular threat for pregnant women. In the media, pregnant women are portrayed as happy and smiling, where shapely breasts and a small bump without excessive fat tissue are the only visible signs of pregnancy, which sets the bar high for expecting mothers. Many women try to rise to these unrealistic expectations by restricting their caloric intake, embarking on physical exercise programs that are not adapted to their physiological needs, or even taking diuretics and laxatives to control their body weight during pregnancy and quickly return to the pre-pregnancy weight [15, 19].

Pregnancy is one of the few periods in a woman's life when extra weight is not perceived as a negative factor, but has positive connotations with healthy fetal development, which runs against the modern cult of a perfect, slim and healthy body [13]. Most research studies, including studies of pregnant women, focus on excessive rather than insufficient weight gain in successive trimesters of pregnancy [1, 19]. The term "pregorexia" (a portmanteau of "pregnancy" and "anorexia") [16] was coined

in 2008 to emphasize that eating disorders can also affect pregnant women. Pregorexia is defined as a strong desire to control weight and maintain the perfect body image during pregnancy [26]. Pregorexia is difficult to diagnose because it not formally recognized as a disease and has not been classified in the ICD-10 or DSM-IV. As a result, pregnant women suffering from pregorexia are often diagnosed as anorectic, despite their specific physiological condition [31]. Pregorexia has identical symptoms to anorexia, and the affected women observe highly restrictive diets, become engaged in vigorous exercise programs, and take nutritional supplements and pharmaceuticals (laxatives and/or diuretics) to control their pregnancy weight [3, 10]. This disorder has been also described as an obsession with healthy eating combined with excessive focus on the ideal body size [31]. Women suffering from pregorexia are extremely anxious about gaining weight during pregnancy and being unable to return to their pre-pregnancy weight [22, 31]. Similarly to other eating disorders, pregorexia is not easy to diagnose. Some symptoms cannot be distinguished from natural physiological processes that occur during pregnancy, such as changes in food and taste preference, nausea or individual differences in the rate of weight gain. These changes should be evaluated with caution to avoid overinterpretation, but worrying signs should be detected as early as possible because unhealthy eating behaviors and pharmaceuticals can negatively affect maternal and fetal health and lead to pregnancy complications [10].

Research objective

The aim of this study was to evaluate the knowledge about healthy pregnancy, specific nutritional needs and adherence to dietary recommendations during pregnancy in women of reproductive age, and to evaluate changes in body weight and eating habits in pregnant women and mothers.

MATERIALS AND METHODS

Study population

The study consisted of an online survey, and a link to the questionnaire was posted in social media groups for pregnant women, women who are planning to become pregnant and/or mothers. Patients of a gynecological clinic were also invited to participate in the study, and they were provided with the relevant information and a link to the online questionnaire during their appointments. Data were collected between 20 January and 20 April 2020, and between 1 November and 31 December 2020. The questionnaire was completed by a total of 342 respondents, but only 325 correctly and completely filled in questionnaires were considered in the study. The surveyed subjects were women aged 18 to 52. The inclusion criteria were female sex, motherhood or plans to have children. In the statistical analysis of the results, the respondents

were divided into two groups: women without children (44.92%) and pregnant women and mothers (55.08%). The respondents' knowledge about eating behaviors during pregnancy was compared between the two groups. The responses given by mothers were used to evaluate weight gains during pregnancy and the nutritional status of pregnant women.

Methods

The study involved a self-designed questionnaire containing single-choice, multiple-choice and open-ended questions. The research tool was an original 40-item questionnaire that was developed for the Polish population based on the questionnaire to measure the level of nutritional and weight gain knowledge in pregnant women [18]. The questionnaire was composed of three main parts. Part I consisted of questions assessing the respondents' knowledge about pregnancy, physiological changes in pregnant women, specific nutritional needs and dietary guidelines for pregnant women, the consequences of maternal undernutrition and low weight gains during pregnancy. Part II was addressed to mothers, and it included questions about their nutritional status and weight gains in successive trimesters of pregnancy, the desire to control or restrict weight gain, and body image during pregnancy. Information about the respondents' body weights was obtained from maternity records based on the medical examinations performed in the first trimester (up to the 10th week of pregnancy), the second trimester (15th to 20th week of pregnancy) and the third trimester (33rd to 37th week of pregnancy). Part III was designed to collect personal information from the respondents, including age, height, pre-pregnancy weight, education and place of residence.

The *Shapiro-Wilk* test was used to test the normality of distributions. Differences between groups were determined with the use of Pearson's chi-squared (χ^2) test. Two-tailed p-values <0.05 were considered statistically significant in all tests. Analyses were performed using Statistica software (version 13.1 PL; StatSoft Inc., Tulsa, OK, USA; StatSoft, Krakow, Poland).

RESULTS

Part I of the questionnaire

Changes in body weight during pregnancy were regarded as acceptable, and pregnancy weight gain was considered a normal process by 92% of the respondents, in the groups of both pregnant women and mothers, and women without children. According to 6% of the surveyed subjects, changes in body weight during pregnancy were undesirable, and most respondents in this group had excessive pre-pregnancy weight (p<0.05). Pregnant women had greater knowledge about the need for increased caloric intake in successive trimesters (p=0.0012). The respondents were most likely to overestimate the recommended caloric intake in the first and third trimester (max. 500 kcal in the first trimester, 1000 kcal higher in the third trimester).

The distribution of answers to the question concerning the effects of low birth weight (LBW) on the child's future health was an important consideration. More than a third of the respondents who did not have children (p=0.002) and nearly a half of mothers and pregnant women were of the opinion that LBW had no effect on the child's health if the infant quickly gained weight, whereas women without children were more likely to indicate that LBW increased the risk of lifestyle diseases (p=0.001). The respondents' age was not a differentiating factor, but women with university education were more often of the opinion that LBW could have negative implications for the child's future health (p=0.002), in particular by increasing the risk of heart disease. Only 10% of pregnant women and mothers and 14% of women without children recognized that LBW was directly associated with overweight and obesity in adulthood.

The respondents were asked to assess the influence of maternal weight during pregnancy on fetal development. In both groups, the highest number of respondents were of the opinion that pregnancy weight affects the child. The number of respondents who voiced a contrary opinion was three times higher among mothers than among women who did not have children (p=0.0021). The number of respondents who disagreed that healthy maternal weight gain in successive trimesters can influence fetal weight gain (p=0.001), healthy fetal development (p=0.002) and increase the risk of intrauterine growth restriction (IUGR) (p=0.001) and preterm birth (p=0.0035) was also twice higher among mothers than among women who did not have children. Physiological changes during pregnancy increase the demand for some nutrients, which is why expecting mothers modify their diets. The majority of the respondents were of the opinion that pregnant women should modify their diets (more than 80% in each group; p>0.05). However, the number of respondents who disagreed that dietary modifications are needed during pregnancy, in particular in the second and third trimester, was significantly higher among pregnant women and mothers (p = 0.023). Food cravings and the elimination of specific food groups were acceptable for 1/3 of the surveyed subjects, and pregnancy/motherhood was not a differentiating factor (p>0.05).

The respondents were asked to indicate nutrients that are essential during pregnancy (Table 1). On average, pregnant women and mothers listed 2.31±0.34 nutrients, whereas women without children listed

Table 1. Answers to the question: Which nutrients (vitamins, minerals, etc.) play the most important role during pregnancy?

Pregnant women and mothers n=146 [%]		Women without children n=179 [%]		p
Folic acid	87 [59.6]	Folic acid	107 [59.7]	ns
Iron	80 [54.8]	Calcium	103 [57.5]	0.0021
Vitamin D	65 [44.5]	Vitamin D	80 [44.7]	ns
Magnesium	48 [32.8]	Iron	73 [40.8]	0.0014
DHA	27 [18.5]	B vitamins	34 [19.0]	0.0027

2.34±0.28 nutrients, and the difference between groups was not significant (p>0.05). However, differences were observed in the type of nutrients and the order in which they were listed.

The respondents were asked whether they had been diagnosed with iron deficiency, low hemoglobin levels or low red blood cell counts during pregnancy, and whether they had been taking dietary supplements, including iron supplements, during pregnancy. In the group of pregnant women and mothers, 65% of the respondents had been taking iron supplements, and more than 50% had been anemic, in particular in the third trimester. Pregnancy weight was not correlated with iron deficiency anemia (p<0.05). Vitamin D deficiency was more frequently indicated by pregnant women (p=0.002) and women with university education (p=0.04), whereas vitamin D supplements were used by more than 40% of pregnant women and mothers. These respondents rarely used other dietary supplements to compensate for nutrient deficiency in pregnancy. Frequent leg cramps and nocturnal shin cramps associated with magnesium deficiency were significantly more often reported by pregnant women (p=0.002), and beginning from the second trimester, 1/3 of these respondents were taking magnesium supplements upon their physicians' advice. The respondents were asked about maternal undernutrition and its impact on pregnancy. In both groups, the majority of the surveyed subjects agreed that maternal undernutrition was a significant risk factor in pregnancy (95.21% of women without children vs 90.50% of mothers, p=0.023). The number of respondents who argued that maternal undernutrition was an important, but not a critical factor was more than three times higher among pregnant women and mothers (p=0.002). The respondents were also asked to rank the consequences of undernutrition for the mother and child. The responses differed significantly between groups (p<0.05). Anemia and vaginal bleeding were most frequently identified as maternal consequences of undernutrition (these answers were significantly more often given by pregnant women and mothers, p=0.002), followed by lactation problems (p=0.03) and depression (p=0.02). Low birth weight (this answer was significantly more often given by pregnant women and mothers, p=0.03) and anemia (this answer was most prevalent among women without children, p=0.01) were selected as the major consequences of maternal undernutrition for the fetus.

The respondents' knowledge about maternal health and medical tests that are required to confirm healthy pregnancy and healthy fetal development was assessed with the use of 10 true or false questions. The average score was 6.3±1.8 points, and no significant differences were found between mothers/pregnant women and women without children (6.6±1.3 vs 6.2±1.7, p>0.05). The respondents scored the lowest number of points in questions concerning normal arterial blood pressure during pregnancy (p=0.01) and risk factors for pre-eclampsia (p=0.001), and earned the highest scores in questions concerning fasting glucose levels and diet-dependent complications in the third trimester, such as constipation (p=0.03), muscle cramps (p=0.05) and iron deficiency anemia (p=0.067).

Part II of the questionnaire

The second part of the questionnaire was addressed only to women in the third trimester of pregnancy and mothers with maternity records, and it was filled out by 147 respondents. In the first question, the respondents were asked whether they felt comfortable with the physiological changes during pregnancy. This question was designed to identify women who were at risk of pregorexia, where body dissatisfaction could trigger pathological behaviors. More than half of the respondents claimed that they regarded physiological changes during pregnancy as a normal phenomenon. Despite the above, every third respondent in that group did not feel comfortable with these changes, and 13% noted that weight gain during pregnancy made them feel unattractive. In the group of women who did not feel comfortable with pregnancy changes, ¾ were of the opinion that their weight had increased excessively during pregnancy (Table 2). Pregnancy weight gain was excessive in all women who had been overweight or obese before becoming pregnant (p=0.002). Mean weight gain was 12.4 kg, and maximum weight gain was 24.1 kg. According to recommendations, pregnancy weight gain should not exceed 7-11.3 kg for overweight women and 5-9 kg for obese women.

Table 2. Pregnancy weight gain in successive stages of pregnancy

	1st trimester	2nd trimester	3rd trimester
Average body weight	61.3 ±27.9 (min 48.7 – max 89.4 kg)	66.2 ± 31.3 (min 49.5 – max 97.1 kg)	69.3 ± 36.9 (min 53.5 – max 106.3 kg)
Underweight respondents (BMI < 19.9), n [%] Average body weight	27 [18.3] 52.4±4.9 (min 47.7-max 56.1)	24 [16.3] 53.8±6.4 (min 49.5-max 57.6)	21 [14.3] 55.5±3.9 (min 53.5-max 58.6)
Respondents with a healthy BMI (20-24.99), n [%] Average body weight	66 [44.9] 58.1±9.3 (min 52.5– max 71.1)	61 [41.5] 60.1±7.7 (min 55.7 – max 74,1)	67 [45.6] 62.7±5.3 (min 58.1 – max 75.6)
Overweight respondents (BMI>25), n [%] Average body weight	54 [36.8] 69.1±10.3 (min 59.7 – max 89.4)	62 [42.2] 73.9±9.4 (min 64.7– max 97.1)	59 [40.1] 78.3±11.8 (min 68.9–max 106.3)

Eleven underweight women (40%) had not achieved the recommended weight in the second or third trimester (p=0.003 vs p=0.001). Underweight women are advised to gain 12.5-18 kg during pregnancy, but mean weight gain was only 8.6 kg, and minimum weight gain was 5.5. kg in this group. The respondents were asked whether they had attempted to modify their weight before becoming pregnant. Three out of every five women had not made such attempts; every third responded had attempted to lose weight, whereas only 5% had attempted to gain weight before becoming pregnant.

Women with pregorexia hope to minimize or completely avoid weight gain during pregnancy. The respondents were asked whether they had attempted to modify their weight during pregnancy (Figure 1). Three-quarters of the studied population had not made such attempts; only three women, classified as underweight (BMI < 18.5), had attempted to gain weight. The group of women who were concerned about weight gain included respondents with a normal BMI, and these subjects accounted for 57% of the women who had attempted to control their weight gain during pregnancy. The majority of these respondents (84%) controlled their weight by avoiding unhealthy foods such as sweets, chips, chocolate, instant foods and fried foods. More than 50% of the surveyed subjects modified their food choices, 1/3 decreased food portions, 10% avoided eating late at night, and ¼ went for walks despite not being professionally active (on medical leave). (Table 3)

Compensatory behaviors such as self-induced vomiting or the use of laxatives (in the absence of constipation) were not reported by any women. However, 15.5% of the subjects had used constipation and laxative treatments other than home remedies. The majority of these respondents were pregnant women in the third trimester (p=0.002), mostly overweight women (p=0.001) and women with a pre-pregnancy BMI < 20 (p=0.003). Five pregnant women in the

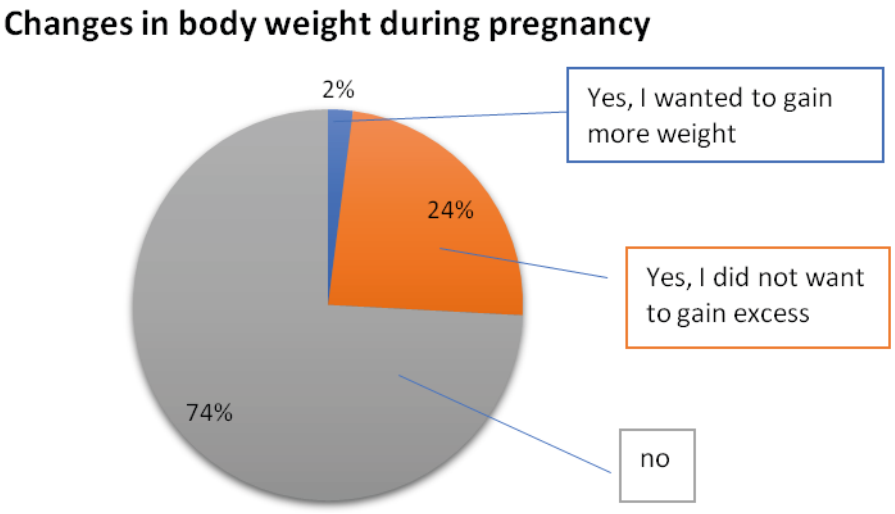

Figure 1. Response to the question: Did you attempt to change your body weight during pregnancy?

Table 3. Components of changes in eating behavior during pregnancy

	Pregnant women with BMI<18.5 before pregnancy n (%)	Pregnant women with BMI 18.5-24.99 before pregnancy n (%)	Pregnant women with BMI>25 before pregnancy n (%)	p
Increased consumption of vegetables and fruits	16 (59.3)	39 (59.1)	28 (51.8)	0.071
Increased consumption of dairy products	13 (48.1)	27 (40.1)	24 (44.4)	0.059
Increased consumption of whole grains	16 (59.3)	33 (50)	28 (51.8)	0.039
Eating breakfast regularly	14 (51.8)	47 (71.2)	31 (57.4)	0.0031
Eating 5-6 meals a day	14 (51.8)	27 (40.1)	30 (55.5)	0.023
Decreased food portion	9 (33.3%)	3 (4.5)	9 (16.6)	0.0017
Reduced intake of fast food	20 (74.1)	19 (28.8)	23 (42.6)	0.0001
Eliminating sugar or sweets	23 (85.2)	23 (34.8)	29 (53.7)	0.0026
Eliminating coffee	9 (33.3)	36 (54.5)	34 (63.0)	0.0048
Eliminating salty snacks	23 (85.2)	29 (43.9)	39 (72.2)	0.002
Used constipation and laxative treatments	7 (25.9)	2 (3.0)	11 (20.4)	0.0037
Reading food labels	20 (74.1)	36 (54.5)	24 (44.4)	0.0018

second trimester had used diuretics without medical consultation (p=0.002).

DISCUSSION

Maternal weight control has significant implications for pregnancy outcomes [30]. Maternal weight should be controlled every 4-6 weeks during prenatal care appointments [32]. Most of the respondents had their weight checked during regular pregnancy visits (56%), whereas more than ¼ of the surveyed subjects had not had their weight checked by a physician or a midwife during the appointment, but had monitored their weight at home and reported the results during the visit. According to 10% of the women, they had been weighed only during the first visit, and weight gain in successive stages of pregnancy was not monitored by the physician.

Pregorexia is not a formal medical term, but it is increasingly used to define eating disorders in pregnant women [6,17]. Weight gain during pregnancy is a major concern for women with pregorexia. Many women are faced with the dilemma of choosing between a positive body image and healthy fetal development [4]. For some women with an eating disorder, pregnancy is the only time when weight gain is acceptable [20]. According to *Możdżonek* and *Antosik* [21], the detection rate for pregorexia is low, mostly because women suffering from this disorder feel ashamed and hide the problem in fear of being judged. In the present study, the respondents were very reluctant to admit that they had an eating disorder. None of the women who reported prolonged vomiting (11 subjects) admitted that it was self-induced. All women who had experienced prolonged vomiting reported low maternal weight gain in the first trimester, which could be attributed to problems with holding down food. However, half of the respondents who had suffered from prolonged vomiting were underweight before pregnancy (BMI<18.5 kg/m^2). None of the pregnant women reported symptoms of pregorexia, even if symptoms of an eating disorder were present. Every fourth respondent had attempted to modify or control her weight to avoid excessive weight gain. In a study by *Wójcik* et al. [31], 38% of the surveyed women had attempted to lose weight during pregnancy.

Despite a steady increase in education levels, nutrition knowledge has not changed significantly in the general population [7]. Women of reproductive age tend to repeat poor dietary behaviors from childhood and adolescence. In a subjective nutritional assessment conducted by *Myszkowska-Ryciak* et al. [23], more than 2/3 of the surveyed women claimed that they ate healthy diets during pregnancy, whereas 74% of the respondents declared that they had changed their diets after becoming pregnant. In the present study, 67% of the respondents were of the opinion that they followed a healthy diet, 14% claimed that they did not eat right during pregnancy, but were not motivated to make any changes or seek advice from a physician or a dietician. The most frequently introduced dietary modifications included a higher number of meals (65%), larger portions (36%), higher intake of fruits (74%), reduced intake of fast foods (31%) and healthier food choices

(25%). *Myszkowska-Ryciak* et al. [23] reported different dietary modifications: the respondents modified their diets by eating a higher number of smaller meals (100%), increasing their intake of vegetables (100%) and fruits (94%), reducing their intake of fast foods (78%) and sweets (69%), and eliminating coffee from their diets (56%). In turn, in the work of *Tymczyna* et al. [28], most pregnant women focused on food quality and increased their consumption of dairy products (20.78%), fruits and vegetables (19.48%) and cereal products (12.99%). Pregnant women from the region of Łódź modified their dietary behaviors by eliminating coffee, fast foods, salty snacks and sweets from their diets [8]. A study conducted in Poznań revealed that pregnant women had insufficient knowledge about the impact of maternal nutrition on pregnancy outcomes. More than half (54%) of the respondents did not change their dietary behaviors after becoming pregnant, and 2% completely ignored nutritional guidelines [11].

Research clearly indicates that eating disorders such as pregnancy-related vomiting and hyperemesis, maternal anemia and infections, increase the risk of pregnancy complications compared with healthy pregnant controls [14]. Eating disorders have negative outcomes, especially nutrition- and growth-related outcomes, for neonates. Maternal anorexia nervosa, including extreme underweight and dietary restriction, has been associated with intrauterine growth restriction, small-for-gestational age infants and low birth weight [24, 27]. Postpartum depression is yet another complication of eating disorders; it affects around 9% of mothers, usually 6-12 weeks after giving birth [29]. In the current study, postpartum depression was reported by 14% of the respondents, and it was significantly more prevalent among women with a low BMI ($p<0.05$) and women with gestational diabetes ($p<0.05$). Recent research suggests that anemia and iron deficiency are among the causes of postpartum depression [2,12]. Iron deficiency can contribute to postpartum depression by disrupting the metabolism of thyroid hormones and decreasing IL-2 production. Iron also participates in the synthesis of neurotransmitters such as dopamine, serotonin and noradrenaline, and low levels of these neurotransmitters can lead to depression. Anemia has been also linked with depression in non-pregnant women. *Goshtasebi* et al. [9] demonstrated that perinatal hemoglobin values lower than 11 g/dl were associated with a higher risk of postpartum depression. However, further research is needed to confirm these observations.

Placental abruption is a serious complication of pregnancy [5]. Low maternal pre-pregnancy BMI has been associated with a high risk of placental abruption, which can be reduced by healthy weight gain during pregnancy [5]. Similar results were reported by *Połocka-Molińska* et al. [25] who observed placental abnormalities in 21.7% of women with low weight gain, but in only 4.8% of women who gained the right amount of weight during pregnancy.

CONCLUSIONS

1. The respondents were aware that weight gain during pregnancy is a normal physiological process, but 1/3 of the respondents did not feel comfortable with the observed changes.
2. Most pregnant women with a normal BMI gained the right amount of weight during pregnancy, but women who were overweight or obese before pregnancy gained excessive weight during pregnancy.
3. The respondents did not have sufficient knowledge about the influence of maternal weight on fetal development, in particular in the group of pregnant women and mothers.
4. The use of diuretics and laxatives by pregnant women without medical consultation is a worrying phenomenon that could compromise maternal nutritional status and give rise to eating disorders during pregnancy.
5. Pregnant women and women planning to start a family should have better access to knowledge about healthy nutrition during pregnancy.
6. Courses focusing on healthy diet and supplementation during pregnancy should be introduced in antenatal care programs.

Acknowledgments

The authors would like to thank all participants who contributed to this study. We are also grateful to gynecologists (Agnieszka Opolska and Karolina Dobrowolska) who facilitated patient participation in the study.

Conflict of interest

The authors declare no conflict of interest.

REFERENCES

1. *Agrawal S., Singh A.*: Obesity or Underweight – What is Worse in Pregnancy? J Obstet Gynaecol India 2016;66(6):448–452 doi:10.1007/s13224-015-0735-4.
2. *Azami M., Badfar G., Khalighi Z., Qasemi P., Shohani M., Soleymani A., Abbasalizadeh S.:* The association between anemia and postpartum depression: A systematic review and meta-analysis. Caspian J Intern Med 2019;10(2):115-124 doi:10.22088/cjim.10.2.115.
3. *Bator E., Bronkowska M., Ślepecki D., Biernat J.:* Anoreksja – przyczyny, przebieg, leczenie [Anorexia – causes, course, treatment]. Now Lek 2011;3(80):184–191 (in Polish).
4. *Claydon E.A., Davidov D.M., Zullig K.J., Lilly C.L., Cottrell L., Zerwas S.C.*: Waking up every day in a body

that is not yours: a qualitative research inquiry into the intersection between eating disorders and pregnancy. BMC Pregnancy Childbirth 2018;18(1):463 doi:10.1186/s12884-018-2105-6.

5. *Deutsch A.B., Lynch O., Alio A.P., Salihu H.M., Spellacy W.N.*: Increased risk of placental abruption in underweight women. Am J Perinatol 2010;27(3):235–240 doi:10.1055/s-0029-1239490.
6. *Franko D.L., Blais M.A., Becker A.E., Delinsky S.S., Greenwood D.N., Flores A.T., Ekeblad E.R., Eddy K.T., Herzog D.B.*: Pregnancy complications and neonatal outcomes in women with eating disorders. Am J Psychiatry 2001;158(9):1461-1466 doi:10.1176/appi.ajp.158.9.1461.
7. *Fraś M., Gniadek A., Poznańska-Skrzypiec J., Kadłubowska M.*: Styl życia kobiet w ciąży [Lifestyle of pregnant women]. Hygeia Publ Health 2012;47(4):412-417 (in Polish).
8. *Godala M., Pietrzak K., Łaszek M., Gawron-Skarbek A., Szatko F.*: Zachowania zdrowotne łódzkich kobiet w ciąży. Cz. I. Sposób żywienia i suplementacja witaminowo-mineralna [Health behaviours of pregnant residents of Łódź. Part I. Diet and vitamin-mineral supplementation]. Probl Hig Epidemiol 2012;93(1):38-42 (in Polish).
9. *Goshtasebi A., Alizadeh M., Gandevani S.B.*: Association between Maternal Anaemia and Postpartum Depression in an Urban Sample of Pregnant Women in Iran. J Health Popul Nut 2013;31(3):398–402 doi:10.3329/jhpn.v31i3.16832.
10. *Harasim-Piszczatowska E., Krajewska-Kułak E.:* Pregoreksja - anoreksja kobiet ciężarnych [Pregorexia – anorexia of pregnant women]. Pediatr Med Rodz 2017;13(3):363–367 doi:10.15557/PiMR.2017.0038**.**
11. *Hyżyk A.K., Sokalska N.*: Ocena zmiany masy ciała u kobiet w ciąży [The evaluation of body mass changes in pregnant women]. Now Lek 2011;80(3):174-177 (in Polish).
12. *Kang S.Y., Kim H.B., Sunwoo S.*: Association between anemia and maternal depression: A systematic review and meta-analysis. J Psychiatr Res 2020;122:88-96 doi:10.1016/j.jpsychires.2020.01.001.
13. *Krisjanous J., Richard J.E., Gazley A.:* The Perfect Little Bump: Does the Media Portrayal of Pregnant Celebrities Influence Prenatal Attachment? Psychology and Marketing 2014;31(9):758–773 doi:10.1002/mar.20732.
14. *Linna M.S., Raevuori A., Haukka J., Suvisaari J.M., Suokas J.T., Gissler M.*: Pregnancy, obstetric, and perinatal health outcomes in eating disorders. Am J Obstet Gynecol 2014;211(4):392-398 doi:10.1016/j.ajog.2014.03.067.
15. *Łepecka-Klusek C., Syty K., Pilewska-Kozak C., Jakiel G.*: Sense of own attractiveness among women in advanced pregnancy. Prog Health Sci 2015;5(1):7–13.
16. *Mandera A., Pawlikowska A., Szuster M., Całkosiński A., Kostrzewska P., Majewski M.*: The pregorexia-anorexia during the pregnancy. J Educ Health Sport 2019;9(5):137–144 doi:10.5281/zenodo.2718477**.**
17. *Mazer-Poline C., Fornari V.*: Anorexia nervosa and pregnancy: having a baby when you are dying to this-case report and proposed treatment guidelines. Int J Eat Disord 2009;42(4):382-384 doi:10.1002/eat.20607.
18. *Mierzejewska E., Honorato-Rzeszewicz T., Świątkowska D., Jurczak-Czaplicka M., Maciejewski T., Fijałkowska A., Szulc-Kamińska J., Czach A., Nałecz H., Szostak-Węgierek D., Szamotulska K.*: Evaluation of questionnaire as an instrument to measure the level of nutritional and weight gain knowledge in pregnant women in Poland. A pilot study. PLoS One. 2020;15(1):e0227682. doi: 10.1371/journal.pone.0227682.
19. *Meštrović Z., Roje D., Vulić M., Zec M.:* Calculation of optimal gestation weight gain in pre-pregnancy underweight women due to body mass index change in relation to mother's height. Arch Gynecol Obstet 2017;295(1):81–86 doi:10.1007/s00404-016-4218-3.
20. *Mitchell-Bieleghem A., Mittelstaedt M., Bulik C.M.*: Eating disorders and childbearing: concealment and consequences. Birth 2002;29(3):182-191 doi:10.1046/j.1523-536x.2002.00186.x.
21. *Możdżonek P., Antosik K.*: Creating of dietary trends in the media and their influence on the development of eating disorders. Public Health Nurs 2017;7(2):159–164 doi:10.17219/pzp/66329.
22. *Müldner-Nieckowski Ł., Cyranka K., Smiatek-Mazgaj B., Mielimaka M., Sobański J.A., Rutkowski K.:* Multiaxial changes in pregnancy: Mental health - A review of the literature. Ginekol Pol 2014;85(10):784–787.
23. *Myszkowska-Ryciak J., Gurtatowska A., Harton A., Gajewska D.*: Poziom wiedzy żywieniowej a wybrane aspekty sposobu żywienia kobiet w okresie ciąży [Nutritional knowledge and selected aspects of the diet of pregnant women]. Probl Hig Epidemiol 2013;94(3):600-604.
24. *Pasternak Y., Weintraub A.Y., Shoham-Vardi I., Sergienko R., Guez J., Wiznitzer A., Shalev H., Sheiner E.*: Obstetric and perinatal outcomes in women with eating disorders. J Womens Health (Larchmt) 2012;21(1):61-65 doi:10.1089/jwh.2011.2907.
25. *Połocka-Molińska P., Plagens-Rotman K., Pawlak M.*: Wpływ masy ciała matki na przebieg ciąży, porodu oraz stan noworodka [The influence of mother body weight on the pregnancy, childbirth and the condition of a new-born child]: Pol Prz Nauk Zdr 2017;4(53):452-463 doi:10.20883/ppnoz.2017.63 (in Polish).
26. *Rzońca E., Bień A., Iwanowicz-Palus G.*: Zaburzenia odżywiania – problem wciąż aktualny [Eating disorders–an ongoing problem]. J Educ Health Sport 2016;6(12):267-273 doi:10.5281/zenodo.198734.
27. *Solmi F., Sallis H., Stahl D., Treasure J., Micali N.*: Low birth weight in the offspring of women with anorexia nervosa. Epidemiol Rev 2014;36(1):49-56 doi:10.1093/epirev/mxt004.
28. *Tymczyna B., Sarna-Boś K., Krochmalska E., Skorupska-Okoń A., Ciukiewicz A.:* Ocena wiedzy ciężarnych na temat wpływu odżywiania na uzębienie dziecka [Assessment of the knowledge of pregnant women about the influence of nutrition on the teeth of a child]. Zdr Publ 2004;114(4):541-544 (in Polish).

29. *Wassef A., Nguyen Q.D., St-André M.*: Anaemia and depletion of iron stores as risk factors for postpartum depression: a literature review. J Psychosom Obstet Gynaecol 2019;40(1):19-28 doi:10.1080/016748 2X.2018.1427725.
30. *Wender-Ozegowska E., Bomba-Opoń D., Brazert J., Celewicz Z., Czajkowski K., Karowicz-Bilińska A., Malinowska-Polubiec A., Meczekalski B., Zawiejska A.*: Polish Gynecological Society. Standardy Polskiego Towarzystwa Ginekologicznego "Opieka położnicza nad ciezarna otyła" [Recommendations of Polish Gynecological Society concerning perinatal care in obese pregnant women]. Ginekol Pol. 2012;83(10):795-9. (in Polish)
31. *Wójcik R., Mojs E., Michalska M.M, Samulak D.:* Podejmowanie odchudzania w okresie ciąży a poporodowe surowicze stężenia żelaza u kobiet – badanie wstępne [Attempts to lose weight during pregnancy and the postpartum serum iron concentration in women – a preliminary test]. Probl Hig Emidemiol 2013;94(4):893-896.
32. www https://1000dni.pl/sites/default/files/guides/poradnik-zywienie-kobiet-w-okresie-ciazy-teoria-i-praktyka.pdf (available: 12.04.2022)

Received: 26.04.2022
Accepted: 14.07.2022
Published online first: 25.07.2022

Rocz Panstw Zakl Hig 2022;73(3):325-332
http://wydawnictwa.pzh.gov.pl/roczniki_pzh/ https://doi.org/10.32394/rpzh.2022.0223

ORIGINAL ARTICLE

MODELS TO PREDICT NON-ALCOHOLIC FATTY LIVER DISEASE LINKED TO OBESITY IN MOROCCO

Habiba Liba[1], *Rekia Belahsen*[1]

[1]Laboratory of Biotechnology, Biochemistry & Nutrition, Training and Research Unit on Nutrition & Food Sciences, Chouaib Doukkali University, Faculty of Sciences, El Jadida, Morocco

ABSTRACT

Background. The prevalence, risk factors and screening for the problem of non-alcoholic fatty liver disease linked to obesity are not well known in Morocco. The diagnosis of this disease by biopsy is invasive and the assessment of its severity by ultrasound shows variability in observation.

Objective. The aim of this retrospective study is to estimate the prevalence of NAFLD linked to obesity, to determine the risk factors associated with it and to develop a non-invasive procedure as a method of diagnosing this disease in Morocco.

Material and Methods. It's a retrospective study. The collection of anthropometric, clinical, biochemical, and radiological data over a period from 2014 to 2018 were captured from registers of patients at the Med VI University Hospital in Marrakech. Data were analyzed using SPSS version 26 software. Descriptive statistics were presented using frequencies and means +/- standard deviation to describe categorical and numeric data respectively. Pearson's chi-square test was used to test the association between categories of two independent samples. Multinomial logistic regression is used to find disease risk factors and models to predict non-alcoholic fatty liver disease (NAFLD) linked to obesity in Morocco.

Results. Gender, increased age, body mass index, alanine aminotransferase, triglycerides, C-reactive protein, alkaline phosphatase, gamma-glutamyl transferase were significantly correlated with NAFLD and its evolvement.

Conclusion. The prevalence of NAFLD linked to obesity is an alarming problem in Morocco. It was 83.5%. Age, gender, body mass index, alanine aminotransferase, triglycerides, C-reactive protein, alkaline phosphatase and gamma-glutamyl transferase are risk factors for NAFLD and its severity. It were used to develop two algorithms that can be used, as a more objective and non-invasive screening method for NAFLD.

Key words: *NAFLD, obesity, prevalence, non-invasive screening method*

INTRODUCTION

Non-alcoholic fatty liver disease (NAFLD) is one of the most common liver diseases in the world. It is characterized by the accumulation of triglycerides in the liver without significant alcohol consumption (<20 g/day for women and <30 g/day for men) [1]. The disease has been associated with several risk factors including metabolic syndrome, overweight or obesity, diabetes mellitus, insulin resistance, drug use and environmental factors [2]. It is a syndrome that encompasses several liver pathologies ranging from simple steatosis, when fat accumulates in the liver, to non-alcoholic steatohepatitis (NASH), when the fat accumulated in the liver causes inflammation of the latter, to fibrosis and cirrhosis, when chronic inflammation progresses to advanced scarring of the liver [3]. NAFLD is most often clinically silent, but may be manifested by the presence of symptoms such as asthenia or a feeling of discomfort in the right upper quadrant [4]. Liver enzymes are found to be normal in over 75% of cases. However, an increase in alanine aminotransferase (ALAT), triglycerides (TG) and gamma-GT (GGT) and an ASAT/ALAT ratio of less than 1 is found in about a quarter of cases. This ratio tends to become greater than 1 with the progression of the disease and the development of cirrhosis [4]

So far, liver biopsy has been the ultimate standard for the detection of fatty liver disease and the differentiation between its stages. However, the relatively high costs, patient discomfort, sampling variability, inter- and intra-observer variability and risk of complications as well as invasiveness make it unsuitable for screening for NAFLD [4]. Thus, despite the variability in the interpretation of images even in the same person, ultrasound is the first-line imaging examination used in clinical practice, for the diagnosis of NAFLD due to its wide availability and its lower

Corresponding author: Rekia Belahsen, Training and Research Unit on Nutrition & Food Sciences, Chouaib Doukkali University, Faculty of Sciences, El Jadida, 24000 Morocco, e-mail: b.rekia@gmail.com or rbelahsen@yahoo.com

cost. The presence and grade of NAFLD was defined by ultrasound on a scale of 0 to 3 (0 = no abnormality, 1 = mild, 2 = moderate, 3 = severe) [5]. However, NAFLD can be detected by computed tomography without contrast, but it is less used as a screening test due to its lower sensitivity and the patient's exposure to radiation [6], whereas transient elastography (TE) is used for assessment of fibrosis [7]. On the other hand, magnetic resonance imaging (MRI) has a better sensitivity for the assessment of NAFLD but this modality cannot differentiate NAFLD from NASH. In addition, MRI combined with elastography (MRE) is a better method of identifying degrees of fibrosis in patients with NAFLD. However, MRI with or without MRE is expensive [8].

Due to the lack of a simple and non-invasive diagnostic test, the prevalence of NAFLD in the general population is uncertain and difficult to assess accurately [9].

Epidemiological data show that the global prevalence of NAFLD in different populations is estimated at 30% in the United States, 32% in the Middle East, 30% in South America, 27% in Asia, 24% in Europe and 13% in Africa [2]. This disease is associated with obesity and metabolic disturbances such as insulin resistance (IR), type 2 diabetes mellitus (T2DM) and dyslipidemia [9, 10]. NAFLD is itself an independent risk factor for cardiovascular disease, leading to increased all-cause mortality and increased liver-related mortality [10]. In the obese population, the prevalence of NAFLD is estimated at around 60 to 95% and that of NASH at 18.5% in obese patients against 3% in non-obese [11].

Furthermore, the global prevalence of obesity has been estimated at 51.34% and 81.83% in patients with NAFLD and NASH, respectively [2]. In this study, overweight and general obesity were defined by BMI and abdominal obesity, representing the percentage of abdominal fat mass, was measured by waist circumference according to the World Health Organization (WHO) [12]. In the NAFLD patients in this study, the determination of the prevalence of comorbidities showed that the metabolic syndrome (MetS) was prevalent in 42% of the NAFLD subjects; 42% had hyperlipidemia; 51% were obese; 39% were hypertensive and 22% had diabetes [2, 9].

In Morocco, while there is no data on the prevalence of NAFLD in Morocco, the country has 53% of its adult population over 18 years of age with overweight, 20% obesity, 10.5% with high blood cholesterol, 29.3% hypertension, 10.6% a diabetes and 10.4% are pre-diabetic [13,14]. In addition, ad hoc surveys carried out in different regions of the country have also reported an increasing trend in the prevalence of all these risk factors as well as that of the metabolic syndrome [14, 15]. With a view to improving the quality of care for people with non-alcoholic fatty liver disease linked to obesity, the objective of this retrospective study is to estimate the prevalence of NAFLD linked to obesity, to determine the risk factors associated with it and to develop a non-invasive procedure as a method of diagnosing this disease in Morocco.

MATERIAL AND METHODS

After informed consent, the collection of anthropometric, clinical, biochemical, and radiological data over a period from 2014 to 2018 were captured from registers of patients at the Med VI University Hospital in Marrakech. Incomplete records, records of patients with tuberculosis or seropositive hepatitis B or C, those with a history of another type of hepatitis or *Wilson's* disease, and those with a history of alcohol consumption were excluded.

The variables selected are age, sex, body mass index, waist circumference, having high blood pressure, diabetes and dyslipidemia. Parameters concerning laboratory analyzes, in particular serum levels of alkaline phosphatase (ALP), gamma-glutamyl transpeptidase (GGT), alanine aminotransferase (ALAT)), aspartate aminotransferase (ASAT), fasting glucose (FG), total cholesterol (TC), triglycerides (TG), high density lipoprotein (HDL) and C-reactive protein (CRP) were also collected. However, measurement of insulin resistance was not cited due to lack of data in the study sample. The weight status of the study population is assessed by calculating the body mass index (BMI) by dividing the weight in kilograms by the square of the height in meters (kg/m^2). Overweight was defined by a BMI greater than or equal to 25 and less than 30 and obesity by a BMI greater than or equal to 30 [12]. Abdominal obesity, reflecting abdominal fat mass, is measured by waist circumference (WC) in cm. Men with a waist circumference <94 were classified as normal weight and WC 94-101.9 overweight and those with WC ≥ 102 cm obese. Women were classified into the same obesity categories based on WC <80, 80–87.9 and ≥ 88 cm [12].

Blood pressure was measured after rest with a mercury sphygmomanometer. Based on WHO guidelines, a threshold of 140/90 mmHg for hypertension has been used [16]. Participants whose blood pressure was 140/90 mmHg or less were considered normotensive, while those with higher values or who reported taking antihypertensive drugs were classified as hypertensive [17]. Diabetes mellitus is characterized by hyperglycemia. Glycated hemoglobin is a simple marker of blood sugar and its value is usually expressed as a percentage. A level greater than or equal to 6.5% determined by high performance liquid chromatography (HPLC), twice, has been included in the diagnostic criteria for

diabetes by the American Diabetes Association [18]. Normal blood glucose values are less than 100 mg/dl in the fasting state and less than 140 mg/dl at the second hour of oral hyperglycemia according to WHO recommendations [18].

Laboratory analyzes of blood taken by venous blood puncture, mainly after overnight fast, provided information on the various functions of the liver. The increase in these indicators is a sign of an anomaly. The ALAT and ASAT transaminases remain renowned for their sensitivity; the specificity for the liver becomes excellent beyond their increase by a factor of 10, ie 300 to 400 U L. For usual values <35 (men) and <32 (women) U / L for ALAT; <43 (male) and <32 (female) U / L for ASAT; <45 (male) and <32 (female) U / L for GGT; <115 U / L (male and female) for serum ALP; 4.25-6.55 (man) and 4.05-5.55 (woman) mmol/l for cholesterol (serum) [19].

Statistical analyses

Data entry and analysis was performed using SPSS version 26 software. Descriptive statistics were presented using frequencies and means +/- standard deviation to describe categorical and numeric data respectively. Pearson's chi-square test was used to test the association between categories of two independent samples. Multinomial logistic regression is used to find disease risk factors that characterize a group of obese subjects with grade 1 NAFLD and those related to grade 2 and 3 NAFLD. Odds ratio (OR) and their confidence intervals (CI) were used to assess the risk of NAFLD if a certain factor is present. The significance level used is 0.05 (p-value).

RESULTS

The demographic and laboratory characteristics of the study sample are summarized in Table I. The results show that approximately 50% of the study participants presented NAFLD, among them 42.5% were with steatosis, 3.5 with fibrosis and 4.4 have cirrhosis. According to gender, women were more affected than men by steatosis (82.3% vs. 17.7%) and cirrhosis (70.6% vs. 29.4%). As for fibrosis, it was more present in men (64.3%) than in women (35.7%). In general, the average age of the participants was 45 ± 15.03. The subjects without NAFLD have a mean age of 40.68 ± 14.56 years, those with NAFLD grade 1 were 48.65 ± 14.53 years old, and those with grade 2-3 have mean age of 43.00 ± 11.93 and 59.94 ± 10.19 respectively.

Among people with steatosis, 51.9% were over 50 years old, 36.8% between 18 and 50 years old and 20% were under 18 years old. A statistically significant association is found between age and the disease. *Pearson's Chi*-square test is indeed 34.55 and the likelihood ratio is 36.64 with a value of p = 0.000 <0.05.

The body mass index (BMI) means for the steatosis grades 0, 1, 2 and 3 were 40.12 ± 7.83; 39.78±11.09; 32.97±8.23 and 28.14±6.36 respectively. The prevalence of simple NAFLD increased from 6.1% in people with a normal BMI to 10.4% in overweight people and to 83.5% in obese people. Fibrosis was prevalent in 14.3% of the subjects with normal weight or overweight while this prevalence achieved 71.4% in obese people. Concerning the cirrhosis, it was present in 35.3% of the normal weight, 29% of the overweight and in 35.3% of the obese people. The results also show that the BMI decreases for fibrosis and cirrhosis (Table 2). The value of the *Pearson's Chi*-square test shows a statistically significant association between BMI and NAFLD (of 52,162; p=0.000 <0.05) with a *Chi*-squared likelihood test value of 37,286 (p = 0.00 <0.05).

The average waist circumference according to steatosis grades 0, 1 and 2-3 was respectively 115.63 ± 17.41, 113.03 ± 19.48 and 90.64 ± 13.20 in women and 116.87 ± 19.31, 124.83 ± 32.10, 95.28 ± 18.13 respectively in men . A significant association between waist circumference and NAFLD was found (*Pearson's Chi*-square tests were 38.7 and 18.18 with p<0.05) (Table 1).

The distribution of the sample investigated according to the presence of chronic diseases shows a coexistence of NAFLD and type 2 diabetes in 47.2% of the subjects. However, the association of both diseases is not statistically significant (p = 0.327) according to the value of *Pearson's Chi*-square test (2.236). Furthermore, the dyslipidemia present in 13.3% of NAFLD patients was significantly associated with NAFLD (p-value = 0.002; *Pearson's Chi*-square value = 12.084). On the other hand, although not significantly associated with NAFLD, anemia was present in 8.2% of NAFLD patients (*Pearson's Chi*-square test = 3.295; p = 0.193>0.05). Added to these chronic diseases, more than half of people with NAFLD (52%) had high blood pressure, the association between these two diseases (NAFLD and hypertension) was however not statistically significant (chi-square test of *Pearson* = 3.579; p = 0.167> 0.05%).

Risk factors for NAFLD

Multinomial logistic regression was used to determine the risk factors for NAFLD characterizing a group of obese subjects with grade 1 NAFLD and those related to NAFLD with grade 2 and 3, from anthropometric and biochemical data on a sample of 386 Moroccans. Thus, increased age, C-reactive protein and triglycerides (TG) had significant effects on the risk of NAFLD regardless of the degree of the disease. Male gender, body mass index, ALAT, ALP,

Table 1. Distribution of study patients according to NAFLD grades and the demographic, clinical and biochemical data

Grades of NAFLD N (%)					
	Grade 0 191(49.5)	Grade 1 164(42.5)	Grade 2 14(3.5)	Grade 3 17(4.4)	Total 386
Age	40.68±14.56	48.65±14.53	43.00 ± 11.93	59.94±10.19	45±15.03
Age categories					
<18yrs	12(80)	3(20)	0(0.0)	0(0)	15(3.9)
[18-50]	121(57.9)	77(36.8)	9(4.3)	2(1)	209(54.00)
≥50	58(35.8)	84(51.9)	5(3.1)	15(9.3)	162(42.10)
Gender Female Male	 168(88) 23(12)	 135(82.3) 29(17.7)	 5(35.7) 9(64.3)	 12(70. 6) 5(29. 4)	 320 66
BMI (kg/m2)	40. 12 ± 7.83	39.78 ± 11,09	32.97 ± 8.23	28.14±6.36	
BMI categories					
Normal	4(2.1)	10(6.1)	2(14.3)	6(35.3)	22(5.7)
Overweight	9(4.7)	17(10.4)	2(14.3)	5(29.4)	33(8.5)
Obesity	178(93.2)	137(83.5)	10(71.4)	6(35.3)	331(85.8)
WC (cm)					
Males	116.87±19.31	124.83±32.10	95.56±18.32	95±17.93	
Females	115.63±17.41	113.03±19.48	91.20±19.10	90.08±7.30	
WC categories **Males**					
<94	2(14.3)	6(42.9)	2(14.3)	4(28.6)	14(21.21)
94-102	3(25)	5(41.7)	4(33.3)	0(0)	12(18.18)
≥102	18(45)	18(45)	3(7.5)	1(2.5)	40(60.61)
Total	23(34.8)	29(43.9)	9(13.6)	5(7.6)	66(100)
Females					
<80	3(25%)	7(58.3%)	1(8.3%)	1(8.3%)	12(3.75%)
80-88	1(5.6%)	12(66.7%)	2(11.1%)	3(16.7%)	18(5.6 2%)
≥88	164(56.6%)	116(40%)	2(0.7%)	8(2.8%)	290(90.63%)
Total	168(52.5%)	135(42.2%)	5(1.6%)	12(3.8%)	320(100%)
ASAT (unit/l)	22.05±12.14	31.43±23.37	58.37±33.75	45.22±36.35	
ALAT (unit/l)	22.15±14.10	29.48±21.15	96.89±45.54	37.40±28.15	
TG (g/l)	1.26±0.51	1.76±1.27	1.98±0.22	1.78±0.24	
HDL-C (g/l)	0.46±0.12	0.51±0.34	0.50±0.29	0.50±0.002	
LDL (g/l)	1.14±0.36	1.09±0.43	1.23±0.14	1.27±0.11	
TC (g/l)	0.31±0.02	1.91±0.72	2.31±0.07	1.92±0.17	
GGT UI/L	45.53± 86.07	68.87±74.94	81.02±49.32	80.21±85.37	
CRP mg/l	10.45±9.25	52.16±70.98	70.41±98.24	20.21±38.66	
Albumin g/l	41.62±5.20	40.45±4.71	32.30±6.36	32.88±3.09	

NAFLD ranks: grade 0: No FAFLD; grade 1: Steatosis; grade 2: Fibrosis; grade 3: Cirrhosis. BMI: body mass index. ASAT: Aspartate aminotransferase. ALAT: Alanine aminotransferase. TG: Triglycerides. TC: Total cholesterol. HDL: High density lipoprotein; LDL: low density lipoprotein. GGT: gamma-glutamyl transferase; CRP: C-reactive protein.

and GGT were significantly correlated with NAFLD grade 2 and 3. Table 3.

In addition, the multinomial log-probabilities of having steatosis and degrees 2 and 3 for people in the age category between 18 and 50 years compared to people aged ≥ 50 years should decrease by 0.573 and 1.868 units respectively. In other words, people aged ≥ 50 years are more likely to have NAFLD than people in the 18-50 age group. As for gender, men are more at risk of developing the advanced stages of steatosis than women (OR = 0.262; CI (0.071-0.96)

Table 2. Distribution of the study subjects according to their weight status and NAFLD grades

BMI categories				
NAFLD grades	Normal weight N (%)	Overweight N (%)	Obesity N (%)	Total N (%)
Grade 0	4(2.1)	9(4.7)	178(93.2)	191(100)
Grade 1	10(6.1)	17(10.4)	137(83.5)	164(100)
Grade 2	2(14.3)	2(14.3)	10(71.4)	14(100)
Grade 3	6(35.3)	5(29.4)	6(35.3)	17(100)
Total	22(5.7)	339 (8.5)	331 (85.8)	386(100)

Grades of NAFLD: grade 0: No NAFLD; grade 1: Steatosis; grade 2: Fibrosis; grade 3: Cirrhosis

Table 3. Summary of the multinomial logistic regression analysis results

NAFLD	Variables	B	p	Exp(B)=OR	IC at 95 % for Exp(B)	
					lower bound	upper bound
Grade 1	CRP (mg/l)	0.049	0.000	1.05	1.031	1.07
	Age≥50 (between 18-50)	-0.573	0.038	0.564	0.328	0.97
	Triglycerides	1.005	0.000	2.733	1.752	4.264
Grade 2 &3 (fibrosis & cirrhosis)	CRP (mg/l)	0.047	0.000	1.048	1.028	1.069
	Triglycerides	1.11	0.004	3.036	1.433	6.432
	ALAT (UI/L)	0.068	0.000	1.07	1.034	1.108
	ALP UI/L	0.011	0.003	1.011	1.004	1.019
	GGT UI/L	-0.016	0.005	0.984	0.973	0.995
	Females vs males	-1.339	0.044	0.262	0.071	0.967
	Age≥50 (between 18-50)	-1.868	0.004	0.154	0.044	0.542
	BMI≥30 (between 18-25)	3.062	0.000	21.371	3.987	114.537
	BMI≥30 (between 25-30)	2.874	0.000	17.701	4.051	77.333

IC: intervalle de confiance ; OR: rapport de cotes. CRP:C reactive protein, ALAT: Alanine aminotransferase, ALP: Alkaline phosphatase, GGT: gamma-glutamyl transferase, BMI: body mass index

Models to predict NAFLD in the obese population

The prediction of NALFD and its severity is estimated, based on anthropometric and biological data from a sample of 386 Moroccans. Indeed, using the SPSS program, obesity is treated as a reference group and two models are estimated to predict steatosis and its advanced degrees, relative to obesity (Figure 1) .

DISCUSSION

The results of the present study report an association between NAFLD and obesity. The prevalence of simple non-alcoholic fatty liver disease increased from 6.1% in people with a normal BMI to 10.4% in overweight people and to 83.5% in obese people.

$$\ln(\pi1/\pi0) = -1.678+0.049*CRP+1.005*TG -0.573*age$$
$$\ln(\pi2/\pi0) = -5.356+0.047*CRP+1.110*TG+0.068*ALAT+0.011*ALP-0.016*GGT-1.339*gender-1.868 *age+3.062*BMI$$

ln: logarithm
$\pi0$: the probability of not having NAFLD
$\pi1$: the probability of having grade1 NAFLD
$\pi2$: the probability of having grade 2-3 NAFLD
$\pi0 + \pi1 + \pi2 = 1$

Figure 1. Algorithm

The same is true for the severity and complications of NAFLD, which shows a prevalence of fibrosis of 14.3% in people of normal weight or overweight and 71.4% in obese. Cirrhosis was present in 35.3% of people with normal weight, 29% in overweight and 35.3% in obese. A significant association between BMI, waist circumference and NAFLD was also found. Consistent with these results, similar research in North America reported the presence of steatosis in 70% and NASH in 18.5% of obese people and severe fibrosis in 13.8% of obese patients [3]. Likewise, in the world population, the prevalence of NAFLD is estimated to be between 60% and 95% in obese people [19]. In addition, the global prevalence of obesity has been estimated at 51% among NAFLD patients and 81% in patients with NASH [2]. In addition, hyperlipidemia has been estimated to be 50% in the NAFLD population and has been observed as an increase in triglycerides and cholesterol in fatty liver disease [20]. In addition, another study stipulated that the global prevalence of non-alcoholic steatohepatitis in patients with type 2 diabetes was 37.3%. Also, among patients with NAFLD and type 2 diabetes who have had a liver biopsy, 17% have advanced fibrosis [21].

All these data converge towards a coexistence of NAFLD with obesity and the metabolic syndrome. However, NAFLD is not developed by all people with this syndrome, and vice versa [22, 23]. Comparably, data from the present study reports that 93.2% of the study sample were obese people but not all of them had NAFLD. Almost half of the obese (47.2%) had NAFLD and type 2 diabetes and just over half (52.8%) who had NAFLD but were not diabetic. On the other hand, while a statistically significant association was found between NAFLD and dyslipidemia, a proportion of 13.3% of this population had both NAFLD and dyslipidemia but in return 86.7% of patients with NAFLD did not have dyslipidemia. In addition, approximately 52% of subjects with NAFLD had hypertension, against 48% without hypertension but without revealing a statistically significant association between NAFLD and this disease.

Another determining factor in this study is age. This is because the prevalence of NAFLD increases with increasing age. People aged ≥ 50 are more likely to have steatosis and its advanced grades compared to younger people in the 18 and 50 age group. The multinomial log probabilities of having steatosis and grades 2 and 3 for people aged 18 to 50 compared to people ≥ 50 are expected to decrease by 0.573 and 1,868 units, respectively. This finding is consistent with data from several studies reporting that NAFLD occurs with increasing age[14, 20, 24]. Interestingly, both sexes could have NAFLD, but males were more likely than females to have fibrosis (grade 2). As in the present study, the prevalence of NAFLD in grades 1, 2 and 3 in females was 82.3%, 35.7%, 70.6% and that of males in the same grades was 17.7%, 64.3% and 29.4% respectively. These data are comparable to the results of several studies [14, 19, 24]. The multinomial log-probability of having advanced grades of steatosis for females was decreased by 1,339 units compared to males. One study reported that the relationship between gender and fibrosis may be influenced by menopausal status in women and that the incidence of NAFLD increases after the age of 50 with a peak at 60-69 years, and that NASH is more severe in women than in men [14, 25].

As for the levels of C-reactive protein and triglycerides, their elevations of one unit would respectively increase the log-probabilities of 0.049 and 1.005 for a person to have steatosis and would also respectively increase the log- probabilities of 0.047 and 1.110 for a person to have advanced degrees of NAFLD. In addition, raising the levels of alkaline phosphatase (ALP), alanine aminotransferase (ALT), and BMI by one unit would respectively increase the multinomial log-probabilities of 0.011, 0.068 and 3.062 for a person to have advanced grades of steatosis. These results are consistent with those in the literature which showed an increase in liver enzymes in 50% of patients with grade 1 NAFLD and in 80% in the advanced stages of the disease [22].

In sum, increased age, CRP and TG, gender, body mass index, ALAT, ALP, GGT are predictors of NAFLD and its severity. In this study, these factors were used to develop two algorithms to predict non-alcoholic fatty liver disease and its severity. Alternatively, these methods are rapid and avoid the risk of exposing the person to radiation and invasiveness of the biopsy as well as the relatively high expense of these diagnostic means.

CONCLUSION

Non-alcoholic fatty liver disease is a public health concern as it has become one of the most common liver diseases in the world due to its increasing incidence, largely explained by the increasing prevalence of obesity. The objective of the present study was to investigate the prevalence of non-alcoholic fatty liver disease linked to obesity in Morocco, to determine the associated risk factors as well as to develop a non-invasive procedure that can be assessed more objectively. The diagnosis of non-alcoholic fatty liver disease by biopsy is invasive and expensive, and there is substantial variability among observers in the ultrasound assessment of its severity. In this study two models were created that can be used to predict the degree of fat infiltration in NAFLD based on a person's demographic and biochemical data. They will help clinicians to easily diagnose this disease without the

need for specialized equipment or expertise. The two algorithms developed in this study need, however, to be validated by other studies and would be necessary to improve the quality of care for people with non-alcoholic fatty liver disease.

Acknowledgments

Thanks to the Director of CHU Med VI of Marrakech for authorizing us to consult the files and to the staff for their help. A special thanks to the patients for their participation, we wish them good recovery.

Conflict of interest

The authors declare that there is no conflict of interest.

REFERENCES

1. *García-Compeán D., Villarreal-Pérez JZ., Cavazos MEO., Lavalle-Gonzalez FJ., Borjas-Almaguer OD., Del Cueto-Aguilera AN., González-González JA., Treviño-Garza C., Huerta-Pérez L., Maldonado-Garza HJ.*: Prevalence of liver fibrosis in an unselected general population with high prevalence of obesity and diabetes mellitus. Time for screening? Ann Hepatol 2020;19(3):258-264. doi: 10.1016/j.aohep.2020.01.003.
2. *Younossi ZM., Koenig AB., Abdelatif D., Fazel Y., Henry L., Wymer M.*: Global epidemiology of nonalcoholic fatty liver disease-Meta-analytic assessment of prevalence, incidence, and outcomes. Hepatology 2016;64(1):73-84 doi: 10.1002/hep.28431.
3. *Andronescu CI., Purcarea MR., Babes PA.*: Nonalcoholic fatty liver disease: epidemiology, pathogenesis and therapeutic implications. J Med Life 2018;11(1):20-23.
4. *Castera L., Friedrich-Rust M., Loomba Castera R.*: Noninvasive Assessment of Liver Disease in Patients With Nonalcoholic Fatty Liver Disease. Gastroenterology 2019;156(5):1264-1281 doi: 10.1053/ jgastro 2018; 12.036.
5. 5. *Shannon A., Alkhouri N., Carter-Kent C., Monti L., Devito R., Lopez R., Feldstein AE., Nobili V.*: Ultrasonographic quantitative estimation of hepatic steatosis in children With NAFLD. J Pediatr Gastroenterol Nutr 2011;53(2):190-5 doi: 10.1097/ MPG.0b013e31821b4b61 .
6. *Jennison E., Patel J., Scorletti E., Byrne CD.*: Diagnosis and management of non-alcoholic fatty liver disease. Postgrad Med J. 2019;95(1124):314-322 doi: 10.1136/ postgradmedj-2018-136316.
7. *Loomba R., Cui J., Wolfson T., Haufe W., Hooker J., Szeverenyi N., Ang B., Bhatt A., Wang K., Aryafar H., Behling C., Valasek MA., Lin GY., Gamst A., Brenner DA., Yin M., Glaser KJ., Ehman RL., Sirlin CB.*: Novel 3D Magnetic Resonance Elastography for the Noninvasive Diagnosis of Advanced Fibrosis in NAFLD: A Prospective Study. Am J Gastroenterol 2016;111(7):986-94 doi: 10.1038/ajg.2016.65.
8. *Park CC., Nguyen P., Hernandez C., Bettencourt R., Ramirez K., Fortney L., Hooker J., Sy E., Savides MT., Alquiraish MH., Valasek MA., Rizo E., Richards L., Brenner D., Sirlin CB., Loomba R.*: Magnetic Resonance Elastography vs Transient Elastography in Detection of Fibrosis and Noninvasive Measurement of Steatosis in Patients With Biopsy-Proven Nonalcoholic Fatty Liver Disease. Gastroenterology 2017;152(3):598-607 doi: 10.1053/j.gastro.2016.10.026.
9. *Godoy-Matos AF., Silva Júnior WS., Valerio CM.* NAFLD as a continuum: from obesity to metabolic syndrome and diabetes. Diabetol Metab Syndr 2020;12:60 doi: 10.1186/s13098-020-00570-y.
10. *Pappachan JM., Babu S., Krishnan B., Ravindran NC.* Non-alcoholic Fatty Liver Disease: A Clinical Update. J Clin Transl Hepatol 2017;5(4):384-393 doi: 10.14218/ JCTH.2017.00013.
11. *Anderson EL., Howe LD., Jones HE., Higgins JP., Lawlor DA., Fraser A.* The Prevalence of Non-Alcoholic Fatty Liver Disease in Children and Adolescents: A Systematic Review and Meta-Analysis. PLoS One 2015;10(10):e0140908; doi: 10.1371/journal. pone.0140908.
12. Obesity: preventing and managing the global epidemic. Report of a WHO consultation. World Health Organization. Tech Rep Ser. 2000; 894: i-xii., 1-253.
13. World Health Organization. [Report of the National Survey on Common Risk Factors for Non-Communicable Diseases Morocco (2017 – 2018)]. 2019.
14. *Habiba L., Mohamed M., Rekia., B.:* Non-alcoholic fatty liver disease in Morocco: The situation., the determinants and the challenges for health care. World Journal of Advanced Research and Reviews 2020; 6(1)., 207-217doi: 10.30574/wjarr.2020.6.1.0100
15. *Belahsen R., Mziwira M., Fertat F.* Anthropometry of women of childbearing age in Morocco: body composition and prevalence of overweight and obesity. Public Health Nutr. 2004;7(4):523-30 doi: 10.1079/ PHN2003570.
16. World Health Organization-International Society of Hypertension Guidelines for the Management of Hypertension. Guidelines Subcommittee. J Hypertens1999; 17(2):151-83.
17. *Anwar S., Rashid H., Aleem B., Moslhey G- J., Al Rashdi A- S.:* Correlation between anthropometric measurements and hypertension in Oman. Age (years) 2020; 48(1).53. 42.36-0.97.
18. American Diabetes Association. Executive summary: Standards of medical care in diabetes2012. Diabetes Care 2012; 35:S4-S10 doi: 10.2337/dc12-s004.
19. *Baudin., B.*: Biochemical exploration of the liver in 2017. Francophone Journal of Laboratories 2017(490):25-33.
20. *Younossi ZM.:* Non-alcoholic fatty liver disease - A global public health perspective. J Hepatol 2019;70(3):531-544; doi: 10.1016/j.jhep.2018.10.033.
21. *Dixon JB., Bhathal PS., O'Brien PE.*: Nonalcoholic fatty liver disease: predictors of nonalcoholic steatohepatitis and liver fibrosis in the severely obese. Gastroenterology 2001; 121(1):91-100 doi: 10.1053/gast.2001.25540.
22. *Vanni E., Bugianesi E., Kotronen A., De Minicis S., Yki-Järvinen H., Svegliati-Baroni G.* From the metabolic syndrome to NAFLD or vice versa? Dig Liver Dis 2010;42(5):320-30. doi: 10.1016/j.dld.2010.01.016.

23. *López-Velázquez JA., Silva-Vidal KV., Ponciano-Rodríguez G., Chávez-Tapia NC., Arrese M.,* Uribe M., Méndez-Sánchez N.: The prevalence of nonalcoholic fatty liver disease in the Americas. Ann Hepatol. 2014;13(2):166-78; https://doi.org/10.1016/S1665-2681(19)30879-8
24. *Estes C., Anstee QM., Arias-Loste MT., Bantel H., Bellentani S., Caballeria J., Colombo M., Craxi A., Crespo J., Day CP., Eguchi Y., Geier A., Kondili LA., Kroy DC., Lazarus JV., Loomba R., Manns MP., Marchesini G., Nakajima A., Negro F., Petta S., Ratziu V., Romero-Gomez M., Sanyal A., Schattenberg JM., Tacke F., Tanaka J., Trautwein C., Wei L., Zeuzem S., Razavi H.*: Modeling NAFLD disease burden in China., France., Germany., Italy., Japan., Spain., United Kingdom., and United States for the period 2016-2030. J Hepatol 2018; 69(4):896-904 doi: 10.1016/j.jhep.2018.05.036.
25. *Goossens N., Bellentani S., Cerny A., Dufour JF., Jornayvaz FR., Mertens J., Moriggia A., Muellhaupt B., Negro F., Razavi H., Semela D., Estes C.*: Nonalcoholic fatty liver disease burden - Switzerland 2018-2030 Swiss Med Wkly 2019;149:w20152 doi: 10.4414/smw.2019.20152.

Received: 02.04.2022
Accepted: 12.08.2022

Rocz Panstw Zakl Hig 2022;73(3):333-340
http://wydawnictwa.pzh.gov.pl/roczniki_pzh/ https://doi.org/10.32394/rpzh.2022.0220

ORIGINAL ARTICLE

EFFECT OF COVID-19 PANDEMIC ON GENDER ASSOCIATED WITH RISK FACTORS: A RETROSPECTIVE DATA ANALYSIS, THAILAND

Jadsada Kunno[1], Busaba Supawattanabodee[1], Chavanant Sumanasrethakul[2], Budsaba Wiriyasirivaj[3], Sathit Kuratong[4], Chuthamat Kaewchandee[1], Pataraporn Yubonpunt[5]

[1]Department of Research and Medical Innovation, Faculty of Medicine Vajira Hospital, Navamindradhiraj University, Bangkok, Thailand
[2]Department of Urban Medicine, Faculty of Medicine Vajira Hospital, Navamindradhiraj University, Bangkok, Thailand
[3]Department of Obstetrics and Gynecology, Faculty of Medicine Vajira Hospital, Navamindradhiraj University, Bangkok, Thailand
[4]Department Medicine, Faculty of Medicine Vajira Hospital, Navamindradhiraj University, Bangkok, Thailand
[5]Department of Public Health, Faculty of Public and Environmental Health, Huachiew Chalermprakiet University, Samut Prakan, Thailand

ABSTRACT

Background. The COVID-19 pandemic is having a serious impact around the world. Many countries have experienced a two or three wave pattern in reported cases. The virus's spread in Thailand was a cluster event distributed over multiple locations, multi-spender, and multiple waves of outbreaks.

Objective. This study aims to study gender associated with age, risk factors, and nationality during coronavirus pandemic in Thailand.

Material and methods. A retrospective cohort study was conducted from January 2020 to May 2021 (17 months) to determine the number of confirmed cases and identify gender associated with, age, various risk factors and nationality were analyzed by chi square test and binary logistic regression analyses.

Results. The results show that the number of cases increased by over 100,000 over the course of three waves of outbreaks. The logistic regression analysis revealed that genders were significantly related with age, various risk factors, and nationality across different waves ($p < 0.01$). Across the primary risk factors were community risk, community cluster and close contact with a previously confirmed patient on confirmed cases during COVID-19 pandemic

Conclusion. Significant differences between genders were significantly associated with age, various risk factors, and nationality may be due to weak social distancing policies and the lack of public health interventions. A COVID-19 vaccination plan is needed for people who are at risk of suffering severe symptoms as well as the general population in outbreak areas to increase immunity.

Key words: *COVID-19, pandemic waves, gender, age, various risk factors*

INTRODUCTION

Coronavirus (COVID-19) is a pandemic found all over the world today. Globally, there have been more than 177 million cases, with an average of around 490,000 cases reported each day and over 50,000 deaths per week [1, 2]. As COVID-19 continues its rapid global spread, increased understanding of the underlying levels of transmission and infection severity are crucial for guiding the pandemic response [3].

According to the literature, many countries have experienced multiple waves of COVID-19 outbreaks. During the 2020 pandemic, empirical data show that characteristics varied between waves [4]. In comparison with the second wave, the proportion of local clusters (24.8% vs. 45.7%) was lower in the third wave, and personal contact transmissions (38.5% vs. 25.9%) and unknown routes of transmission (23.5% vs. 20.8%) were higher [5]. Consequently, many governments and health authorities, including the World Health Organization (WHO), have been

Corresponding author: Busaba Supawattanabodee, Department of Research and Medical Innovation, Faculty of Medicine, Vajira Hospital, Navamindradhiraj University, Bangkok, Thailand, Tel.: +66-2244-3125 e-mail: sbusaba@nmu.ac.th

actively educating people to take preventive measures to reduce the spread of the virus, including lockdown measures [6, 7, 8].

Multiple waves in Thailand [9, 10, 11] have been traced to super-spreading events at entertainment establishments, pubs, bars, karaoke lounges, and various types of gambling venues in different regions of the country. These events led to an expansion of spread of COVID-19 to many provinces since the risk locations were sites that attracted crowding, extended interactions, and high turnover. For example, clusters of outbreaks during the first wave of coronavirus have been traced to super-spreader events in sports venues or indoor entertainment establishments. An outbreak in March 2020 was associated with attendees at a boxing stadium in Bangkok, who spread the virus to other provinces as they travelled home or on business. The origin of the second wave has been traced to a wholesale shrimp market. Most of the initial infections beginning in mid-December 2020 were among Thai residents and non-Thai migrants who live and work in that locality.

The impact of gender and age may as a dependent risk factor, one study indicated that men are at higher risk of contracting the novel coronavirus than women [12, 13]. Males have lagged females in taking up social distancing measures. Males' poorer compliance with public health containment measures may help explain their higher COVID-19 mortality. Moreover, the relative share of infections among young, older, and debilitated people may shape the observed proportion of deaths in different demographic groups and the overall fatality rate in the total population [18]. When adjusting for sex, age and the presence of comorbidities, that study found that mortality significantly increased among elderly men, which is consistent with other regional studies [14]. Different age groups have experienced the pandemic in distinct ways [15, 16]. Physical distancing means that children must spend more time within the household, and it sharply reduces opportunities for young children to play with their peers, engage in typical rough-and-tumble learning, or develop empathy [15, 17].

Thus, the COVID-19 pandemic is a multi-spender, and outbreaks affect everyone living in Thailand, including citizens, migrant workers, and other foreign nationals. Various risk factors associated with multiple waves of coronavirus outbreaks include close contact with a previously confirmed patient, community risk, and clustered communities as well as socio-demographic factors such as nationality, gender, and age. Hence, the aim of this study was to investigate the effect of COVID-19 pandemic on gender associated with age, various risk factors and nationality in Thailand.

MATERIAL AND METHODS

Study design

We conducted a retrospective data study of all cases of COVID-19 in Thailand. Information was recorded between January 2020 and May 2021 [9, 10, 11]). All records were fully anonymized before the researchers accessed them. This study was approved by the ethics committee of Faculty of Medicine Vajira Hospital, Navamindradhiraj University, Bangkok, Thailand, (COE: 011/2021X).

Data collection

COVID-19 data gathered by the Department of Disease Control, Ministry of Public Health, Thailand were collected, including time period, number of confirmed cases by PCR test for SARS-CoV-2, and sociodemographic characteristics, specifically gender, age, various risk factors and nationality. The study period was stratified based on the months of test screenings and diagnoses to identify temporal trends in cases. Population data were divided between five phases: Phase I: January–February 2020, Phase II: March–May 2020 (First wave), Phase III: June–November 2020, Phase IV: December 2020–March 2021 (Second wave), and Please V: April–May 2021 (Third wave).

Study population

The COVID-19 infection database was queried to identify all recorded ages, gender, nationality, and various risk factors. Cases in which such information was missing were excluded.

We stratified the population based on age (< 20, 21 – 40, 41 – 60, and > 60 years). Gender (males and females). We stratified nationality into Thai and non-Thai including migrant worker (Myanmar, Khmer, Laotian), and foreigner categories. Population various risk factors were stratified on the basis of close contact with a previously confirmed patient (risk 1), community cluster (risk 2), community risk (risk 3, such as enclosed space), active-community surveillance (risk 4), and other risk factors (risk 5) (Figure 1).

Statistical analysis

We summarized the characteristics of the categorical data. Characteristics were compared using descriptive statistics, and categorical data were compared using a chi square test. We used binary logistic regression to test the association between gender and age, various risk factors, and nationality on confirmed cases of COVID-19. The level of statistical significance was set at p-value < 0.05 was considered to indicate statistical significance. Statistical analysis

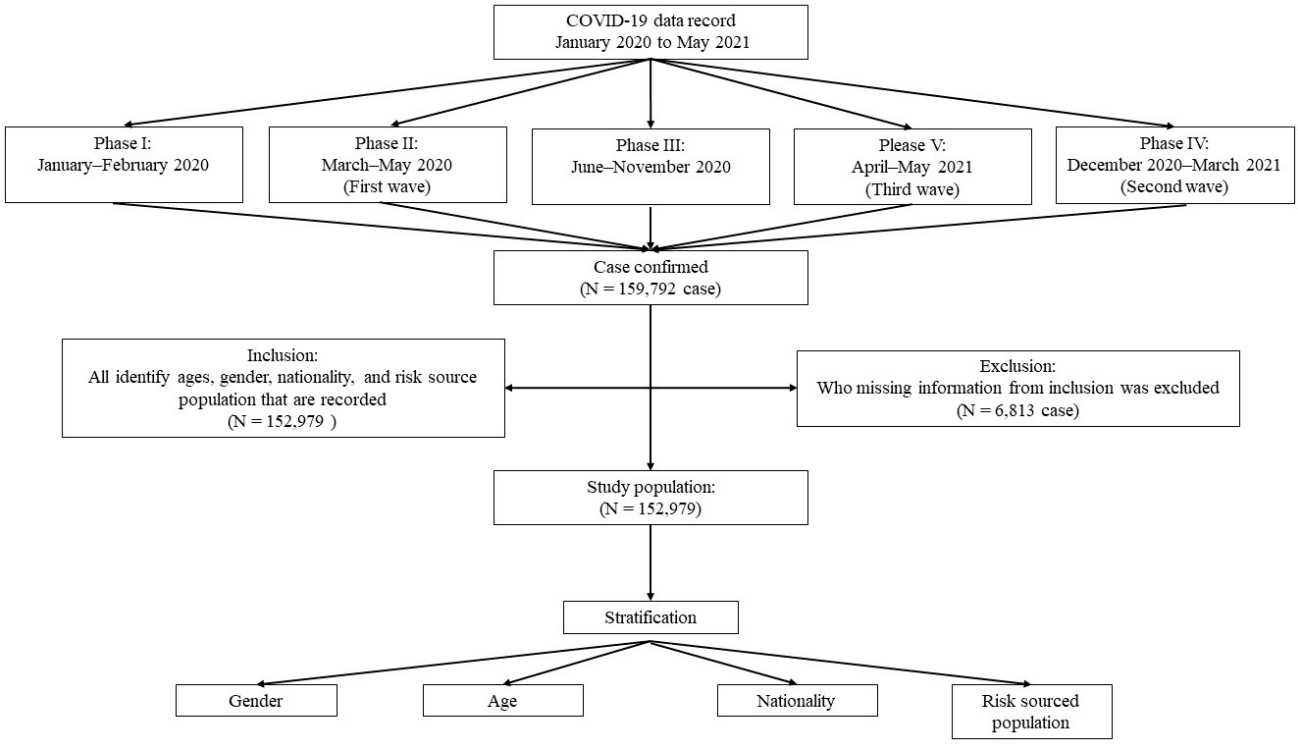

Figure 1. Derivation of study population

was performed using the Statistical Package for the Social Sciences Program (SPSS), version 22.

RESULTS

Socio-demographic characteristics during different phases during COVID-19 pandemic

The Chi-square results showed that the different phases of the COVID-19 pandemic in Thailand were significantly difference with gender, age, various risk factors and nationality (P-value < 0.01) (Table 1).

There were approximately 150,000 confirmed cases within the 17-month period from January 2020 to May 2021. During phase I (January–February 2020), there were approximately 42 confirmed cases. During the first wave in phase II (March–May 2020), the number of new cases increased to approximately 3,040. Phase III (June–November 2020) was characterized by low levels of new cases (approximately 938 cases); however, a second wave occurred during Phase IV (December 2020–March 2021), when the number of new cases increased to more than 20,000. A third wave–which continues into the present–can be observed during Phase V (April–May 2021), when the number of new cases increased to more than 100,000 (Table 1).

The number of cases according to female 74,170 and male 78,809 cases. The mean age was 35 ± 14.01 years. Table 1 presents Thais were the main nationality impacted by the coronavirus, with 118,391 cases, distantly followed by non-Thai nationality with 34,588 cases. Data of various risk factors revealed that most tested individuals had been in risk1 accounted for the largest number of cases (n = 67,214), followed risk2 (n = 39,377), and risk3 (n = 13,002), and risk4 (n = 30,456). In addition, 2,969 cases occurred due to other risks, Hence, the COVID-19 pandemic had a deep impact on all socio-demographic groups living in Thailand, including Thai, migrant workers and foreigners across genders and all stages of life. The most prominent risk factors were close contact with a previously confirmed patient and community risk.

Association between gender and variable factors during COVID-19 pandemic within Phase I - Phase V

The binary logistic regression test showed gender were significantly associated with age (*P*-value < 0.01**)** (Table 2). Therefore, gender was significantly associated with risk factors inducing community cluster, community risk and active-community surveillance (*P*-value < 0.01), while gender were likely significant association with close contact with a previously confirmed patient (*P*-value > 0.05). However, gender was not significantly associated with nationality (*P*-value > 0.05).A multivariate analysis (Table 2), All ages were significantly associated with gender (*p*-value < 0.01), whereby male prosperity risk more than female (OR = 1.103 – 1.140, 95%CI 1.054 – 1.200). Risk factor at community clusters were significantly associated with gender (*p*-value < 0.01), whereby male prosperity risk more than female (OR = 1.971, 95%CI 1.828 – 2.125). Factors

Table 1. Socio-demographic characteristics during different phases of the COVID-19 pandemic in Thailand

Characteristics	Phases of the COVID-19 pandemic across 17 months												
	Phase I - January - February 2020		Phase II First wave March – May 2020		Phase III - June - November 2020		Phase IV Second wave December 2020 - March 2021		Phase V Third wave April – May, continues 2021		Total		*P*-value
Gender	n (%)												
Female	19	0.0	1,374	1.9	375	0.5	12,745	17.2	59,657	80.4	74,170	100.0	< 0.01
Male	23	0.0	1,666	2.1	563	0.7	9,228	11.7	67,329	85.4	78,809	100.0	
Total	42	0.0	3,040	2.0	938	0.6	21,973	14.4	126,986	83.0	152,979	100.0	
Age (years)													
< 20	3	0.0	228	1.4	82	0.5	1,458	8.7	15,072	89.5	16,843	100.0	< 0.01
21 – 40	18	0.0	1,554	1.7	530	0.6	14,964	16.8	72,083	80.9	89,149	100.0	
41 – 60	8	0.0	964	2.6	264	0.7	4,698	12.5	31,576	84.2	37,510	100.0	
> 60	13	0.1	294	3.1	62	0.7	853	9.0	8,255	87.1	9,477	100.0	
Total	42	0.0	3,040	2.0	938	0.6	21,973	14.4	126,986	83.0	152,979	100.0	
Risk factors													
Risk 1	32	0.0	1,599	2.4	276	0.4	2,773	4.1	62,534	93.0	67,214	100.0	< 0.01
Risk 2	0		0		0		17,587	44.7	21,790	55.3	39,377	100.0	
Risk 3	8	0.1	1,146	8.8	16	0.1	567	4.4	11,265	86.6	13,002	100.0	
Risk 4	0		55	0.2	0		332	1.1	30,069	98.7	30,456	100.0	
Risk 5	2	0.1	240	8.1	661	18.2	718	24.2	1,348	45.4	2,969	100.0	
Total	42	0.0	3,040	2.0	938	0.6	21,973	14.4	126,986	83.0	152,979	100.0	
Nationality													
Thai	17	0.0	2,734	2.3		0.6	7,922	6.7	107,029	90.4	118,391	100.0	< 0.01
Others	25	0.1	306	0.9		0.7	14,051	40.6	19,957	57.7	34,588	100.0	
Total	42	0.0	3,040	2.0		0.6	21,973	14.4	126,986	83.0	152,979	100.0	

Risk1: close contact with a previously confirmed patient; Risk2: community cluster; Risk3: community risk; Risk4: active-community surveillance Risk5: others. Statistical analysis was performed by chi square test. *P*-value < 0.05 was considered to indicate statistical significance.

Table 2. Bivariate and multivariate analysis of gender associated with age, risk factors and nationality on confirmed cases of COVID-19 within Phase I - Phase V

Variable factors	Gender		Bivariate		Multivariate	
	%		OR (95%CI)	*P*-value	OR (95%CI)	*P*-value
Age (years)	Female	Male				
< 20	11.3	10.7	1.115 (1.060 – 1.172)	< 0.01	1.140 (1.084 – 1.200)	< 0.01
21 – 40	57.4	59.1	1.213 (1.162 – 1.265)	< 0.01	1.113 (1.066 – 1.162)	< 0.01
41 – 60	24.5	24.5	1.182 (1.130 – 1.236)	< 0.01	1.103 (1.054 – 1.154)	< 0.01
> 60	6.7	5.7	Ref.		Ref	
Risk factors						
Risk 1: close contact with a previously confirmed patient	48.3	39.8	1.056 (0.980 – 1.136	0.151	1.069 (0.992 – 1.151)	0.079
Risk 2: community cluster	20.5	30.6	1.910 (1.772 – 2.059)	< 0.01	1.971 (1.828 – 2.125)	< 0.01
Risk 3: community risk	8.7	8.3	1.218 (1.124 – 1.319)	< 0.01	1.226 (1.132 – 1.328)	< 0.01
Risk 4: active-community surveillance	20.3	19.6	1.239 (1.149 – 1.337)	< .0.01	1.263 (1.171 – 1.363)	< 0.01
Risk 5: others	2.2	1.7	Ref.		Ref	
Nationality						
Thai	77.4	77.3	1.006 (0.982 – 1.031)	0.611	0.890 (0.868 – 0.913)	< 0.01
Others	22.6	22.7	Ref.		Ref	

Female = 0, Male = 1; Ref = reference group; OR = odds ratio; CI = confidence interval. Significant at *P*-value < 0.05.

community risk were significantly associated with gender (*p*-value < 0.01), whereby male might risk more than female (OR = 1.226, 95%CI 1.132 – 1.328). Factors on active-community surveillance were significantly associated with gender (*p*-value < 0.01), whereby male might risk more than female (OR = 1.263, 95%CI 1.171 – 1.363). In addition, who close contact with a previously confirmed patient were neatly significantly associated with gender (*p*-value =0.079). However, Thai nationality were significantly associated with gender (*p*-value < 0.01), whereby male prosperity risk lower than female (OR = 0.890, 95%CI 0.868 – 0.913). Hence, gender associated with age, risk factors and nationality on confirmed cases during COVID-19 pandemic.

DISCUSSION

The current reports the results of a retrospective cohort study of all confirmed cases of the COVID-19 pandemic in Thailand from January 2020 to May 2021. The results show that there were over 152,979 confirmed cases of COVID-19 during that 17-month period, including approximately 74,170 cases among females and 78,809 cases among males. Our study did not investigate socio-demographic differences in mortality rates due to limitations in data and time; however, age and gender are well-established risk factors for COVID-19; for example, over 90% of deaths in the UK to date have occurred among people aged over 60 years, and men account for 60% of deaths [12].

This study found significant differences between the three waves of the COVID-19 pandemic in Thailand. Our results indicate that the third wave is more serious than previous waves, which may be due to a lack of strong social distancing policies and public health interventions. Our findings differ from other studies that found that first wave of COVID-19 pandemic had the most negative impact on public health, whereas the second wave evinced more stable evolutionary dynamics [18]. Sufficient epidemiologic investigations and contact tracing could not be performed during the third wave, and there was a marked increase in the proportion of unknown routes of transmission [10, 12].

Association between gender associated with age, risk factors and nationality on confirmed cases of COVID-19 within Phase I - Phase V

In fact that gender was significantly associated with age, risk factors and nationality (*P*-value < 0.01), and more cases occurred in males than females. We found that clusters risk might play an important role in modifying transmission patterns of COVID-19. The presence of gender was associated with age (*P*-value <0.01), based on distribution of COVID-19 fatalities remained steady across the three waves, this was

difference with previous study showed that females and age were found to be at greater risk for being COVID-19 infected [19].

In this study reported that elderly individuals over 60 years of age accounted for approximately 9,477 COVID-19 cases. Although COVID-19 has an extremely steep risk gradient for death across age groups [12], increasing age has been strongly associated with risk, with people aged 80 or over having a more than 20-fold-increased risk compared with 50–59-year-olds (fully adjusted HR 20.60; 95%CI 18.70–22.68) [12]. However, strategies specifically focused on protecting high-risk elderly individuals should be considered in managing the pandemic [14]. Case reports indicate a mean age range for fatalities of 50–60 years, and studies have demonstrated that younger patients tend to exhibit milder symptoms [20]. One study found that the median age of the infected was 42 in men and 39 in women, and the most affected age group was that aged 19–50 years, which represented 59.6% of the entire cohort, almost double the prevalence among the same age group in Italy (24.0%) and very similar to the age distribution in China [14, 21].

To the best of our knowledge, gender was significantly associated with risk factors inducing community cluster, community risk, active-community surveillance, and close contact with a previously confirmed patient (*P*-value <0.01). As the same our presented one study indicated that men are at higher risk of contracting the COVID-19 than women [12, 13]. Another study found that men accounted for 55.4% (n = 5,247) of all cases with an incidence rate of 60.5 per 100,000, whereas women accounted for 44.6% of cases (n = 4,221) and an incidence of 47.2 per 100,000 [21]. Males have lagged females in taking up social distancing measures. Males' poorer compliance with public health containment measures may help explain their higher COVID-19 mortality.

Our study considered ethnicity as an independent risk factor. This study found gender were significantly associated with nationality (*P*-value <0.01). However, a systematic review and meta-analysis could not confirm ethnicity as an independent risk factor for negative outcomes in COVID-19 patients [22]. It is time to learn from the lessons of past disease outbreaks. Given the low-to-high-quality evidence indicating that ethnicity is not an independent risk factor, COVID-19 risk assessments should only consider ethnicity in conjunction with other risk factors such as age or comorbidities. Following the second wave of the pandemic in Thailand, screening and surveillance have been expanded to try to detect potential outbreaks before they could ignite, and more outreach testing has been conducted, especially among the migrant worker population and their contacts. It is important to learn from prior experiences and strategies to reduce observed disparities [22], and resources must be invested in the hardest-hit communities [23].

There are currently limited retrospective studies addressing the nationality and risk factors were not directly estimated, and some gender and age information are missing. Therefore, our results must be taken with caution. However, we believe that the findings are relevant, as they represent a national-level study of risk factors and socio-demographic impacts across Thailand, and limited information is currently available on this issue.

CONCLUSIONS

The best results of the present study show an effect of different waves during the COVID-19 pandemic on gender associated with age, risk factors and nationality in Thailand, which may be attributable to a lack of strong social distancing policies and public health interventions. The main risk factors were close contact with a previously confirmed patient and community risk. We recommend an acceleration of screening among workers so that they can enter the health service system as soon as possible and confirmed cases can be isolated. The likelihood of replication and mutation increases when a virus is widely circulating and causing many infections in a population [24, 25, 26, 27]. Priority should be given to vaccinating high-risk groups to maximize global protection against new variants and minimize the risk of transmission [28]. As more people get vaccinated, virus circulation is expected to decrease, which will in turn result in fewer mutations [26]. Generally, control measures have been tightened around the country, and people have been strongly admonished to wear masks, practice hand-washing hygiene, and socially distance while outside the home.

Abbreviations

Coronavirus disease (COVID-19); Risk1 (previously confirmed patient) referred to as "direct contact", this is when one person is physically close enough to an infected individual to come into direct contact with their bodily fluids, including respiratory droplets; Risk2 (community cluster) referred to cluster occurs when there is a concentration of infections in the same area at the same time such as occurs at village, district, district and province; Risk3 (community risk) referred to workplace, not public-facing, bar, childcare facility, health care, restaurant etc. or other hand refer to enclosed space; Risk4 (active-community surveillance) referred to when members of a community actively participate in detecting, reporting, responding to and monitoring health events in their community; Risk5 (other risk factors) referred to state quarantine; WHO: World

Health Organization; OR: Odds Ratio; CI: Confidence Interval.

Acknowledgments

The authors would like to acknowledge the COVID-19 data gathered by the Department of Disease Control, Ministry of Public Health, Thailand. Information was recorded between January 2020 and May 2021 (17 months).

Ethics approval and consent to participate

This study was approved by the ethics committees of Faculty of Medicine Vajira Hospital, Navamindradhiraj University, Bangkok, Thailand. (Approval no. COE: 011/2021X).

Funding

None

Consent for publication

Not applicable

Declaration of competing interest

The authors declare that they have no conflicts of interest.

Availability of data and materials

The data sets generated and analyzed during the current study are not publicly available due to identifiable information but are available from the corresponding author on reasonable request answering the survey. The secondary analyses from announcement regarding the COVID-19 from the Department of Disease Control in Thailand data used to support the findings of this study are included within the article. Data are available from announcement regarding the COVID-19 from the Department of Disease Control in Thailand for researchers who meet the criteria for access to confidential data. The data used to support the findings of this study have been deposited in the Department of Disease Control in Thailand repository from: https://ddc.moph.go.th/viralpneumonia/eng/file/main/en_Thailand%20Covid-19%20plan_MOPH_2021.pdf. For more information, https://ddc.moph.go.th/viralpneumonia/eng/announcement.php.

Authors' contributions

JK., contributed to study design, data collection, data analysis, interpretation, writing and revision of the manuscript. BS., contributed to study design, data analysis, interpretation. CS., contributed to study design, data analysis, interpretation. BW., contributed to study design, data analysis, interpretation. SK., contributed to study design, data analysis, interpretation. CK., contributed to study design, data analysis, interpretation. PY., contributed to data analysis, interpretation, and writing. All authors read and approved the final manuscript.

REFERENCES

1. WHO, Coronavirus disease (COVID-19) Weekly Epidemiological Update and Weekly Operational Update. 2021: World Health Organization, https://www.who.int/emergencies/diseases/novel-coronavirus-2019/situation-reports.
2. WHO, Coronavirus disease (COVID-19). 2020, World Health Organization, https://www.who.int/emergencies/diseases/novel-coronavirus-2019.
3. *O'Driscoll, M.*, et al., Age-specific mortality and immunity patterns of SARS-CoV-2. Nature, 2021. 590(7844):140-145.
4. *Iftimie, S.*, et al., First and second waves of coronavirus disease-19: A comparative study in hospitalized patients in Reus, Spain. PLOS ONE, 2021. 16(3): e0248029.
5. *Seong, H.,* et al., Comparison of the second and third waves of the COVID-19 pandemic in South Korea: Importance of early public health intervention. Int J Infectious Diseases, 2021;104:742-745.
6. *Godman, B.,* et al., Rapid Assessment of Price Instability and Paucity of Medicines and Protection for COVID-19 Across Asia: Findings and Public Health Implications for the Future. Public Health Front. 2020;8:585832-585832.
7. *Farooq, H.Z.,* et al., Real-world SARS CoV-2 testing in Northern England during the first wave of the COVID-19 pandemic. J. Infect, 2021.
8. *Alrasheedy, A.A.*, et al., Knowledge, Attitude and Practice About Coronavirus Disease (COVID-19) Pandemic and Its Psychological Impact on Students and Their Studies: A Cross-Sectional Study Among Pharmacy Students in Saudi Arabia. Risk Manag Healthc Policy, 2021;14:729-741.
9. Thailand, M.P.H., Strategy: Managing the new wave of the Covid-19 Epidemic. 2021, https://ddc.moph.go.th/viralpneumonia/eng/situation.php: Department of Disease Control,
10. Thailand, D.D.C., Thailand Situation, (Report) 2021, https://ddc.moph.go.th/viralpneumonia/eng/situation.php: Department of Disease Control, Thailand, https://ddc.moph.go.th/viralpneumonia/eng/situation.php.
11. Thailand, D.D.C., Thailand Situation, (COVID-19). 2021: Department of Disease Control, Thailand, https://ddc.moph.go.th/viralpneumonia/eng/index.php
12. *Williamson, E.J.*, et al., Factors associated with COVID-19-related death using OpenSAFELY. Nature, 2020. 584(7821): p. 430-436.
13. *Williamson, E.*, et al., OpenSAFELY: factors associated with COVID-19-related hospital death in the linked electronic health records of 17 million adult NHS patients. medRxiv, 2020: p. 2020.05.06.20092999.
14. *Ioannidis, J.P.A.*, C. Axfors, and D.G. Contopoulos-Ioannidis, Population-level COVID-19 mortality risk for non-elderly individuals overall and for non-elderly individuals without underlying diseases in pandemic epicenters. Environ Res 2020;188:109890-109890.

15. *DeGrace, S.*, et al., Sex differences in maladaptive emotional and behavioral responses to COVID-19: What is the role of personality? Personality and Individual Differences, 2021:178:110834.
16. *Díaz, A., Á. Beleña, and J. Zueco,* The Role of Age and Gender in Perceived Vulnerability to Infectious Diseases. International Journal of Environ Res Publ Health, 2020;17(2):485.
17. *Mendoza-Jiménez, M.J., Hannemann T.V., Atzendorf J.:* Behavioral Risk Factors and Adherence to Preventive Measures: Evidence From the Early Stages of the COVID-19 Pandemic. Frontiers in Public Health, 2021. 9(679).
18. *Coccia, M.,* The impact of first and second wave of the COVID-19 pandemic in society: comparative analysis to support control measures to cope with negative effects of future infectious diseases. Environ Res 2021;197:111099.
19. *Kushwaha, S.*, et al., Biological attributes of age and gender variations in Indian COVID-19 cases: A retrospective data analysis. Clin Epidemiol Global Health, 2021;11:100788.
20. *Verity, R.*, et al., Estimates of the severity of coronavirus disease 2019: a model-based analysis. Lancet Infect Dis, 2020;20(6):669-677.
21. *Ortiz-Prado, E.*, et al., Epidemiological, socio-demographic and clinical features of the early phase of the COVID-19 epidemic in Ecuador. PLOS Neglected Tropical Diseases, 2021;15(1): e0008958.
22. *Raharja, A., Tamara A., Ko L.T.:* Association Between Ethnicity and Severe COVID-19 Disease: a Systematic Review and Meta-analysis. J Racial Ethn Health Disparities 2020;1-10
23. *Bibbins-Domingo, K.*, This Time Must Be Different: Disparities During the COVID-19 Pandemic. Annals of Internal Medicine, 2020;173(3):233-234.
24. *Plante, J.A.*, et al., The variant gambit: COVID-19's next move. Cell Host & Microbe, 2021;29(4):508-515.
25. *Samadizadeh*, S., et al., COVID-19: Why does disease severity vary among individuals? Respiratory Medicine, 2021;180:106356.
26. Peiffer-Smadja, N., et al., COVID-19 vaccines: A race against time. Anaesthesia Critical Care & Pain Medicine, 2021;40(2):100848.
27. *Jhaveri, R.,* The Next Set of COVID-19 Vaccines: Leveraging New Development Platforms to Increase Access for More People Around the World. Clin Ther 2021.
28. *Forman, R.*, et al., COVID-19 vaccine challenges: What have we learned so far and what remains *to be done?* Health Policy, 2021;125(5):553-567.

Received: 27.04.2022
Accepted: 21.07.2022
Published online first: 12.08.2022

Rocz Panstw Zakl Hig 2022;73(3):341-349
http://wydawnictwa.pzh.gov.pl/roczniki_pzh/ https://doi.org/10.32394/rpzh.2022.0224

ORIGINAL ARTICLE

SOCIO-ECONOMIC CHARACTERISTICS, HEALTH STATUS AND ACCESS TO HEALTH CARE IN AN ELDERLY MOROCCAN COMMUNITY: STUDY OF THE GENDER FACTOR

Mohamed Mziwira[2], *Azzelarab Ahaji*[1], *Kaoutar Naciri*[1], *Rekia Belahsen*[1]

[1]Laboratory of Biotechnology, Biochemistry and Nutrition, Training and Research Unit on Nutrition and Food Sciences, Department of Biology, Faculty of Sciences, Chouaib Doukkali University, El Jadida, Morocco
[2]Laboratory of Bio-Geosciences and Materials Engineering, Higher Normal School, Hassan II University of Casablanca, Casablanca, Morocco

ABSTRACT

Background. . In Western societies, gender differences in health and health behavior are extensively documented, but less is known about gender health disparities in Morocco. Aging is not yet a research topic or a source of concern. However, the country will face significant demographic aging in the future.

Objective. The purpose of this study was to investigate gender differences in indicators associated with socioeconomic status, health status, and access to health care among the elderly population of the El Jadida region in Morocco.

Material and methods. It is a cross-sectional study on a random sample of 537 persons, aged 60 and older (136 women and 401 men) from the El Jadida region.

Results. When compared to their male counterparts, older Moroccan women face a number of disadvantages, including lower levels of education and literacy, lower levels of employment, rising rates of widowhood and living alone, and a lower likelihood of receiving formal pension benefits. In terms of health status, half of older women do not have medical coverage, almost all feel more tired, and, in discomfort, half suffer from total and central obesity, with more visual, oral, and memory health problems. Older Moroccan men, on the other hand, have greater hypertension, smoke and consume more tobacco and alcohol, and are more anxious, depressed, and insomniac. Overall, for both sexes, the perception of self-rated health status was deemed poor, with three health problems reported per person. Many other demographic, psychosocial, and economic indicators were not significantly related to gender.

Conclusions. In Morocco, older people face a variety of problems that have a negative impact on their perception of aging. Furthermore, there are gender differences in socioeconomic status, prevalence, symptoms, and correlates of chronic diseases, health service use, and lifestyle. Longitudinal studies and immediate implementation of medical policy for this population are needed

Key words: *elderly people, gender gaps, socioeconomic factors, health status, health care access, chronic disease, Morocco*

INTRODUCTION

Many countries are currently experiencing, to varying degrees, the so-called phenomenon of "population aging." Seniors accounted for 8% of the global population in 2009, and this percentage is expected to rise to 22% by 2050 [26]. However, the degree of aging, and thus the degree of aging, varies from continent to continent. Europe, North America, and Oceania are the continents with the oldest populations, with 17.4%, 15.1 %, and 12.5 % of the population over 65, respectively. Asia currently holds 7.9% of the world's elderly population, but this number is expected to rise considerably as the population ages. Except for the North African countries, Africa, which is on the verge of having 3.5% of its population over 65 years old, remains a young continent due to high fertility rates [41]. As a result, the aging of the population will have a variety of social, economic, and cultural consequences [4]. Rapid population

Corresponding author: Rekia Belahsen, Laboratory of Biotechnology, Biochemistry & Nutrition. Training and Research Unit on Nutrition & Food Sciences., Chouaib Doukkali University, Faculty of Sciences, El Jadida, 24000 Morocco, Phone: 212 523342325 or 212 664971616; Fax: 212 523342187/4449, e-mail: rbelahsen@yahoo.com or b.rekia@gmail.com

aging is accompanied by growing concern about individual and population health as a result of the increased incidence and prevalence of chronic non-communicable diseases, which can reduce a person's sense of well-being and quality of life in later life [8].

Gender differences in the experience of aging, on the other hand, have become a source of concern for policymakers around the world. They are also visible in lifestyle risk factors for chronic diseases like alcohol consumption, smoking, and physical activity, which can reflect personal health beliefs and values as well as underlying gender norms and contribute to gendered patterns of health care utilization. Women are less likely than men to engage in risky lifestyle behaviors such as drinking and smoking, but they are also less physically active than men [25].

Morocco is, in fact, in an advanced stage of demographic transition, with a life expectancy of more than 72 years, a continuous decline in fertility and a longer life expectancy, and an elderly percentage that is increasing at an unprecedented rate. Indeed, the proportion of people over 60 increased from 7.2% (less than one million) in 1960 to 8.1 percent in 2004 and 9.4% (3.2 million) in 2014 [17]. Predictions are growing, as this proportion is expected to rise from 11.5% of the Moroccan population in 2020 to 15.4% by 2030.

Morocco's strategy on old age may be recent, and as a result, the term first emerged in April 2002, in the national report on aging by Solidarity's Minister of Employment, Vocational Training, and Social Development [20]. Many social welfare institutions have been established, but age dimensions have not been taken into account because the elderly are treated on an equal footing with other people such as the disabled and the homeless. Health care systems that have not been oriented from the start to the myriad of health problems and long-term care needs of the elderly, as well as to disease prevention, must respond to the new demographic reality and associated changes in population health. Despite the growing prevalence of older adults and Morocco's commitment to providing accessible health care, little is known about the factors influencing older adults' health-seeking behaviors and the gender differences in these behaviors.

The main goal of this study is to investigate gender differences in indicators associated with the socioeconomic status, health status, and access to health care of the elderly population of the El Jadida region in Morocco, with the goal of making recommendations and proposals for implementing a health care policy for this population category of population.

MATERIAL AND METHODS

The study was based on a sample of 537 elderly individuals (136 women and 401 men) aged, 60 years old and over, of both genders, who visited public health centers in El Jadida, a Moroccan agricultural province.

The survey was conducted by teams of professionals (one anthropometrist and one interviewer) who had been trained in standardized interviews and anthropometric measurements. The sample recruitment was based on the person's age. Individuals with confirmed Alzheimer's disease and other cognitive disorders that could jeopardize the procedures, as well as those who could not understand the written formal consent, were excluded from participating in the study. The study protocol has been accepted by the dedicated Moroccan authority of ministry of health.

A structured questionnaire was used to collect socio-demographic and health information during a face-to-face interview. Individual and household characteristics such as date of birth, gender, rural/urban residence, education, marital status, number of children, marital status, working status, and disease background (diagnosed chronic disease and reported health problem) were included in the questionnaire. For analysis purposes, age is divided into four categories for analysis: 60 to 69 years, 70 to 79 years, and 80 years and older.

Self-reports were used to collect data on health issues. Respondents were asked if they had any of the following medical health issues: visual impairments, hearing impairments, memory problems, or oral problems.

We have set aside a section of the questionnaire to assess the quality of life of these elderly people based on their health insurance coverage, the type of medical consultation, and the nature of the stress (probable anxiety, depression, fatigue, insomnia, discomfort, melancholy).

Body mass index (BMI) was calculated by dividing body weight (kg) by the square of height (m), and WHO cutoff standards for overweight (BMI≥25) and obesity (BMI≥30) were used as indices of general obesity [43].

To quantify central obesity, a flexible tape was used to measure waist circumference (WC) at the midpoint between the lowest rib and the iliac crest and hip circumference at the greater trochanter to the nearest millimeter. In the analysis, the mean of two different measurements was used. WC is used as a marker for intra-abdominal fat deposition or visceral fat deposition. A waist circumference of 88 cm for women and 102 cm for males is considered as a cardiovascular risk factor [34, 44].

A mercury sphygmomanometer was used to measure blood pressure (BP) in a sitting position after

at least 10 minutes of rest. According to the Adult Treatment Panel III criteria, high blood pressure was defined as a systolic blood pressure of 130 mmHg and/or a diastolic blood pressure of 85 mmHg and/or self-reported treatment of hypertension with antihypertensive medication [13]. Furthermore, to estimate the prevalence of hypertension, blood pressure was measured three times at one-minute intervals, with the average used as the final blood pressure. Furthermore, blood samples were collected after a 12-hour overnight fast; plasma was separated by centrifugation and stored at -90 °C until analysis.

Hyperglycemia was defined as fasting blood glucose levels greater than 6.1 but less than 6.9 mmol/l, and diabetes as levels greater than 7 mmol/l [31]. Tobacco use was considered present in individuals who reported smoking up until the day of the interview. Alcohol consumption was measured using yes/no questions about consuming alcoholic beverages. All statistical analyses were carried out using the Statistical Package for the Social Sciences (SPSS Inc., Chicago, IL, USA) version 23.0. The data was descriptively analyzed by calculating absolute (n) and frequency values (percent), arithmetic mean, and standard deviation (SD). The categorical variables are expressed as percentages. The Chi-square test was used to examine relationships between different groups and variables. *Tukey's* test was used to compare the means of the variables. A p-value of less than 0.05 was considered statistically significant.

RESULTS

Table 1 shows that the participants' average age was 68.45 4.34 years old, with significant gender differences. The most of them were men (74.7%), in their 70s (58.1%), literate (81.5%), retired (70.3%), and married (70.3%). (94.8%). Men were, on average, older than women (69.9 ± 3.43 years).

As shown in Table 2, the majority of participants had health insurance (70.4%). Men (77.6%) had greater access to health insurance than women (49.3 percent) ($p<0.001$). Half of the participants (56.1%) went to a general practitioner, while only 16% went to a specialist. The proportions of people experiencing stress as melancholy/discomfort, insomnia, anxiety/depression, and tiredness were 26.8%, 25.5%, 16.6%, and 23.3 %, respectively. Men are more vulnerable to anxiety/depression and insomnia than women, while the latter are more tired and uncomfortably so.

Table 3 shows that 6.7% of participants were current smokers and 10.1% consumed alcohol in the 30 days preceding the survey. The perception of their self-rated health status as bad was declared by 35% of those who used health services; these were primarily retired people at the time of the survey. Only 8.8% of those polled thought their health was in good shape.

According to the BMI classes, none of the participants are underweight. The majority of participants (49.3%) were classified as "overweight," while 24.2% were classified as "obese". Obesity is diagnosed in half of the women studied.

There were significant differences in self-rated health status, BMI status, smoking status, and alcohol consumption between men and women ($p< 0.05$); men have poorer perceptions of their health, consume more alcohol, and smoke more than women. Men, like women, tend to gain weight, whereas women are more likely to be obese.

Among the diagnosed chronic diseases, hypertension was the most frequently reported (66.1%), more so in men (75%), followed by central obesity (25.3%), and total obesity (25.3%) (24.2%). Memory problems (65.7%), visual impairments (50.7%), and oral problems were the most common health issues (46.4%). A very small number of cancer cases were also reported. Except for diabetes mellitus, there are significant differences in the reporting of all diagnosed chronic diseases and health problems by gender, with women reporting a higher proportion. Women were significantly more likely than men to report "total obesity," "central obesity," "visual and hearing impairment," and "memory and oral problems."

As shown in Table 4, comorbid conditions were also common in the studied age category. Based on self-reported data, 93.7% of the participants reported at least one health problem experienced during the 3 months prior to the interview; 14.7% of respondents had one, 21.6% had two, and 57.4% had three or more of the selected chronic conditions. On average, approximately three health problems per person were reported, with men reporting more health problems than women ($p<0.05$) and urban areas having more than rural areas of residence (4 vs 2.8 per person; $p<0.05$).

A stratified analysis by area of residence and gender revealed that older men in the urban area reported a greater number of problems, with a higher proportion claiming to have four or more health problems than their counterparts in the rural region. Rural women reported more health problems and chronic diseases than urban women. The study also found that older people who have a negative perception of aging have 2.5 times more health problems and are more likely to have more than three chronic diseases.

DISCUSSION

As the elderly population grows, the debate over how to maintain their physical and mental health,

Table 1. Main socio-demographic characteristics of elderly people according to gender

Variables	% Total (n=537)	Men (n=401)	Women (n=136)	p-Value
Age (mean ± SD)	68.45 ± 4.34	69.9 ± 3.43	64.17 ± 3.92	0.000
Age range (years)				0.000
60	53.8	41.9	89	
70	45.1	56.6	11	
Over 80	1.1	1.5	0	
Area of residence (%)				0.535
Rural area	53.6	53.6	53.7	
Urban area	46.4	46.4	46.3	
Years of schooling (%)				0.000
Illiterate	27	18.5	52.2	
Literate	73	81.5	47.8	
Marital status (%)				0.008
Married	92.9	94.8	87.5	
Single/Divorced/widowed	7.1	5.2	12.5	
Family structure (%)				0.081
Small family	43	41.1	48.5	
Extended family	57	58.9	51.5	
Occupation (%)				0.000
Unemployed/Housewife	13.6	0	53.7	
Active	22.9	29.7	2.9	
Retired	63.5	70.3	43.4	
Number of children (%)				0.106
2 children and less	30	28.4	34.6	
More than 3 children	70	71.6	65.4	

The mean difference is significant at *p<0.05. Data are expressed as Mean ± SD (standard deviation).

Table 2: Health Services utilization among elderly people according to gender

Variables	% Total (n=537)	Men (n=401)	Women (n=136)	p-Value
Active health insurance				0.000
No	2.6	22.4	50.7	
Yes	70.4	77.6	49.3	
Type of medical consultation				0.5481
general doctor	56.1	57.9	50.7	
Specialist doctor	16.4	16.2	16.9	
Pharmacist	10.1	9.5	11.8	
Traditional medicine	17.5	16.5	20.6	
nature of the stress				0.000
Anxiety/Depression	16.6	21.7	1.5	
Tired	23.3	14.5	49.3	
Insomnia	25.5	31.9	6.6	
Irritation any type	7.8	10	1.5	
melancholy/Discomfort	26.8	21.9	4.2	

The mean difference is significant at *p<0.05.

Table 3. Health status and chronic disease prevalence in both genders

Variables	%Total (n=537)	Men (n=401)	Women (n=136)	p-Value
Self-rated health status				0.000
Good	8.8	10.7	2.9	
Normal	56.6	51.6	71.3	
Bad	34.6	37.7	25.7	
BMI (mean ± SD)	27.15 ± 3.55	26.30 ± 2.84	29.63± 4.22	0.000
Category BMI (kg/m^2)				0.000
Underweight (BMI < 18.5)	0	0	0	
Normal weight (BMI 18.5 to 24.99)	26.4	28.7	19.9	
Overweight (BMI 25 to 29.99)	49.3	55.4	31.6	
Obese (BMI ≥ 30)	24.2	16	48.5	
Tobacco use				0.002
Non-smoker	93.3	91.5	98.5	
Current smoker	6.7	8.5	1.5	
Alcohol consumption				0.000
No	89.9	87	98.5	
Yes	10.1	13	1.5	
Diagnosed chronic disease				
Hypertension	66.1	75.6	38.2	0.000
Diabetes mellitus	14.9	16.2	11	0.09
Total obesity	24.2	16	48.5	0.000
Central obesity	25.3	16.7	50.7	0.053
Reported health problem				
Visual impairments	50.7	46.9	61.8	0.002
Hearing impairments	34.3	41.6	12.5	0.000
Memory problems	65.7	58.4	87.5	0.000
Oral problems	46.4	39.2	67.6	0.000

The mean difference is significant at *p<0.05. BMI: body mass index. Data are expressed as Mean ± SD (standard deviation).

Table 4. Prevalence of chronic conditions, by socio-demographic and perception of health status

	Mean health problems (Mean ± SD)	p-Value	Number of chronic conditions (%)				p-Value
			0	1	2	≥ 3	
Total	3.35 ± 2.07		6.3	14.7	21.6	57.4	
Age range		0.000					0.000
60s	2.69 ± 1.68		8.7	17.3	26.3	47.8	
70s	3.16 ± 1.16		3.7	11.6	16.5	68.2	
Over 80	4.14 ± 2.23		0	16.7	0	83.3	
Gender		0.036					0.000
Men	3.46 ± 2.09		3.2	15	25.2	56.6	
Women	3.02 ± 2.00		15.4	14	11	59.6	
Area of residence		0.000					0.000
Urban area	3.99 ± 2.39		8.4	12.4	11.2	67.9	
Rural area	2.79 ± 1.55		4.5	16.7	30.6	48.3	
Self-rated health status		0.000					0.000
Good	1.89 ± 0.75		2.1	25.5	55.3	17	
Normal	2.6 ± 1.85		10.2	20.7	26.3	42.8	
Bad	4.9 ± 1.7		1.1	2.2	5.4	91.4	

The mean difference is significant at *p<0.05. Data are expressed as Mean ± SD (standard deviation).

independence, and well-being, as well as their financial security, is becoming more heated [37].

In Morocco, a demographic transition is underway, with people aged 60 and over accounting for 8.1% of the population, up from 7.2% in 1962, according to the General Population and Housing Census of 2004. With the projected decline in fertility and future lengthening of the lifespan, the country will face an increasing burden from this age group. This proportion of the elderly would rise from 11.5% in 2020 to 15.4 % by 2030 [16].

The data presented on the present study population are part of a large cross-sectional study conducted at the household level on a total sample of 1019 participants in the El Jadida region. This information relates to sociodemographic, health, and nutritional status. People over the age of 60 constituted 52.7% of the population studied. This rate indicates that aging is more prevalent in this region than the national average, which was 9.4% in 2014 [17]. This current cross-sectional study is one of the few among Moroccan elderly people.

In terms of socioeconomic status, both men and women have a stronger relationship between socioeconomic status and wellbeing as a result of social integration and functional capacity building [1, 27]. Many women face poverty in old age as a result of inequalities in income, education, and employment throughout their lives, which are aggravated by limitations on pension benefits and a lack of control over financial resources. In comparison to their male counterparts, older Moroccan women experience a number of disadvantages, including higher rates of illiteracy (47,8%), unemployment (53,7%), increased rates of widowhood and living alone, and a reduced likelihood of receiving formal pension benefits. Previous research undertaken in low- and middle-income countries has supported these findings [7, 15, 46].

The rate of illiteracy remains lower than that recorded in Africa (78%) and Asia (53%). The same observation can be made about the average levels of illiteracy for older men, which also remains lower than in Africa and Asia (58% and 29%, respectively [39]. Literacy opens up more possibilities in terms of information. Employment options, and hence financial status and regular income, are influenced by education.

Women are less likely than men to hold positions of power, have job security, authority, autonomy, and advancement opportunities. Even educated women's access to authority, autonomy, available, albeit limited, public pensions, and opportunities for advancement has been restrained by low employment rates [32, 36]. Nevertheless, because older Moroccan women and men are more likely to live in child-headed households, intergenerational co-residence is more frequent in Moroccan families. This solution may allow them to break the cycle of isolation, maintain a sense of usefulness, and reduce feelings of incapacity, improve their quality of life, maintain physical and motor activity, and thus avoid the psychological complications of aging, such as withdrawal, distrust, isolation, and loneliness, which can lead to mental and physical pathology. Furthermore, previous research has shown that older women are more likely to be living alone, single, or widowed in developing countries [40, 42]. For these women, the social and economic consequences of widowhood may be more severe.

The study also found that the percentage of older women who are socially isolated is considerably higher than the percentage of older males, given the fact that older men are more likely than women to remarry following divorce or widowhood. As a result, older women frequently refuse to remarry since social systems do not favor older people marrying, and they are afraid of social pressure, public judgment, insults, and even humiliation [33, 35].

On the other hand, one's health status can have a significant impact on one's happiness and quality of life. The majority of those in the study had health insurance. Although this rate is higher than that reported by the National Health Insurance Agency (70% vs. 26% of the Moroccan population) [17], it should be noted that half of the older women, who are more vulnerable to poverty, do not have medical insurance and will have more difficulties in accessing health care than men.

In fact, the place of residence can be viewed as the primary entry point or barrier to receiving needed health care [19]. In terms of place of residence and gender, nearly 53% live in rural areas because they rely on agricultural activities, compared to 41% at the national level. This figure was reduced to account for the urbanization rate, which increased from 29.1% in 1960 to 60.3% in 2014 [17].

Gender disparities in self-rated health exist in almost all countries and across geographical regions, to the detriment of women [9]. This research showed a higher proportion of the elderly with poor self-rated health in urban areas than in rural areas. In contrast, previous research has found that rural residents have lower health status, quality of life, socioeconomic status, and a higher unemployment rate than city dwellers, which reduces access to health care and good nutrition [5, 12, 18].

In this study, the majority of elderly people had multiple chronic diseases and health problems, with a higher prevalence and significant difference between women and men. Although some local, regional, or national studies have already reported the prevalence rates of one or more chronic diseases in Morocco [3, 28, 30], this study provides a primary overview of

the scope of the most chronic diseases in a sample of Moroccan elderly people in this region.

Our study data revealed that living in an urban area is associated with chronic conditions, which is consistent with previous findings [38, 45]. This higher prevalence in urban areas compared to rural areas may also be associated with a higher prevalence of combined risk factors such as dietary changes, physical inactivity, and obesity, confirming the effect of urbanization as one facet of the global transition occurring in Morocco. Furthermore, the low proportion observed in rural areas could be due to a lack of access to diagnosis [6]. Nevertheless, the use of care in this category of the population is greater than in people under 60 years old. This margin is even more pronounced for people affected by at least one chronic disease. In this age group, the coexistence of three chronic diseases is common, regardless of gender or location. Chronic diseases are a considerable financial burden on health insurance; in 2015, they accounted for 48.2% of total cost for a population with at least one chronic condition (2.8%) [14].

Depression was mentioned as another health issue in this study's participants. In fact, one out of every four Moroccans will experience a major depressive episode at some point in their lives, with significantly higher rates among women and in urban regions [2]. In our study, we observed that elderly men have a significant prevalence of depression, anxiety, insomnia, and discomfort. Negative affective symptoms, especially the prevalence of depression symptoms, are becoming a major issue as people become older, contributing considerably to a decrease and degradation in elders' quality of life and perhaps being a risk factor for physical problems [10, 21, 22, 23, 24]. Our work discusses the issue of the elderly population's quality of life decline, as it has in other nations with varied levels of transition trends and using different approaches [11, 29]. The information obtained could be used as a baseline for future research on this age group.

CONCLUSIONS

Gender gaps exist in socio-economic, health, well-being and psychological status. Our study indicated to the problem of decreased the quality of life and well-being in the elderly population. The data obtained might be a reference for future studies on this age group of the population.

Acknowledgments

The authors would like to express their gratitude to all of the participants for their time and support.

There was no external support for this publication. The final version of the manuscript was approved by all authors.

Conflict of interest

There are no conflicts of interest to record for the authors.

REFERENCES

1. *Angelini V., Cavapozzi D., Corazzini L., Paccagnella O.* : Age, health, and life satisfaction among older Europeans. Soc Indic Res 2012;105:293–308. doi: 10.1007/s11205-011-9882-x
2. *Asouab F., Agoub M., Kadri N., Moussaoui D., Rachidi S., Tazi MA., Toufiq J., Chaouki N.* : Prevalences of mental disorders in the general moroccan population. D.E.L.M. bulletin épidémiologique 2005;1–7. (In French).
3. *El Ayachi M., Mziwira M., Vincent S., Defoort C., Portugal C., Lairon D., Belahsen R* .: Lipoprotein profile and prevalence of cardiovascular risk factors in urban Moroccan women. Eur J Clin Nutr ; 2005;59:1379–1386. doi: 10.1038/sj.ejcn.1602251
4. *Baali A., Lahmam A., Amor H., Aboussad A., Boestch G., Chapuis-Lucciani N.* : Colloquiums of the Group of Anthropologists of the French Language (GALF) Perception of aging, state of health and socio-demographic environment of a group of elderly people Marrakech, Morocco. 2012.
5. *Baernholdt M., Yan G., Hinton I., Rose K., Mattos M.* : Quality of Life in Rural and Urban Adults 65 Years and Older: Findings From the National Health and Nutrition Examination Survey. J Rural Heal 2012;28:339–347. doi: 10.1111/j.1748-0361.2011.00403.x
6. *Belahsen R.* : Nutrition transition and food sustainability. In: Proceedings of the Nutrition Society. Cambridge University Press 2014;385–388.
7. *Benksim A., Ait-Addi R., Khalloufi E., Habibi A., Amine M., Cherkaoui M.* : Chronic Diseases, Depressive Symptoms and Socio-economic Characteristics Among Older Adults in Morocco: A pilot Study on Gender Differences. Eur J Geriatr Gerontol 2020;2:18–23. doi: 10.4274/ejgg.galenos.2020.274
8. *Bloom DE., Chatterji S., Kowal P., Lloyd-Sherlock P., McKee M., Rechel B., Rosenberg L., Smith JP.*: Macroeconomic implications of population ageing and selected policy responses. Lancet 2015;385:649–657.
9. *Boerma T., Hosseinpoor AR., Verdes E., Chatterji S.*: A global assessment of the gender gap in self-reported health with survey data from 59 countries. BMC Public Health 2016;16. doi: 10.1186/S12889-016-3352-Y
10. *Bryła M., Burzyńska M., Maniecka-Bryła I.* : Self-rated quality of life of city-dwelling elderly people benefitting from social help: Results of a cross-sectional study. Health Qual Life Outcomes 2013;11:181. doi: 10.1186/1477-7525-11-181
11. *Campolina AG., Lopez RVM., Nardi EP., Ferraz MB.* : Quality of life in a sample of Brazilian adults using the generic SF-12 questionnaire. Rev. Assoc. Med. Bras 2018;64:234–242.
12. *Chen SH., Cheng HY., Chuang YH., Shao JH.* : Nutritional status and its health-related factors among

older adults in rural and urban areas. J Adv Nurs 2015;71:42–53. doi: 10.1111/jan.12462
13. *Cleeman JI.:* Executive summary of the third report of the National Cholesterol Education Program (NCEP) expert panel on detection, evaluation., and treatment of high blood cholesterol in adults (adult treatment panel III). J Am Med Assoc 2001;285:2486–2497. doi: 10.1001/jama.285.19.2486
14. Compulsory Health Insurance. Annual report global 2015. Available http://www.anam.ma/wp-content/uploads/2015/06/RAG2018-VF.pdf.
15. *Dong XQ., Chen R.* : Gender differences in the experience of loneliness in U.S. Chinese older adults. J Women Aging 2017;29:115–125. doi: 10.1080/08952841.2015.1080534
16. HCP High Commissioner of Plans. National survey on the elderly in Morocco. Investigation report 2011.
17. HCP High Commissioner of Plans. Social indicators of Morocco 2013-2014.
18. *Van Der Hoeven M., Kruger A., Greeff M.*: Differences in health care seeking behaviour between rural and urban communities in South Africa. Int J Equity Health 2012;11. doi: 10.1186/1475-9276-11-31.
19. International Labour Office. *:* Global evidence on inequities in rural health protection. New data on rural deficits in health coverage for 174 countries. 2015.
20. *Jacquet I.*: Old age in Morocco, Academia-B 2012. Available https://www.editions-academia.be/livre-la_vieillesse_au_maroc-9782872099399-38973.html
21. *Kabátová O., Uríčková A., Botíková A.* : Factors affecting the incidence of depression in the elderly. Cent Eur J Nurs Midwifery 2014;5:105–111. doi: 10.15452/CEJNM.2014.05.0004
22. *Khalatbari-Soltani S., Marques-Vidal P., Imamura F., Forouhi NG.* : Prospective association between adherence to the Mediterranean diet and hepatic steatosis: The Swiss CoLaus cohort study. BMJ Open 2020;10. doi: 10.1136/bmjopen-2020-040959
23. *Kim H., Thyer BA., Munn JC. :* The relationship between perceived ageism and depressive symptoms in later life: Understanding the mediating effects of self-perception of aging and purpose in life, using structural equation modeling. Educ Gerontol 2019;45:105–119. doi: 10.1080/03601277.2019.1583403
24. *Layte R., Sexton E., Savva G.* : Quality of life in older age: Evidence from an Irish cohort study. J Am Geriatr Soc 2013;61. doi: 10.1111/jgs.12198
25. *Linardakis M., Papadaki A., Smpokos E., Kafatos A., Lionis C.* : Prevalence of multiple behavioral risk factors for chronic diseases in medical students and associations with their academic performance. J Public Heal 2020;28:383–392. doi: 10.1007/s10389-019-01030-2
26. *Lutz W., Sanderson W., Scherbov S.* : The coming acceleration of global population ageing. Nature 2008;451:716–719. doi: 10.1038/nature06516
27. *Meggiolaro S., Ongaro F. :* Life satisfaction among older people in Italy in a gender approach. Ageing Soc 2015;35:1481–1504. doi: 10.1017/S0144686X14000646
28. *Mizouri R., Sebai I., Boukhayatia F., Zahra H., Khiari M., Zribi S., Othman Rym R Ben., Mahjoub F., Berriche O., Jamoussi H.* : Arterial hypertension and diabetes association in the elderly. Arch Cardiovasc Dis 2019;Suppl 11:e337. doi: 10.1016/j.acvdsp.2019.05.014
29. *Montazeri A., Vahdaninia M., Mousavi SJ., Asadi-Lari M., Omidvari S., Tavousi M. :* The 12-item medical outcomes study short form health survey version 2.0 (SF-12v2): A population-based validation study from Tehran, Iran. Health Qual Life Outcomes 2011;9. doi: 10.1186/1477-7525-9-12
30. *Mziwira M., Ayachi M El., Lairon D., Belahsen R.* : High blood pressure in urban Moroccan women from an agricultural region. Med J Nutrition Metab 2011;4:111–116. doi: 10.1007/s12349-010-0047-2
31. *National Agency for Accreditation and Health Evaluation.* : Methods of screening and laboratory diagnosis of dyslipidemia in primary prevention. J Mal Vasc 2000;7:226–230. doi: AE-02-2001-62-1-C1-0003-4266-101019-ART7.
32. OECD (Organisation for Economic Co-operation and Development): The Pursuit of Gender Equality, An Uphill Battle. OECD iLibrary 2017.
33. *Oh S.* : Remarried men and remarried women: How are they different? J Divorce 1987;9:107–113. doi: 10.1300/J279v09n04_08
34. *Olinto M., Nacul L., Gigante D., Costa J., Menezes A., Macedo S.*: Waist circumference as a determinant of hypertension and diabetes in Brazilian women: a population-based study. Public Health Nutr 2004 ;7:629–635. doi: 10.1079/phn2003582.
35. *Osmani N., Matlabi H., Rezaei M.* : Barriers to Remarriage Among Older People: Viewpoints of Widows and Widowers. J Divorce Remarriage 2018;9:51–68. doi: 10.1080/10502556.2017.1375331
36. *Proctor BD., Semega JL., Kollar MA.* : Income and poverty in the United States: 2015, Current Population Reports. U.S. Census Bureau 2016;60-256.
37. *Puvill T., Lindenberg J., De Craen AJM., Slaets JPJ., Westendorp RGJ.*: Impact of physical and mental health on life satisfaction in old age: a population based observational study. BMC Geriatr 2016;16:1–9. doi: 10.1186/s12877-016-0365-4
38. *Song H., Feng D., Wang R., Yang J., Li Y., Gao J., Wang Z., Yan Z., Long C., Zhou J., Feng Z.*: The urban-rural disparity in the prevalence and risk factors of hypertension among the elderly in China - a cross-sectional study. PeerJ 2019. doi: 10.7717/peerj.8015.
39. United Nations - Population Division Department of Economic and Social Affairs. Between Gender and Ageing The Status of the World's Older Women and Progress since the Madrid International Plan of Action on Ageing. UN Women Coordination Division, 2012.
40. United Nations - Population Division Department of Economic and Social Affairs. : World Population Ageing 2017. In: United Nations New York. Available https://www.un.org/en/development/desa/population/publications/pdf/ageing/WPA2017_Highlights.pdf. Accessed 27 Jan 2022
41. *Wan He., Goodkind D and Kowal P.* : An Aging World: 2015. U.S. Department of Health and Human Services, National Institute On Aging 2016.
42. *West LA., Cole S., Goodkind D., He W.* : 65+ in the United States: 2010. US Cencus Bureau 2014;23–212.

43. World Health Organization. Obesity: preventing and managing the global epidemic : report of a WHO consultation 2000. Available https://apps.who.int/iris/handle/10665/42330
44. World Health Organization. Obesity: preventing and managing the global epidemic. World Health Organization 2003.
45. *Wu F., Guo Y., Kowal P., Jiang Y., Yu M., Li X., Zheng Y., Xu J.* : Prevalence of Major Chronic Conditions among Older Chinese Adults: The Study on Global Ageing and Adult Health (SAGE) Wave 1. PLoS One 2013 ; 8:e74176. doi: 10.1371/journal.pone.0074176
46. *Yount KM., Sibai AM. :* Demography of Aging in Arab Countries. In: International Handbook of Population Aging. Springer Netherlands 2009;277–315.

Received : 28.04.2022
Accepted : 09.08.2022

Rocz Panstw Zakl Hig 2022;73(3):351-364
http://wydawnictwa.pzh.gov.pl/roczniki_pzh/ https://doi.org/10.32394/rpzh.2022.0215

ORIGINAL ARTICLE

HEALTH STATUS AND FACTORS INFLUENCING ACCESS TO HEALTHCARE SERVICES BY WORKERS IN PETROL STATIONS IN RAYONG PROVINCE, THAILAND

Anamai Thetkathuek[1], *Chan Pattama Polyong*[2]

[1]Department of Industrial Hygiene and Safety, Faculty of Public Health, Burapha University, Chonburi, 20131, Thailand
[2]Occupational Health and Safety Program, Faculty of Science and Technology, Bansomdejchaopraya Rajabhat University, Bangkok, 10600, Thailand

ABSTRACT

Background. Petrol station (PS) workers are categorized as "unskilled labor", which leads to low wages, economic instability, and a lack of adequate access to healthcare services (HCS) despite exposure to dangerous chemicals. A lack of information on the HCS access of PS workers is concerning.

Objective. This study was undertaken to elucidate factors that influenced access to health care for fuel station workers in gas stations in Rayong province, Thailand.

Material and methods. Two-hundred PS workers (100 serving at fuel dispensers and 100 working at other locations) were interviewed to evaluate their health conditions and factors affecting HCS access. The study cohort comprised 137 (68.5%) women and 63 (31.5%) men. The mean age was 30.29±10.97 years and the average monthly salary was 341.16 ± 124.72 USD.

Results. The average distance between a respondent's residence and government hospital was 10.49 ± 8.571 km. Most respondents (63.5%) were in good physical health, and 79.5% reported having "positive mental health". However, 73% reported neurological disorders and 57% reported respiratory symptoms. During the previous 12 months, 44.5% of respondents had HCS access; 80.9% of participants reported that they had accessed HCS to obtain treatment for an illness, and 18% went for a health checkup. Government centers were rated as "satisfactory" by 78.8% of PS workers, and 75.5% received medical coverage under a social-security scheme. Moreover, 38.5% obtained HC information from their colleagues. HCS access was attributed to the cost of receiving HCS as well as the sex, marital status, illness severity of PS workers.

Conclusions. Workers should have access to comprehensive HCS. Policy frameworks and systems for HCS should take account of employees' sex, marital status, and the type and severity of their illnesses.

Key words: *health status, health services, gas stations, Eastern Economic Corridor, Thailand*

INTRODUCTION

Because they fuel ever-increasing numbers of vehicles of various types, petrol attendants in petrol stations (PS) contribute to the economy of Thailand [1]. However, categorization of this occupational group as "unskilled labor" leads to low wages, economic instability, and inadequate social welfare, as well as reduced access to healthcare services (HCS) [2]. Access to primary HC is one of the most pressing issues confronting developing countries, including Thailand [3]. Although individual PS have been reported to employ an average of ~7 people, their skills have not been utilized appropriately [4]. Timely access to HCS has been found to reduce morbidity and mortality due to preventable diseases [3].

HCS access is particularly important for PS workers because they are exposed to a wide array of hazards [3, 5], including physical, chemical, and biological hazards, as well as stress [6, 7, 8, 9, 10]. Only 14.6% of PS employees experience safe conditions while working [11]. Chemical hazards to which these workers may be exposed to regularly include substances containing benzene, toluene, ethylbenzene, and xylene (BTEX). Inadequate protective gear and very close proximity to the fuel head can have adverse acute and chronic effects upon health, including neurological effects [8, 12]. BTEX can cause headaches and dizziness [11,12],

Corresponding author: Anamai Thetkathuek, Department of Industrial Hygiene and Safety, Faculty of Public Health, Burapha, University, Chonburi, 20131, Thailand, Tel.: 66-1-6921013, e-mail: anamai@buu.ac.th

respiratory symptoms (e.g., coughing and throat irritation), and irritation to the eyes and skin (e.g., itching) [13]. BTEX compounds are likely responsible for long-term chronic health effects, including blood-related diseases (e.g., myeloid leukemia and aplastic anemia), potentially irreversible damage to the kidneys, cardiovascular disorders, or nervous-system disruptions. Benzene is especially dangerous, being classified as carcinogenic to humans (IARC group 1) [14]. The World Health Organization (WHO) has reported that exposure to benzene (1.7 $\mu g/m^3$) may be associated with an excess lifetime risk of leukemia of 10 people per million [15]. Thus, there is an urgent need for the provision of adequate HCS access for PS workers, but this is a complex concept.

The importance of service distribution is reflected in measurement of the performance of HCS access [16, 17]. After passing of the National Health Security Act B.E. 2002, progress has been made in the protection of human rights in public health in Thailand. Almost all Thai citizens are entitled to receive medical care based on their rights in one of three systems: the welfare of civil servants, the social security system, and universal health insurance system. They also have the right to receive services in case of accidents or emergencies without having to reserve money in advance (Universal Coverage for Emergency Patient Project) [18].

The conformity of HCS into geographic, economic, or social dimensions permits operational measurements of HCS access [16]. Economic development in Rayong Province (RP) may be one of the factors influencing HCS access. The cost of medical services is quite high, which may affect people on a low income [16]. The HC utilization is conceptual and aims to demonstrate the factors that lead to HCS use. The first factor is population characteristics. This factor can be broken down into predisposing factors (sex, age, education), social structure (e.g., education, occupation, beliefs), resource factors (e.g., income, health insurance, the ability to pay various expenses, distance from residence to the healthcare establishment, channels for receiving healthcare information) [19], and healthcare needs (e.g., severity of existing disease). The second major factor is environmental (i.e., the social, economic, and political) factors affecting HCS access. The third major factor is health behavior (e.g., exercise, smoking, and initial self-treatment) affecting HCS access [20, 21, 22]. Moreover, the degree of coordination, efficiency, and quality of care are key factors influencing the satisfaction of patients relating to HCS access [17].

Previously, scholars have focused only at HCS access by migrant workers [23, 24, 25]. However, information on the health status and factors affecting HCS access among PS workers is lacking. Insights emerging from such studies can aid development of HCS targeting this specific class of workers. Acknowledging this knowledge gap, we investigated the health conditions and factors influencing HCS access among PS workers in RP.

MATERIALS AND METHODS

Ethical approval of the study protocol

The study protocol was approved by the Human Research Committee of Burapha University (Chonburi, Thailand). Protecting the rights of study participants was an important concern of our study. The research objectives were explained to all PS workers, who were free to choose whether to participate in this study. Coercion was not applied, and PS workers were free to withdraw from the trial at any time. Inclusion criteria People were included in our study if they: (i) were aged 18–60 years; (ii) could read, understand conversations, and write in Thai; (iii) had never been diagnosed with a blood disease.

Study design

This cross-sectional study was carried out in RP (located 179 km from Bangkok) from October 2020 to May 2021. The study cohort was 200 people who worked in PS in RP. According to our survey, we found that the PS in RP had similar tasks: fuel services, cashier-based work, as well as working in offices, coffee shops, restaurants, and convenience stores. Therefore, the workers in each PS were homogeneous. We employed a cluster random sampling method according to the inclusion criteria stated above. If the required number of individuals was not obtained, then data were collected from all of the workers who volunteered at that PS. Then, the next PS was chosen and the process continued until the projected sample size was obtained.

Determination of the sample size

The sample size was determined using a formula applied to populations in previous surveys conducted in Thailand [4]. Each PS had an average of seven employees. The level of confidence was determined to be 1.96 at 95%, with a 5% margin of error for each of the 100 PSs. Moreover, 76.4% of Asian laborers have been reported to have HCS access (p=0.764) [28]. The calculated sample size was 198.70. Two-hundred PS employees were selected: 100 who worked at fuel dispensers and 100 who served outside fuel dispensers.

Research instruments and methods of data collection

The research instrument used to collect data in this study was a questionnaire consisting of four sections.

Part one was based on demographic characteristics and health behavior (seven questions). It focused on

sociodemographic characteristics: age, sex, marital status (single/married/widowed, other), highest education level attained (below secondary school/ above secondary school or the equivalent), individual income, smoking habits, and alcohol consumption. The responses to these questions entailed scoring options and filling in words in the questionnaire.

Part two was based on health status and needs (15 questions). It focused on physical and mental health conditions in the past few years. Respondents selected scoring options for physical illness, mental illness, and their severity (healthy/minor illness/severe illness) and filled-in words. Questions related to: sources of HCS access; whether workers could continue working in the previous 12 months if they were slightly ill; the location at which they were most likely to receive HCS if they were seriously ill or unable to continue working in the previous 12 months; HCS access during the 12 months; treatments received for illnesses.

Part three was based on enabling factors (eight questions with multiple sub-questions). This covered: monthly income (in USD); the cost of receiving HCS each time (in USD); inability to pay (payments in installments/pay what you can/contact social services/ borrow from employers or others); distance from residence to HC establishment where services are received regularly (in km); channels for receiving information on HC and ratings of welfare rights relating to HCS access; satisfaction levels regarding a government facility where treatment was provided.

Part four was based on HCS access (two questions). The focus was on whether PS employees had HCS access in a medical facility at least once in year (yes/ no).

Data collection

Data were collected between October and November 2020. At the onset, a memorandum was sent to PS managers, and they were asked for permission to collect data at each PS. After receiving permission to collect the data, research assistants were trained to provide them greater understanding of the questions. The managers and coordinators at each PS were asked subsequently to collect the data. Finally, each respondent was interviewed, with each interview in the office area of each PS lasting 15 min.

Statistical analyses

After checking the accuracy of the data, the data were coded and saved on a personal computer. Statistical analyses were undertaken using SPSS 20 (IBM, Armonk, NY, USA). The statistical analysis consisted of two parts. The first part contained descriptive statistics (frequencies, percentages, means, standard deviations, medians, minimum values, and maximum values) presented in tabular form. The second part comprised inferential statistics obtained by univariate logistic regression analysis to determine the correlation between each variable and HCS access. Factors that were significant upon univariate analysis were entered into a multivariate logistic regression model. Independent variables that were associated with HCS access (including sex, age, marital status, levels of minor and severe illnesses, cost of HCS access, distance from residence to HC establishment) were analyzed as adjusted odds ratios (ORs) and 95% confidence intervals (CIs). $p < 0.05$ was considered significant.

Critique of research findings

After the report had been written, a meeting was held with experts to hear their opinions and make corrections before writing the final report. The researcher presented the study results to 15 experts (physicians, occupational-health experts, safety engineers, epidemiologists, statisticians, researchers, toxicologists, occupational-health nurses, personnel from the Ministry of Health, and information-technology personnel). Everyone was provided an opportunity to make suggestions. Each session took ~120 min. Subsequently, the report was amended based on the advice of these experts.

RESULTS

The results relating to the sociodemographic characteristics and health behavior of PS workers (Table 1) showed that they were more female workers (68.5%) than male workers, and that the mean age (± SD) was 30.29 ± 10.97 years. Most employees were younger than 30 years, with 57% having an educational status of high school or above. Most respondents were non-smokers (66%) and 54.5% consumed alcohol (Table 1). Our assessment focusing on health conditions revealed that 63.5% and 79.5% of respondents were physically and mentally healthy, respectively. Among the 65% of respondents who had had a mild illness, 43.1% sought treatment at a government hospital and 19.2% at a private hospital. Also, 32.0% of respondents had experienced a serious illness. However, of respondents who had experienced a serious illness, most (57.8%) received HCS at a government hospital. If respondents received HCS in a hospital, 79.5% did not have to pay, and 20.5% had to pay in full. If a PS worker received HCS in a hospital, the cost of receiving this service on each visit was 15.85± 17.52 USD.

Of the respondents who received obtained access to health care service on each occasion with an average distance ± SD.10.49 ± 8.571 kilometers from accommodation to health care facilities, receiving the most information, 38.5 percent of colleagues, followed by family, 32 percent, with welfare rights in

Table 1. Demographic characteristics and health behavior indicating numbers and percentages of workers in gas stations in Rayong Province, Thailand

Demographic characteristics and health behavior factors of respondents	Workers dispensing fuel (n = 100)		Workers not dispensing fuel (n = 100)		Total	
	n	%	n	%	N	%
Demographic factor						
Population						
Sex						
Female	56	56.0	81	81.0	137	68.5
Male	44	44.0	19	19.0	63	31.5
Age (years)						
≥30	35	35.0	51	51.0	86	43
< 30	65	65.0	49	49.0	114	57
Mean ± SD	27.81±9.56		32.77±11.75		30.29±10.97	
Min–Max	18-58		18-66		18-66	
Marital status						
Married/widowed, other	38	38.0	57	57.0	95	47.5
Single	62	62.0	43	43.0	105	52.5
Educational background						
Less than secondary school	39	39.0	25	25.0	64	32
Higher than secondary school (or equivalent)	61	61.0	75	75.0	136	68
Health behavior						
Smoking habit						
Yes	44	44.0	24	24.0	68	34
No	56	56.0	76	76.0	136	66
Alcohol consumption						
Yes	49	49.0	42	42.0	91	45.5
No	51	51.0	58	58.0	109	54.5

Table 2. Needs factor, and enabling factor during the past year within the group handling the fuel dispenser and the group working outside the dispenser area

	Workers dispensing fuel (n = 100)		Workers not dispensing fuel (n = 100)		Total	
	n	%	n	%	n	%
Needs factor						
Physical health						
Good physical health	66	66.0	61	61.0	127	63.5
Slight illness	33	33.0	39	39.0	72	36.0
Serious illness	1	1.0	0	0.0	1	0.5
Mental health						
Good mental health	85	85.0	74	74.0	159	79.5
Slight illness	14	14.0	25	25.0	39	19.5
Serious illness	1	1.0	1	1.0	2	1
Illness within 12 months						
No	67	67.0	60	60.0	127	63.5
Yes	33	33.0	40	40.0	73	36.5
Severity of the illness						
Continued working	19	57.6	30	75.0	49	67.1

	Workers dispensing fuel (n = 100)		Workers not dispensing fuel (n = 100)		Total	
	n	%	n	%	n	%
Time of work for treatment, not require hospitalization	13	39.4	8	20.0	21	28.8
Required hospitalization	1	3.0	2	5.0	3	4.1
Slightly ill but able to continue working in the last 12 months						
No	43	43.0	27	27.0	70	35
Yes	57	57.0	73	73.0	130	65
Places to get treatment when slightly ill (select more than one item)						
Private hospitals	6	10.5	19	26.0	25	19.2
Public hospitals	29	50.9	27	37.0	56	43.1
Clinics	11	19.3	11	15.1	22	16.9
Local *tambol* health hospitals	1	1.8	3	4.1	4	3.1
Drugstores	10	17.5	13	17.8	23	17.7
Severe illness or unable to continue working during the past 12 months						
No	72	72.0	64	64.0	136	68
Yes	28	28.0	36	36.0	64	32
Places visited for treatment when seriously ill (select more than one item)						
Private hospital	4	14.3	11	30.6	15	23.4
Government hospital	19	67.9	18	50.0	37	57.8
Clinic	3	10.7	5	13.9	8	12.5
District health- promotion hospital	2	7.1	0	0.0	2	3.1
Drug store	0	0.0	2	5.6	2	3.1
Enabling factor						
Monthly income (in USD)						
<283	47	47.0	40	40.0	87	43.5
≥283	53	53.0	60	60.0	113	56.5
Mean ± SD	318.24±28.699		364.36± 154.05		341.16± 124.72	
Min–Max	226.44–849.14		141.52–1,132.18		141.52–1,132.18	
Distance from residence to medical care service (in km)	9.91 ± 7.956		11.01 ± 9.114		10.49 ± 8.571	
Non-treatment-related expenses during each visit						
Free of charge	73	73.0	63	63	136	68
Full payment	27	27.0	37	37.0	64	32
Mean ± SD	320.77 ± 488.524		531.62 ± 1613.165		444.60 ± 1272.083	
Channels for receiving information on health care						
Family	33	33.0	31	31.0	64	32
Co-workers	47	47.0	30	30.0	77	38.5
Health officials	11	11.0	23	23.0	34	17
Others	26	26.0	25	25.0	22	25.5
Cost of receiving health services on each visit (in USD)						
Free of charge	83	83.0	76	76.0	159	79.5
Full payment	17	17.0	24	24.0	4	20.5
Mean ± SD	12.93± 17.85		17.83± 17.41		15.85 ± 17.52	
Inability to pay						
No	90	90.0	88	88.0	178	95.7
Yes	3	3.0	5	5.0	8	4.3

	Workers dispensing fuel (n = 100)		Workers not dispensing fuel (n = 100)		Total	
	n	%	n	%	n	%
Not specified	7	7.0	7	7.0	14	
Solution for those unable to pay medical expenses						
Payment by installments	1	33.3	3	60.0	4	56
Borrowing from employers	0	0.0	2	40.0	2	25
Others	2	66.7	0	0.0	2	25
Welfare rights relating to health care						
Life insurance	2	2.0	2	2.0	4	2
Social Security Scheme	81	81.0	70	70.0	151	75.5
Health insurance	2	2.0	5	5.0	7	3.5
Employer	1	1.0	1	1.0	2	1.0
Personal funds	12	12.0	15	15.0	27	13.5
Other	2	2.0	7	7.0	9	4.5
Satisfaction with the services provided at public medical care establishment						
Very satisfied	7	19.4	11	20.8	18	20.2
Satisfied	29	80.6	41	77.3	70	78.7
Unsatisfied	0	0.0	1	1.9	1	1.1

Table 3. Numbers and percentages of workers with a current history of illness, classified according to systemic symptoms

	Workers dispensing fuel (n = 100)		Workers not dispensing fuel (n = 100)		Total	
	n	%	n	%	n	%
Eye disorders						
- No	70	70.0	58	58.0	128	64
- Yes	30	30.0	42	42.0	72	36
Ear disorders						
- No	85	85.0	79	79.0	164	82
- Yes	15	15.0	21	21.0	36	18
Respiratory disorders						
- No	40	40.0	45	45.0	85	42.5
- Yes	60	60.0	55	55.0	115	57.5
Skin disorders						
- No	83	83.0	81	81.0	164	82
- Yes	17	17.0	19	19.0	36	18
Neurological symptoms						
- No	25	25.0	29	29.0	54	27
- Yes	75	75.0	71	71.0	146	73
Mental and emotional disorders						
- No	51	51.0	49	49.0	100	50
- Yes	49	49.0	51	51.0	100	50
Hepatobiliary disorders						
- No	81	81.0	82	82.0	163	81.5
- Yes	19	19.0	18	18.0	37	18.5
Urinary bladder disorders						
- No	48	48.0	47	47.0	95	47.5
- Yes	52	52.0	53	53.0	105	52.5

	Workers dispensing fuel (n = 100)		Workers not dispensing fuel (n = 100)		Total	
	n	%	n	%	n	%
Anemia symptoms						
- No	91	91.0	93	93.0	184	92
- Yes	9	9.0	7	7.0	16	8
Chronic diseases (DM/thyroid/heart disease/hypertension)						
- No	97	97.0	93	93.0	190	95
- Yes	3	3.0	7	7.0	10	5

Health care from social security the most 75.5 %. The monthly income reported by 68% of respondents was ≥283 USD, and 56.5% had a mean income of 341.16 ± 124.72 USD (Table 2). Specific information on various systemic symptoms (e.g., neurological, psychological, respiratory) is detailed fully in Table 3.

Of these PS workers, 40.3% had an annual health check at a government hospital, and 29.2% at a private hospital. A total of 44.5% of respondents received a hospital visit, of which 80.9% were a medical visit, 18% were a medical examination, and 7.9% were associated with drug disbursement. A total of 36% of PS workers had an annual health check-up (Table 4). Multiple logistic regression analyses of independent variables and their effect on HCS access are shown in Table 5. For instance, the effect of being female on HCS access carried an OR of 2.348 (95%CI 1.067–5.165), yet it was 2.128 (95%CI 1.054–4.296) if the respondent was married/widowed/other.

Table 4**.** Numbers **(**percentages**)** of gas station workers with access to health care services

Access to health care services	Workers operating at the fuel dispensers (n = 100)		Workers operating outside the fuel dispensers (n = 100)		Total	
	n	%	n	%	n	%
Access to health care services in a health facility over the past 12 months						
No	64	64.0	47	47.0	111	55.5
Yes	36	36.0	53	53.0	89	44.5
Treatment of illnesses	29	80.6	43	81.1	72	80.9
Vaccination against disease	2	5.6	2	3.8	4	4.5
Attending a lecture on health knowledge	1	2.8	2	3.8	3	3.4
Health check-up	4	11.1	12	22.6	16	18
Contraception	2	5.6	1	1.9	3	3.4
Receiving medicine	2	5.6	5	9.4	7	7.9
Chest x-rays	1	2.8	3	5.7	4	4.5
Annual health check-up						
No	70	70.0	58	58.0	128	64
Yes	30	30.0	42	42.0	72	36
Sources of health services						
Private hospitals	6	20.0	15	35.7	21	29.2
Public hospitals	13	43.3	16	38.1	29	40.3
Clinics	6	20.0	5	11.9	11	15.3
Local *tambol* health-promotion hospitals	3	10.0	0	0.0	3	4.2
Drugstore	2	6.7	6	14.3	8	11.1

Table 5. Factors affecting gas station workers' access to health services

Factor	Health services		Crude		Adjusted			
	No n = 111 (55.5%)	Yes n = 89 (44.5%)	OR	*p*-value	OR	95% CI		Coefficient (β)
						Lower	Upper	
Sex								
Female	66 (48.2)	71 (51.8)	2.689 (1.416,5.106)	0.002	2.348	1.067	5.165	0.854
Male	45 (71.4)	18 (28.6)	Ref	Ref	Ref	Ref	Ref	Ref
Marital status								
Married/widowed/other	40 (42.1)	55 (57.9)	2.871 (1.612,5.113)	0.000	2.128	1.054	4.296	0.755
Single	71 (67.6)	34 (32.4)	Ref	Ref	Ref	Ref	Ref	Ref
Slightly ill but working in the last 12 months								
No	62 (88.6)	8 (11.4)	Ref	Ref	Ref	Ref	Ref	Ref
Yes	49 (37.7)	81 (62.3)	12.811 (5.66,29.00)	0.000	7.978	3.264	19.499	2.077
Seriously ill								
No	93 (68.4)	43 (31.6)	Ref	Ref	Ref	Ref	Ref	Ref
Yes	18 (28.1)	46 (71.9)	5.527 (2.87,10.629)	0.000	3.394	1.581	7.286	1.222
Cost of receiving health services each time (baht)								
No	96 (60.4)	63 (39.6)	Ref	Ref	Ref	Ref	Ref	Ref
Yes	15 (36.6)	26 (63.4)	2.641 (1.298,5.375)	0.007	2.439	1.03	5.775	0.892
Distance from residence to the medical care establishment where they regular receive services (in km)								
≤ 5	69 (63.9)	39 (36.1)	Ref	Ref	Ref	Ref	Ref	Ref
>5	42 (45.7)	50 (54.3)	2.106 (1.194,3.716)	0.010	1.82	0.898	3.678	0.597
Other expenses , not spending money								
No	84 (61.8)	52 (38.2)	Ref	Ref	-	-	-	-
Yes	27 (42.2)	37 (57.8)	2.214 (1.209, 4.053)	0.010	-	-	-	-
Age (years)								
≥30	41 (47.7)	45 (52.3)	1.746 (.991,3.078)	0.054				
< 30	70 (61.4)	44 (38.6)	Ref	Ref	-	-	-	-

Factor	Health services		Crude		Adjusted			
	No n = 111 (55.5%)	Yes n = 89 (44.5%)	OR	*p*-value	OR	95% CI		Coefficient (β)
						Lower	Upper	
Educational background								
Less than secondary school	36 (56.2)	28 (43.8)	0.956 (0.526,1.740)	0.884	-	-	-	-
Higher school (or equivalent)	75 (55.1)	61 (44.9)	Ref	Ref	-	-	-	-
Income (baht/month)								
< 10,000	51 (58.6)	36 (41.4)	Ref	Ref	-	-	-	-
≥10,000	60 (53.1)	53 (46.9)	1.251 (0.712, 2.200)	0.436	-	-	-	-
Physical health								
Good health	77 (60.6)	50 (39.4)	Ref	Ref	-	-	-	-
Slight illness Serious illness	34 (46.6)	39 (53.4)	0.055 (0.99,3.16)	0.055	-	-	-	-
Mental health								
Good health	110 (55.6)	88 (44.4)	Ref	Ref	-	-	-	-
Slight illness serious illness	1 (50.0)	1 (50.0)	1.250 (0.077, 20.69)	0.875	-	-	-	-
Satisfaction with public medical care services								
Very satisfied	-	18 (100.0)	-	-	-	-	-	-
Moderately satisfied	-	70 (100.0)	-	-	-	-	-	-
Not satisfied	-	1 (100.0)	Ref	Ref	-	-	-	-
Social benefits.relating to health care								
Employer/at own expense/ other	22 (57.9)	16 (42.1)	0.887 (0.434,1.812)	0.711	-	-	-	-
Life insurance/social security/ health insurance	89 (54.9)	73 (45.1)	Ref	Ref	-	-	-	-
Sources of health information (can select more than one response)								
No	74 (54.4)	62 (45.6)	Ref	Ref	-	-	-	-
Yes (Family, coworkers, health officials)	37 (57.8)	27 (42.2)	0.871 (0.478,1.587)	0.652	-	-	-	-

Cox & Snell R Square 0.339, Nagelkerke R Square 0.453

DISCUSSION

The percentage of people accessing HCS is <50% even though a universal health insurance system is in operation in Thailand [18]. In our cross-sectional study, we looked at the population characteristics, environmental factors, and health behavior of PS workers in RP. Multiple logistic regression analysis revealed that the patterns of HCS access were similar to those described previously. Scholars have documented a significant relationship between HCS access and some sociodemographic characteristics, enabling factors, and healthcare needs: sex, marital status, illness severity, the cost of receiving HCS in each visit, and distance from residence to the HC establishment where services are received regularly [29, 30]. Women had HCS access that was 2.348-times greater than that of men. This observation reveals the challenge of ensuring HCS access which meets the requirements of different sexes as well as socioeconomically disadvantaged and vulnerable populations. This is an important challenge because different groups may judge appropriateness and quality differently [16]. However, our data are not in accordance with the results of a study by *Sangkhamkul* et al. [31] who investigated social networks. They reported that the level of disability and being female could predict HCS access. However, their study group differed in relation to demographic factors (e.g., age, occupation) compared with our study cohort.

HCS access of respondents with a status of married/divorced/widowed was 2.128-times greater than that of unmarried respondents, data which are consistent with the findings of a study by Pandey and colleagues [13]. They analyzed the relationship between marital status and HCS access. In a cohort comprising 12,929 people, 6,473 (50.3%) were married, of whom 58% (compared with a population of unmarried people of 36%) had greater HCS access provided at hospital outpatient departments. Thus, social factors influence HCS access. Our findings can help to explain the relationship between marital status and HCS access, in addition to providing evidence that can contribute to HC development.

Illness severity

We found that illness severity affected HCS access. Those with a minor illness and severe illness were, respectively, 7.978-times and 3.394-times more likely to access HCS than those who were not ill. The reasons why workers wanted HCS access was (i) to obtain treatment for illness (80.9%) and (ii) to have a health checkup (18%). Respondents with a mild illness or a severe illness were more likely to receive HCS than those who were not ill. However, this finding is not consistent with that of Lee and colleagues [25]. They found that workers who had a mild illness did not themselves avail HCS. However, the design and cohort of our study differed from those of Lee and colleagues because the participants in the latter were migrant workers working in Singapore. Several studies have found that workers who are not sick rarely avail themselves of HCS in hospitals or other HC centers. We discovered that 40.3% had an annual health check at a government hospital, and 29.2% at a private hospital. Different from the findings of the study by *Lee* et al. [25] on migrant workers, other studies have found that if workers become ill, most go to a private clinic, and fewer go to a government clinic [25, 32].

Health status and medical history

Most of our respondents were in good physical and mental health. This observation may have been because of their age: most were aged <30 (range, 18–60) years. Hence, the chance of having an underlying disease was low. *Chaiklieng* et al. [33] found that the age range of PS employees was 19−58 years, which is lower than that of PS employees working in Brazil (20−70 years) [34]. Hence, workers should be urged to seek HCS regularly. However, we also found that 73% of respondents reported previously identified neurological symptoms, 57% reported respiratory symptoms, and 50% reported mental and emotional problems. These data are consistent with results in several studies showing that 47.0% of PS workers experienced headache and 22.0% experienced fatigue [35, 36, 37]. Of the 57% of PS workers who developed respiratory symptoms, their symptoms may have been caused (at least in part) by smoking (34% of PS workers smoked tobacco). This prevalence of smoking is higher than that in the general population in Thailand (19.1%) [38]. Health promotion by changing workers' behavior to stop smoking can reduce the risk factors for respiratory diseases. A comprehensive approach raises awareness of the huge impact of chronic respiratory diseases, and highlights the risk factors as well as ways to prevent and treat these diseases [39]. In addition, health surveillance by prevention and control of chronic disease from BTEX exposure as well as good Occupational, Social and Health and Safety practices should be used by employers and employees to control risk, minimize exposure, and protect the health of all people at risk of exposure working in PS [40].

Data for respondents with liver abnormalities were consistent with behavioral-health data: 45.5% of PS workers consumed alcohol. Alcohol consumption can lead to various liver abnormalities, including inflammation and related disorders [41]. PS owners and public-health officials should participate in campaigns urging these workers to stop consuming alcohol, enhancing good liver health, as well as reducing the cost of receiving HCS in hospital.

Cost of receiving HCS

This study revealed that the payment of health care service was associated with 2.439 time more accessing health services than without having to pay for health care. We observed that PS workers who paid for HCS had greater access to HCS than those who did not pay for HCS. Our results appeared to reverse the causality between the cost of receiving HCS and access to HCS because it was a cross-sectional study in which participants accessed HCS before we collected their data. In addition, 75.9% of respondents were found to be eligible for health insurance through social security, with the average cost of one-time access to HCS being not very high (12.58± 36.01USD). Therefore, such costs should not be a major barrier to HCS access by PS workers. In our study, the cost of HCS increased with illness severity, but we did not examine this relationship. Also, we did not assess the type of expense for each time healthcare was accessed. *Langton* et al. [42] found that HCS access with regard to hospitalization in two study cohorts increased, with the greatest increase seen in the last 2 months of life (67% increase in the cohort who died from cancer and 80% increase in the comparison cohort). Also, the percentage of dispensed medicines per month increased by 36% in the cohort who died from cancer and 19% in the comparison cohort.

Distance from the respondents' residence to the HC establishment in which services are received regularly

The average distance between a respondent's residence and government hospital was 10.49 ± 8.571 km, so HCS access was convenient for PS workers. A distance from the respondent's residence to the establishment where he/she received HCS >5 km was associated with HCS access that was 1.817-times higher than if the distance from the respondent's residence to the establishment where he/she receives HCS was ≤5 km. We found that respondents had a short distance to travel to access HCS in RP. Our results are in accordance with those of *Jordan* et al. [43]. They found the maximum distance to a hospital to be 9.4 km (travel time = 13.7 min). In the UK, a threshold distance to a specialist hospital is 24–50 miles [44], and is 10 miles to screening services, 4 miles to family-planning clinics , and 2.5 miles to primary care , all of which have been described as "poor access".

Our study data are not in accordance with results from other studies indicating that distance affects HCS access [45]. RP is economically developed, which makes travel to HCS very convenient. Also, the public-transport system in Thailand has improved and become more convenient and comfortable, so distance is no longer an obstacle to HCS access [6, 46]. However, we concentrated purely on urban areas, so we likely underestimated the extent of geographic barriers to HCS access. Our study is similar to many studies that found the distance from the participant's residence to a hospital to be correlated with HCS access. Distance has been found to be a significant driver for HC use and feeds into aspects of HCS access [47, 48]. Moreover, the distance from a "hub" location is a proxy for factors which affect the affordability of good access to HCS. Practices which are further away from their nearest hub have a much lower prevalence of use of the service [49].

Income and welfare rights relating to HC

The average monthly income of PS workers was 341.16 ± 124.72 USD, with most workers having a monthly salary >283 USD. This figure is close to the minimum daily wage of 9.48 USD and 9.51 USD in RP and Chonburi Province, respectively [50]. Although low income has been associated with a wide range of problems related to HCS access [51], we found that income did not influence HC behaviors. RP is a highly developed economic area, so the high cost of living may hinder HCS access. However, we found that income was not related to HCS access. This may have been because PS workers receive social security and government welfare for HC. In 2005, member countries of the WHO (including Thailand) committed to developing health-financing systems such that all people have HCS access known as "universal coverage". Health inequalities are observed in many countries [52, 53].

Our findings, however, differed from those of a long-term study in China which found that income influenced HCS access [54]. Although salaries were found to be associated significantly with visits to a physician within 3 days of illness onset ($p = 0.002$) [26], that study did not evaluate individuals in the same occupational group, and their educational status differed. The lack of association between income and HCS access in our study may have been because 75.5% of respondents had social-security cards.

Channels for receiving HC information

Access to primary HC is key to improving health outcomes [55]. In our study, respondents reported receiving the most support for HC information from their coworkers (38.5%), followed by receiving information from their family (32%), and health officials (17%). If a PS worker has good information related to HCS as well as social support, he/she will have better HCS access that will improve his/her health. More channels of communication are required to ensure that workers receive HC-related information and have better access to providers and outreach services for HC. We are focusing on a lack

of communication related to HC information that influences a person's ability to access HCS [16, 55]. There should be more channels to provide information about HC through colleagues and family. Also, HC workers should be more accessible to PS workers to encourage them to enhance their understanding of the value of having HCS access and achieving positive health outcomes [56].

Satisfaction with HCS

Satisfaction with HCS influences the well-being of a population. Quantifying satisfaction is one way of rating the quality of HCS [57]. A total of 78.8% of respondents were satisfied with the access to HCS provided by hospitals and other sources. However, we did not study causality in detail to assess satisfaction. A study in an elderly population revealed satisfaction with service quality to have a significant relationship with HCS access [31], but our study cohort was much younger.

Limitations and strengths of our study

Our study had two main limitations. First, the interview form for PS workers with systemic disorders did not involve passing a physical examination by a physician. There may have been a discrepancy with regard to documenting abnormal symptoms. Nevertheless, the interview form has been validated by physicians, and is intended for screening for abnormal symptoms only. Second, data were collected during the coronavirus disease 2019 (COVID-19) outbreak in Thailand. Most people avoided leaving their homes/workplace to a hospital because they were afraid of contracting COVID-19. Therefore, some workers may not have been able to access HCS. Studies are needed to monitor employees' access to HCS services under normal working conditions.

Our study had three main strengths. First, information about health conditions as a whole and risk behaviors in PS workers can lead to greater access to HCS and promotion of health among this group of workers. Second, the face-to-face interviews with individual PS workers allowed abnormal symptoms to be assessed appropriately. Third, the *critique* of study results by 15 experts provided recommendations for future, more comprehensive research. As a result, the credibility and knowledge gained from this research can be utilized to encourage employees to seek HCS.

CONCLUSIONS

HCS access was attributed to the cost of receiving HCS as well as the sex, marital status, illness severity of PS workers: these factors should be considered as part of the framework for public-health policy. PS workers should be encouraged to access HCS by undergoing annual health checkups (and not waiting until they become ill). Providing all workers with equal and comprehensive HCS access can enhance their long-term health. Qualitative and quantitative studies are needed to investigate the social and cultural factors associated with increased access to HCS.

Acknowledgments

We would like to thank the staff at Memorial Hospital Sirindhorn for their assistance with data collection in the study area, and the owners and station managers for their cooperation. We would also like to express our appreciation to all of the volunteers who assisted in this study.

Funding

This research was funded by the Health Systems Research Institute at Burapha University, Thailand (grant number 63-070).

Conflicts of interest

The authors declare that they have no competing interests in this work.

REFERENCES

1. Department of Land Transport, Planning Division. [Internet]. Transportation Statistics Group. Annual transport statistics report. 2020. [cited 2021 April 16]. Available from: https://web.dlt.go.th/statistics.
2. *Wannarat T.* Study of informal labour in Thailand. Silpakorn University Journal 2014;34:119–150.
3. WHO. Community health workers: a strategy to ensure access to primary health care services. 2020. [cited 2021 April 16]. Available from: https://apps.who.int/iris/handle/10665/249563.
4. *Chaiklieng S, Nantanuch R.* Factors associated with adverse symptoms related to benzene toxicity among workers at gasoline stations. Srinagarind Medical Journal 2015;30:458–466.
5. *Moolla R, Curtis CJ, Knight J.* Assessment of occupational exposure to BTEX compounds at a bus diesel-refueling bay: a case study in Johannesburg, South Africa. Sci Total Environ 2015;537:51–57. doi: 10.1016/j.scitotenv.2015.07.122malasia.
6. *Zoleikha S, Mirzaei R, Roksana M.* Exposure to chemical hazards in petrol pumps stations in Ahvaz City, Iran. Arch Environ Occup Health 2017;72:3–9. doi: 10.1080/19338244.2015.1058233. Epub 2016 Sep 27.
7. OSH Matters. Gas-ping for safety at the gas station: an OSH Assessment. 2016. [cited 2021 April 16]. Available from: https://oshmatters.wordpress.com/2016/10/04/gas-ping-for-safety-at-the-gas-station-an-osh-assessment.
8. *Chaiklieng S, Suggaravetsiri P, Kaminski N, Autrup H.* Factors affecting urinary tt-muconic acid detection among benzene exposed workers at gasoline station. Int

J Environ Res Public Health 2019;16:4209. doi: 10.3390/ijerph16214209.
9. *Latif MT, Abd Hamid HH, Ahamad F, Khan MF, Mohd Nadzir MS, Othman M,* et al. BTEX compositions and its potential health impacts in Malaysia. Chemosphere 2019;237:124451. doi: 10.1016/j.chemosphere.
10. *Al-Ayyadhi N, Akhtar S.* Prevalence and risk factors associated with self-rated morbidities among South Asian migrant gas station workers in Kuwait. J Immigr Minor Health 2018;20:1324–1331. doi: 10.1007/s10903-018-0701-1.
11. *Tunsaringkarn T, Zapuang K, Rungsiyothin A.* Association between body mass index and liver function among gasoline station workers in Bangkok, Thailand. Medicine Journal of Health Research 2015;29(1):55–61.
12. *Abdel Rasoul GM, Salem EA, Allam HK, Shehata YA, Abu-Salem ME, El-Sayed Zagloul AF.* Neurobehavioral and hematological health disorders among fuel supply station workers. Menoufia Med J 2017;30:1103-1109. doi: 10.4103/mmj.mmj_252_17.
13. *Kim BM, Park Ek, LeeAn SY, Ha M, Kim EJ, Kwon H, Hong YC, Jeong WC, Hur J, Cheong HK, Yi J, Kim JH, Lee BE, Seo JH, Chang MH, Ha EH.J.* BTEX exposure and its health effects in pregnant women following the Hebei Spirit oil spill]. Prev Med Public Health 2009;42(2):96-103. doi: 10.3961/jpmph.2009.42.2.96.
14. International Agency for Research on Cancer (IARC). [Internet]. Benzene. 2021. 2020. [cited 2021 April 16]. Available from: https://monographs.iarc.who.int/agents-classified-by-the-iarc/.
15. WHO. Exposure to benzene: a major public health concern. 2020. [cited 2021 April 16]. Available from: https://www.who.int/publications/i/item/WHO-CED-PHE-EPE-19.4.2
16. *Levesque JF, Harris MF, Russell G.* Patient-centered access to health care: conceptualizing access at the interface of health systems and populations. Int J Equity Health 2013;11:12–18. doi: 10.1186/1475-9276-12-18.
17. *Penchansky R, Thomas JW.* Med Care. The concept of access: definition and relationship to consumer satisfaction 1981;19(2):127–40. doi: 10.1097/00005650-198102000-00001.
18. *Wattanapha P.* Right to enter statement medical care of Thai people in the last century.
19. where you came. 2019. [cited 2021 April 16]. Available from: https://www.constitutionalcourt.or.th/.
20. *Dixon-Woods M, Cavers D, Agarwal S, Annandale E, Arthur A, Harvey J,* et al. Conducting a critical interpretive synthesis of the literature on access to healthcare by vulnerable groups. BMC Med Res Methodol 2006;6:35. doi:10.1186/1471-2288-6-35.
21. *McGrail MR, Humphreys JS.* A new index of access to primary care services in rural areas. Aust N Z J Public Health 2009;33:418–423. doi: 10.1111/j.1753-6405.
22. *Reeve C, Humphreys J, Wakerman J, Carter M, Carroll V, Reeve D.* Strengthening primary health care: achieving health gains in a remote region of Australia. Med J Aust 2015;202:483–487. doi: 10.5694/mja14.00894.
23. *Aspin C, Brown N, Jowsey T, Yen L, Leeder S.* Strategic approaches to enhanced health service delivery for Aboriginal and Torres Strait Islander people with chronic illness: a qualitative study. BMC Health Serv Res 2012;12:143. doi: 10.1186/1472-6963-12-143.
24. *Thetkathuek A, Jaidee W, Jaidee P.* Access to health care by migrant farm workers on fruit plantations in Eastern Thailand. J Agromedicine 2017;22:189–199. doi: 10.1080/1059924X.2017.1317682.
25. *Sanruen C, Kijteerawutipong N.* Health services accessibility of foreign workers before entering the economic development zone and Asian economic. J Public Health Nurs 2015;29: 123–136.
26. *Lee W, Neo A, Tan S, Cook AR, Wong ML, Tan J*, et al. Health-seeking behaviour of male foreign migrant workers living in a dormitory in Singapore. BMC Health Serv Res 2014;14:300. doi: 10.1186/1472-6963-14-300.
27. *Charoenmukayanan S, Sriratanaban J.* Health care service used of Laos patient in Thailand: case study influencing governmental hospital, Thailand. Health System Research Institute. Asian Biomedicine 2012;665–672.
28. *Biswas D, Kristiansen M, Krasnik A, Norredam M.* Access to healthcare and alternative health-seeking strategies among undocumented migrants in Denmark. BMC Public Health 2021;11:560. doi: 10.1186/1471-2458-11.
29. *Seo JY, Chao YY, Yeung KM, Strauss SM.* Factors influencing health service utilization among Asian immigrant nail salon workers in the greater New York City area. J Community Health 2019;44:1–11. doi: 10.1007/s10900-018-0544-7.
30. *Stepurko T, Pavlova M, Groot W.* Overall satisfaction of health care users with the quality of and access to health care services: a cross-sectional study in six Central and Eastern European countries. BMC Health Serv Res 2016;16:342 doi:10.1186/s12913-016-1585-1.
31. *Diagne MF, Ringold D, Zaidi S.,* Governance and public service delivery in Europe and Central Asia: unofficial payments, utilization and satisfaction. World Bank Policy Research Working Paper 2012:5994.
32. *Ngamkham S, hSuwannapong N, Tipayamongkholgul M, Jaruwan Manmee J.* Access to Health Care Services of the Elderly, Thawiwatthana District, Bangkok. Kuakarun Journal of Nursing 2020;5(2):91-104.
33. *Laosai S, Teeravisit A*. Access to health services of Burmese migrant workers in industrial factories in Khon Kaen Province. Graduate research conference. Knonkaen University. 2012. [cited 2021 April 16]. Available from: https://gsbooks.gs.kku.ac.th/55/cdgrc13/files/hmp5.pdf.
34. *Chaiklieng S, Suggaravetsiri P, Autrup H.* Risk assessment on benzene exposure among gasoline station workers. Int J Environ Res Public Health 2019;16:2545. doi: 10.3390/ijerph16142545
35. *Geraldino BR, Nunes RFN, Gomes JB, Giardin I, da Silva PVB., Campos É* et al. Analysis of benzene exposure in gas station workers using trans, trans-muconic acid. Int J Environ Res Public Health 2020;17:5295. doi: 10.3390/ijerph17155295.
36. *Werder EJ, Engel LS, Blair A, Kwok RK, McGrath JA, Sandler DP.* Blood BTEX levels and neurologic

symptoms in Gulf states residents. Environ Res 2019;175:100–107. doi: 10.1016/j.envres.2019.05.004
37. *Alves LP, Vieira DSP, Nunes LSS, Cruz LPS, Reis ACS, Gomes IVS* et al. Relationship between symptoms, use of PPE and habits related to occupational exposure to BTEX compounds in workers of gas stations in Bahia, Brazil. Journal of Environmental Protection 2017;8:650–661. doi: 10.4236/jep.2017.85042
38. *Tunsaringkarn T, Siriwong W, Rungsiyothin A, Nopparatbundit S*. Occupational exposure of gasoline station workers to BTEX compounds in Bangkok, Thailand. Int J Occup Environ Med 2012;3:117–125.
39. The National Statistical Office. Health behaviors/health risk behaviors. 2021. [cited
40. 2021 April 16]. Available from: http://statbbi.nso.go.th/staticreport/page/sector/th/05.aspx
41. WHO. Global surveillance, prevention and control of chronic respiratory diseases. A comprehensive approach. 2021. [cited 2021 April 16]. Available from: http://www.who.int/gard/publications/GARD_Manual/en/018 91.
42. *Kuranchie FA, Angnunavuri PN, Attiogbe F, Nerquaye-Tetteh EN*. Occupational exposure of benzene, toluene, ethylbenzene and xylene (BTEX) to pump attendants in Ghana: Implications for policy guidance. Cogent Environmental Science 2019: 5(Issue 1).
43. *Wang W, Wang C, Xu H, Gao Y.* Aldehyde dehydrogenase, liver disease and cancer. Int J Biol
44. Sci 2020;16:921–934. doi: 10.7150/ijbs.42300
45. *Langton JM, Reeve R, Srasuebkul P, Haas M, Viney R, Currow D, and Pearson SA*. Health service use and costs in the last 6 months of life in elderly decedents with a history of cancer: a comprehensive analysis from a health payer perspective Br J Cancer 2016;114(11):1293–1302. doi: 10.1038/bjc.2016.75
46. *Jordan H, Roderick P,Martin D, Barnett S.* Distance, rurality and the need for care: access to health services in South West England. International Journal of Health Geographics 2020;3(21):1-9.
47. *Cassar K, Godden DJ, Duncan JL*: Community mortality after ruptured abdominal aortic aneurysm is unrelated to the distance from the surgical centre. Br J Surgery 2001;88:1341–1343.
48. *Sanklaleak W, Boromtanarat C, Tiautchasuwan Y.* Factors affecting the utilization of antenatal care service of migrant workers in government hospitals in Samutsakhon Province. The 4th STOU Graduate Research Conference. 2014. [cited 2021 April 16]. Available from: https://www.stou.ac.th
49. *Taneeranan S*. Getting out of the rut of poverty in Thailand through transport accessibility. Journal of Society for Transportation and Traffic Studies 2016;7:30–37.
50. *Haynes R, Bentham G, Lovett A,* et al. Effects of distances to hospital and GP surgery on hospital inpatient episodes, controlling for needs and provision. Soc Sci Med 1999;49:425–433. 10.1016/S0277-9536(99)00149-5 https://doi.org/10.1016/S0277-9536(99)00149-5
51. *Raknes G, Hansen EH, Hunskaar S*. Distance and utilisation of out-of-hours services in a Norwegian urban/rural district: an ecological study. BMC Health Serv Res 2013;13:222. https://doi.org/10.1186/1472-6963-13-222.
52. *Murphy J, Elliot M, Ravindrarajah R, Whittaker W.* Investigating the impact of distance on the use of primary care extended hours. Int J Popul Data Sci 2021;6;6(1):1401. doi: 10.23889/ijpds. v6i1.1401. eCollection 2021.
53. Ministry of Labor, Thailand. [Internet]. The minimum wage under the Notification of the Wages Committee. Re: minimum wage rate (No.10). 2020. [cited 2021 April 16]. Available from: https://www.mol.go.th/wp-content/uploads/sites/2/2020/01/Prakadwage10-6Jan2020.pdf.
54. *Lazar M, Davenport L*. Barriers to health care access for low income families: a review of literature. J Community Health Nurs 2018;35:28–37. doi: 10.1080/07370016.2018.1404832
55. WHO. Closing the gap in a generation. Health equity through action on the social determinants of health. 2008. [cited 2021 April 16]. Available from: https://apps.who.int/iris/bitstream/handle/10665/69832/WHO_IER_CSDH_08.1_eng.pdf;jsessionid=98B9619688C8C9DCECAA2605EDE28FCD?sequence=1
56. Chiang Mai Provincial Labor Protection and Welfare Office, Ministry of Labor. Informal worker. 2018. [cited 2021 April 16]. Available from: http://chiangmai.labour.go.th/index.php/2018-10-31-08-11-28/408-2018-10-31-06-%20%20%2038-13:
57. *Xie M, Huang Z, Zang W.* The inequality of health-income effect in employed workers in China: a longitudinal study from China Family Panel Studies. Int J Equity Health 2020;19:96. doi: 10.1186/s12939-020-01211-6.
58. *Davy C, Harfield S, McArthur A, Munn Z, Brown A*. Access to primary health care services for indigenous peoples: A framework synthesis. Int J Equity Health 2016;15:163. doi: 10.1186/s12939-016-0450-5
59. *O'Sullivan BG, Joyce CM, McGrail MR.* Adoption, implementation and prioritization of specialist outreach policy in Australia: a national perspective. Bull World Health Organ 2014;92:512–519. doi: 10.2471/BLT.13.130385
60. *Lautamatti M, Sumanen RR, Mattila KJ***.** Continuity of care is associated with satisfaction with local health care services. BMC Family Practice 2020;21:181, https://doi.org/10.1186/s12875-020-01251-5.

Received: 30.03.2022
Accepted: 20.06.2022
Published online first: 10.07.2022

Rocz Panstw Zakl Hig 2022;73(3):365-371
http://wydawnictwa.pzh.gov.pl/roczniki_pzh/ https://doi.org/10.32394/rpzh.2022.0226

ORIGINAL ARTICLE

IDENTIFYING MONKEYPOX: DO DENTAL PROFESSIONALS HAVE ADEQUATE KNOWLEDGE AND AWARENESS?

Ambreen Kaur[1], Richa Goel[2], Ravinder Singh[3], Arvind Bhardwaj[4], Raj Kumari[5], Ramandeep Singh Gambhir[6]

[1]Department of Orthodontics, Luxmi Bai Institute of Dental Sciences and Hospital, Patiala, Punjab, India
[2]Department of Public Health Dentistry, Karnavati School of Dentistry, Gandhinagar, Gujarat, India
[3]Department of Oral Medicine and Radiology, MM College of Dental Sciences and Research, MM (Deemed to be University), Mullana, India
[4]Department of Periodontology, MM College of Dental Sciences and Research, MM (Deemed to be University), Mullana, India
[5]Department of Medical Surgical Nursing, Civil Hospital, Shahabad Markanda, Haryana, India
[6]Department of Public Health Dentistry, Rayat and Bahra Dental College and Hospital, Mohali, Punjab, India

ABSTRACT

Background. The emergence of monkeypox has presented a new challenge for health agencies around the globe. There is significant increase in the number of cases that too in non-endemic countries with more than 18000 cases reported worldwide.

Objective. The present study was conducted to assess knowledge and awareness regarding monkeypox among dental professionals.

Materials and Method. The present cross-sectional study among 410 subjects who were residing in a northern state of the country. Informed consent was obtained from all the subjects. Study sample was selected on the basis of Systematic random sampling methodology. A self-designed close-ended questionnaire written in English and verified by experts was utilized for the study. The questionnaire was delivered personally to study subjects to collect the required information. Chi-square test and ANOVA were used for statistical analysis.

Results. One-fourth (24.8%) of the subjects never heard about monkeypox disease. Negative response was given by 44.8% of subjects regarding resemblance of monkeypox with small pox. Only 31.2% of subjects had knowledge regarding oral manifestations of the disease. High knowledge scores were reported by only 28% of subjects. Higher knowledge levels were significantly related to education level and working profile of study subjects. Online media (Internet) was preferred as the main source to obtain more information by 42.2 of subjects.

Conclusion. There was low level of knowledge regarding monkeypox among study subjects. There is an urgent need for dental professionals to keep themselves updated with recent knowledge on new emerging infectious diseases.

Key words: *monkeypox, knowledge, dentists, transmission, infections, India*

INTRODUCTION

Owing to mass vaccinations programs across the globe and development of herd immunity in vulnerable populations, the numbers of COVID-19 cases have reduced considerably. However, the world is still fighting the war against COVID-19 as disease outbreaks continue to occur in one or another part of the globe owing to the emergence of new variants [1]. Recently, Monkeypox, another disease of global public health importance, has started to re-emerge in different countries. It is a zoonotic disease caused by monkeypox virus (MPXV), which is a member of orthopoxvirus genus and severe clinical presentation bearing resemblance to that of smallpox [13]. Though the first case of human monkey pox was reported in 1970 in the Democratic Republic of Congo where the disease is endemic, multiple cases of monkeypox were identified in several non-endemic countries recently [3].

Monkeypox has emerged as the most important orthopoxvirus for public health after the eradication of smallpox in 1980 and cessation of vaccination. Individuals infected with monkeypox experience

Corresponding author: Ramandeep Singh Gambhir, Department of Public Health Dentistry, Rayat and Bahra Dental College and Hospital, Mohali, Punjab, India. PIN-140103, Tel. +91 99156-46007; e-mail: raman2g@yahoo.com

fever, intense headache, lymphadenopathy and myalgia during the initial period. It is followed by skin eruptions in the form of rash on face, palms of the hands and soles of the feet, oral mucous membranes, genitals, conjunctiva and the cornea (Figure 1) [11].

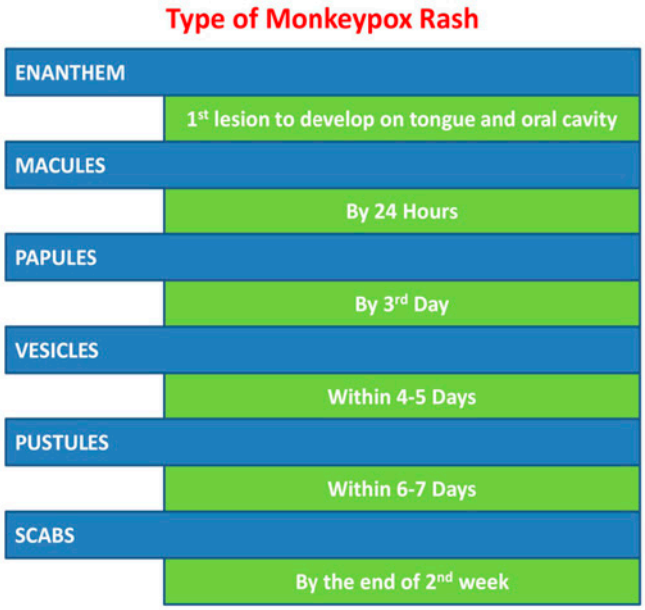

Figure 1. Progression of monkeypox rash

Human to human transmission is possible through direct contact with infectious skin or lesions and predominantly transmitted by men having sex with men [12]. Transmission can also occur from contaminated materials like bed linens, electronics, clothing etc. that have infectious skin particles. Monkeypox is often a self-limiting infection, with symptoms lasting 2-4 weeks with the case fatality ratio around 3%-6% [15].

According to latest reports, 60 countries have reported the outbreaks of the viral disease in which monkeypox is not endemic and total number of cases have crossed 18000 worldwide (75 countries) and the cases are increasing on a daily basis [4]. India has also reported four cases of monkeypox till now. WHO has declared monkeypox disease as a Public Health Emergency of International Concern (PHEIC) owing to significant increase in the number of cases globally and experts see a risk of its further international spread [18]. Being health professionals, dentists should have awareness regarding symptoms of monkeypox. Aerosol-generating procedures on monkeypox-infected patients can put dentists and dental hygienists at risk of contracting the disease. The importance of meticulous hand hygiene and use of Personal Protective Equipment (PPE) cannot be under estimated. There is limited number of research articles in the biomedical literature and barely any studies evaluating knowledge of dental professionals regarding monkeypox. Therefore, the present study was conducted to assess the knowledge and awareness of dental professionals regarding identification of monkeypox among their patients.

MATERIALS AND METHOD

Ethical clearance

Ethical clearance for the present cross-sectional study was obtained from concerned health authorities. The purpose and the methodology of the study was thoroughly explained to each subject. They were assured of data confidentiality/anonymity, and informed that participation was voluntary. Informed consent was taken from all the subjects who were willing to participate in the study. The study was conducted in the month of June 2022 in a north Indian state.

Study population and study sample

Private dental practitioners and dental academicians constituted the study population. List of all private dental practitioners was obtained from Local Indian Dental Association (IDA) bodies. The following formula was used to calculate the required sample size:

$$n = \frac{Z^2 1\text{-}(\alpha / 2) \times S2}{d2}$$

Where:

Z - is the standard normal score with 95% confidence interval (CI) (α=0.05),

S - is the standard deviation of the variable, and d is maximum acceptable error (4%).

After excluding the non-responders, a total of 410 subjects constituted the final study sample. These were enrolled in the study using systematic random sampling methodology.

Tools of data collection / Research Instrument

A self-designed, close-ended questionnaire written in English was employed specifically for the study. The contents of the questionnaire were verified by a medical microbiologist and a family medicine doctor who had a pre-existing research interest in emerging infectious diseases. A pilot study was undertaken on 20 subjects to pre-test the questionnaire and to check the feasibility of the study. Reliability of the questionnaire was assessed using Test-Retest and the values of measured Kappa (k) were 0.82 and Weighted Kappa (k) was 0.78. The questionnaire was divided into two parts: Section A - a 'General Section' which was made to collect socio-demographic details of the subjects (gender, occupation, experience, working profile etc.). Section B comprised of 12 questions on assessing knowledge and awareness regarding monkeypox (heard about monkeypox, current outbreak, signs and symptoms, oral manifestations, availability of vaccine etc). The questionnaire was delivered to the subjects (residence or place of practice) and designated time was given to fill the questionnaire and thereafter it was collected. A reminder was given though phone calls to answer all the questions as some of the subjects may be having a busy schedule. The response of subjects' (positive or negative) towards the questionnaire was assessed on a two-point Likert scale (positive or negative). The total score of the subject was calculated by adding the sum of responses which ranged from 1 to 12, on a Likert Scale. The final knowledge scores were categorized at three levels: low (0-4), medium (5-8) and high (9-12).

Statistical Analysis

Data were collected from the study subjects and entered into Microsoft Excel Spreadsheet version 2019 and was assessed using SPSS statistical package (SPSS, version 25.0, Chicago, IL, USA). Categorical measurements were calculated using descriptive statistical methods (number, percentages, mean etc.). Comparison of qualitative variables was done using Analysis of Variance test (ANOVA) and Chi-square test. The significance level was set at <0.05.

RESULTS

Socio-demographic profile of study population

The analysis of the socio-demographic data depicted in Table 1 revealed that the majority of the subjects were males (56.5%) as compared to females (43.5%) and 42.7% had a post graduate qualification in dentistry. It was also observed in our study that subjects having experience of more than 10 years in academics/private practice were comparatively less (25.1%) as compared to subjects having 5-10 years of experience (42%). More than half of the subjects (52.4%) were involved in private dental practice alone.

Table 1. Socio-demographic characteristics of the study population

Socio-demographic characteristic		Number	Percentage (%)
Gender	Male	232	56.5
	Female	178	43.5
Educational status	Graduate (BDS)	235	57.3
	Postgraduate (MDS)	175	42.7
Years of Experience	Up to 5	135	33.0
	5-10	172	42.0
	More than 10	103	25.1
Working Profile	Private hospital/Clinic	215	52.4
	Academician/Teacher	85	20.7
	Both	110	26.9

Response to the questionnaire on monkeypox

The responses of the subjects on various questions regarding monkeypox are summarized in Figure 2. We were surprised to note that approximately one-fourth (24.8%) of the subjects never heard about monkeypox disease. Almost 40% (39.5%) of subjects were not aware that there was outbreak of monkey pox in different countries. When asked about resemblance of monkey pox with small pox, 44.8% of the subjects gave a negative response. Only 52.6% of the subjects agreed to the fact that human to human transmission of monkeypox is possible. Total of 58.5% of subjects said development of skin rash as the most common sign. Less than one-third of subjects (31.2%) didn't have adequate knowledge regarding oral manifestations of

the disease and importance of hand hygiene and PPE (31.7%) to prevent the disease (31.7%). Only 36.2% of the subjects were aware of advisory given by Govt. of India on monkeypox.

Knowledge/awareness level of subjects

Only 28% of subjects reported high knowledge scores and 33.6% of subjects were having low knowledge scores according to Likert calculations

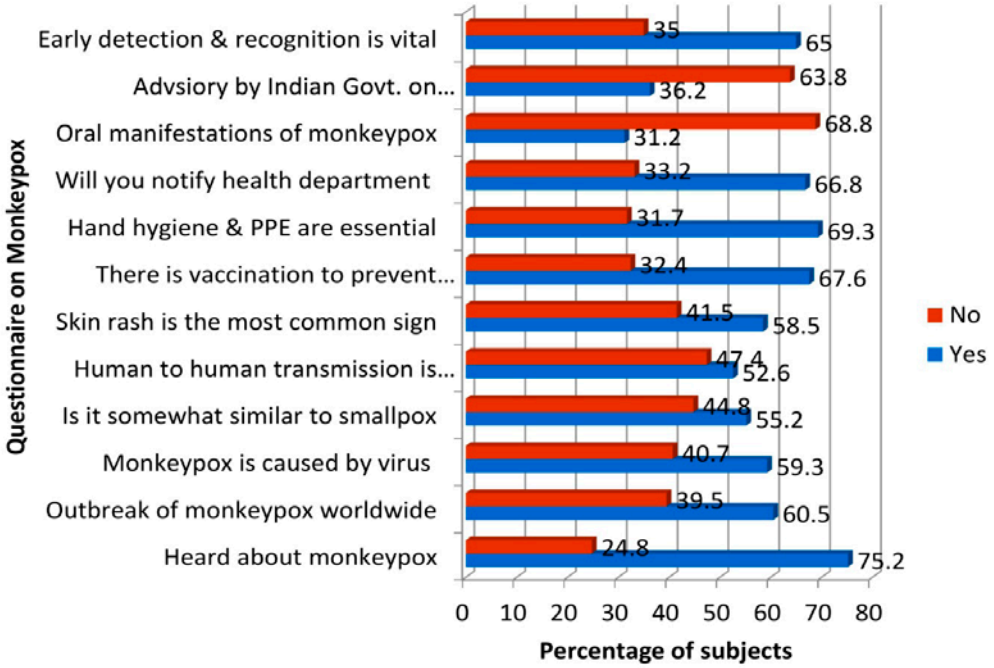

Figure 2. Questionnaire assessing knowledge of subjects on monkeypox

Table 2. Final knowledge scores of study subjects regarding monkeypox (on the basis of Likert scale)

Knowledge score	Number of subjects	Percentage of subjects	95% CI
Low	137	33.6	32.17-46.25
Medium	157	38.4	37.12-42.17
High	116	28.0	22.16-31.72
Total	410	100	

Table 3. Association between socio-demographic characteristics of study subjects with knowledge levels

Socio-demographic variable	Knowledge level						p-value
	Low knowledge		Medium knowledge		High knowledge		
Gender	No.	%	No.	%	No.	%	
Males	86	37.0	84	36.2	62	26.7	0.067
Females	51	28.6	73	41.0	54	30.3	
Educational Status							
Graduate (BDS)	79	35.1	97	41.2	59	25.1	0.024*
Postgraduate (MDS)	58	33.1	60	34.2	57	32.5	
Years of Experience							
Up to 5	50	37.0	49	36.2	36	26.6	0.078
5-10	57	33.1	66	38.3	49	28.4	
More than 10	30	29.1	42	40.7	31	30.0	
Working Profile							
Private hospital/Clinic	70	32.5	82	38.1	63	29.3	0.012*
Academician/Teacher	28	32.9	29	34.1	28	32.9	
Both	39	35.4	46	41.8	25	22.7	

*P<0.05 (Statistically significant), Tests used: Chi-square test, ANOVA

(Table 2). The analysis of knowledge scores according to socio-demographic characteristics of study subjects is mentioned in Table 3. According to the knowledge level analysis, the proportion of subjects having high knowledge scores was higher among postgraduate subjects and the findings were statistically significant (p=0.024). Similarly a higher proportion of subjects exhibiting high knowledge scores was seen among academicians as compared to others and the findings were statistically significant (p=0.012). However, knowledge scores were not significantly related to gender and years of experience. Willingness to receive more information on monkeypox if number of cases increase was shown by 65.4% of subjects and out of which 42.2% of subjects preferred online media as the main source to obtain information (Table 4).

Table 4. Different sources to receive more information if the monkeypox cases increase

Willing to receive information	No.	%
No	142	34.6
Yes	268	65.4
Sources		
Online media	113	42.2
Colleagues	43	16.1
Medical Journals	41	15.3
Newspapers/magazines	27	10.0
Television	44	16.4

DISCUSSION

In the present times, the challenges which human kind is facing are unprecedented like the recent occurrence of COVID-19 pandemic. Recently, an outbreak of monkeypox, a rare self-limiting sporadic disease, has been reported from non-endemic countries and the cases are increasing every day. Dentists, forming an important part of the health care community, should possess adequate knowledge regarding signs and symptoms of the disease which is crucial to the safety of patients and dental professionals. To the best of our knowledge, the present study is first of its kind in the entire country focusing on dental professionals' knowledge regarding monkeypox. The main finding of this study was the generally unsatisfactory levels of knowledge among study subjects.

Astonishingly, almost one-fourth of the subjects in the study never heard about monkeypox and 40% of subjects were not aware of its outbreak globally. This could be due to the reason that till recently, monkeypox outbreaks were rarely reported; badly managed and little described leading to an incomplete picture of the disease's importance [16]. Moreover, only four confirmed cases of monkeypox are reported in India till now, therefore dissemination of information regarding monkeypox through newspapers and social media is very limited. However, more than 90% of subjects had heard about monkeypox in a study conducted among general practitioners in Indonesia [8].

The clinical picture of monkeypox closely resembles with smallpox that starts with fever initially followed by skin rash appearing 1-3 days after the onset of fever and lymphadenopathy, with lesions appearing simultaneously, and evolving at a similar rate [7, 10]. Approximately 45% of subjects in our study had no knowledge regarding this. This is similar to reports of a recent study conducted among students in Jordanian Health Schools [14].

Human-to-human transmission of monkeypox virus can take place through close contact with respiratory secretions, skin lesions of an infected person and contaminated objects, including clothing and bedding [9, 17]. Only 52.6% of subjects in the present study were possessed adequate knowledge on this aspect. Lack of knowledge in these areas can have significant negative public health consequences.

Dental professionals being 'Oral Specialists,' should have knowledge regarding oral manifestations of diseases which are of public health importance [2]. Less than one-third of subjects in our study had knowledge regarding oral manifestations of monkeypox. This could be due to the reason that dental professionals do not encounter cases of monkeypox routinely in their practice as it is an uncommon infectious disease and India falls under non-endemic category.

Effective disease control requires a strong partnership between clinicians and public health personnel. It is the responsibility and duty of medical practitioners to notify public health authorities in case they encounter a patient suspected of having a disease designated as PHEIC to allow prompt and direct public health action if needed [6]. However, findings of the study revealed that 33.2% of subjects felt no need to report such cases (monkeypox) to government authorities.

This study also found that proportion of subjects possessing high knowledge scores were more in case of post graduates as compared to graduates (p=0.024), stressing the positive impact of education on knowledge which can prove vital in the prevention of disease. Moreover, academicians significantly reported higher knowledge scores as compared subjects engaged in private practice (p=0.012). This could be due to the reason that subjects engaged in academic teaching are exposed to more learning experiences which can lead to regular knowledge updating. Online media (Internet) was preferred as the main source to obtain more information on monkeypox as compared to other sources. The use of Internet in the health domain is becoming a major worldwide trend. Internet is

exploding with information and is being used for health purposes by great deal of population through mobile phones, electronic health records, social media etc. [5].

The findings of the present study should be interpreted in light of some limitations. As studies on knowledge of dental professionals regarding monkeypox were almost non-existent in literature, the results of the study have been compared to studies engaging other health professionals. Secondly, as this was a questionnaire based study and relied upon self-reported data, so there can be possibility of social-desirability bias. Moreover, the study population didn't include subjects working in government set up or institutions as obtaining permission from government authorities is a tedious procedure and takes lot of time. Lastly, the study focussed on limited sample size, therefore more studies engaging a larger sample should be conducted in future as this was first of its kind study providing baseline information.

CONCLUSION

The results of the study reveal that level of knowledge regarding monkeypox among study subjects was low as less than one-third of subjects showed high knowledge scores. This was expected as one-fourth of study subjects never heard about monkeypox. I think it is vital for dental professionals to have knowledge regarding new emerging infectious diseases and the public health response that often shifts as we learn new information. So many dental professionals were caught off guard during the COVID-19 pandemic and the changing public health measures. Given the large livestock population and human population that live close to forests and wildlife habitats, India is a hotspot for zoonotic diseases. Currently, Monkeypox may not be a cause for major concern but it is imperative we remain vigilant and there is no room for complacency, considering how rapidly diseases get transmitted across a vastly populous country. To prevent transmission, there is an urgent for round-the-clock surveillance, along with prompt identification and isolation of confirmed cases.

Conflict of interest

Authors declare no conflict of interest.

REFERENCES

1. *Aggarwal A., Nirola A., Singh R., Goel R., Gupta A., Gambhir R.S.:* Omicron sub-variants: Is the world going to witness another wave? Rocz Panstw Zakl Hig. 2022;73:159-162.
2. *Brown K., Leggat P.A.:* Human Monkeypox: Current State of Knowledge and Implications for the Future. Trop Med Infect Dis. 2016;1:8. doi: 10.3390/ tropicalmed1010008.
3. *Bunge E.M., Hoet B., Chen L., Lienert F., Weidenthaler H., Baer L.R., Steffen R.:* The changing epidemiology of human monkeypox-A potential threat? A systematic review. PLoS Negl Trop Dis. 2022;16:e0010141. doi: 10.1371/journal.pntd.0010141.
4. Factbox: Monkeypox cases around the world. Available at: https://www.reuters.com/business/healthcare-pharmaceuticals/monkeypox-cases. Accessed on: 19th July 2022.
5. *Fernández-Luque L., Bau T.:* Health and social media: perfect storm of information. Health Inform Res. 2015;21:67-73. doi: 10.4258/hir.2015.21.2.67.
6. *Ferson M.J.:* Notification and Disease Control: Obligations of the Medical Practitioner Under Public Health Legislation. Legal and Forensic Medicine. 2013;707–25.
7. *Gong Q., Wang C., Chuai X., Chiu S.:* Monkeypox virus: a re-emergent threat to humans. Virol Sin. 2022:S1995-820X(22)00120-1. doi: 10.1016/j.virs.2022.07.006.
8. *Harapan H., Setiawan A.M., Yufika A., Anwar S., Wahyuni S., Asrizal F.W., Sufri M.R., Putra R.P., Wijayanti N.P., Salwiyadi S., Maulana R., Khusna A., Nusrina I., Shidiq M., Fitriani D., Muharrir M., Husna CA., Yusri F., Maulana R., Andalas M., Wagner AL., Mudatsir M.:* Knowledge of human monkeypox viral infection among general practitioners: a cross-sectional study in Indonesia. Pathog Glob Health. 2020;114:68-75. doi: 10.1080/20477724.2020.1743037.
9. *Hatfield S.:* An Overview of Monkeypox for Dental Hygienists & Other Dental Professionals. Available from: https://www.todaysrdh.com. Accessed on: 22nd July 2022.
10. *McCollum A.M., Damon I.K.:* Human monkeypox. Clin Infect Dis. 2014;58:260-7.
11. Monkeypox. World Health Organization. Available at: https://www.who.int/news-room/fact-sheets/detail/ monkeypox. Accessed on: 18th July, 2022
12. *Morand A., Delaigue S., Morand J.J.:* Review of poxvirus: emergence of monkeypox. Med Sante Trop. 2017;27:29-39. doi: 10.1684/mst.2017.0653.
13. *Petersen E., Kantele A., Koopmans M., Asogun D., Yinka-Ogunleye A., Ihekweazu C., Zumla A.:* Human Monkeypox: Epidemiologic and Clinical Characteristics, Diagnosis, and Prevention. Infect Dis Clin North Am 2019;33:1027-43. doi: 10.1016/j. idc.2019.03.001.
14. *Sallam M., Al-Mahzoum K., Dardas L.A., Al-Tammemi A.B., Al-Majali L., AL-Naimat H., Jardaneh L., Al Hadidi F., Al-Salahat K., Al-Ajlouni E., AlHadidi N., Bakri F.G., Mahafzah A., Harapan H.:* Knowledge of Human Monkeypox and Its Relation to Conspiracy Beliefs among Students in Jordanian Health Schools: Filling the Knowledge Gap on Emerging Zoonotic Viruses. Medicina. 2022;58 :924.doi: 10.3390/ medicina 58070924.
15. *Saxena S.K., Ansari S., Maurya V.K., Kumar S., Jain A., Paweska J.T., Tripathi A.K., Abdel-Moneim A.S.:* Re-

emerging human monkeypox: A major public-health debacle. J Med Virol 2022 . doi: 10.1002/jmv.27902.
16. *Sklenovská N., Van Ranst M.:* Emergence of Monkeypox as the Most Important Orthopoxvirus Infection in Humans. Front Public Health 2018;6:241. doi: 10.3389/fpubh.2018.00241.
17. *Tambo E., Al-Nazawi A.M.:* Combating the global spread of poverty-related Monkeypox outbreaks and beyond. Infect Dis Poverty 2022;11:80. doi: 10.1186/s40249-022-01004-9.
18. WHO. WHO declares monkeypox global health emergency. Available at: http://www.tribuneindia.com. Accessed on 24th July 2022.

Received: 28.07.2022
Accepted: 06.08.2022

Rocz Panstw Zakl Hig 2022;73(3):373-376
http://wydawnictwa.pzh.gov.pl/roczniki_pzh/

INSTRUCTION FOR AUTHORS

Quarterly ***Roczniki Państwowego Zakładu Higieny*** / ***Annals of the National Institute of Hygiene*** is the peer reviewed scientific journal that publishes original articles, reviews, short communications, letters to the editor and book reviews. Journal is devoted to the studies concerning scientific problems of food and water safety, nutrition, environmental hygiene, toxicology and health risk assessment, public health and other areas related to health sciences.
Since 2013, Volume 64, number 1 all the papers are published in English.

All articles for publication in Roczniki Panstwowego Zakladu Higieny / Annals of the National Institute of Hygiene (Rocz Panstw Zakl Hig) should be submitted electronically via e-mail: roczniki.pzh@pzh.gov.pl

Fees
Since 1st January 2018 a fee of 650 PLN for Polish Authors (including VAT) and 150 EUR for foreign Authors is introduced for publication of paper in Roczniki Panstwowego Zakladu Higieny / Annals of the National Institute of Hygiene. The fee will be charged after acceptance of the manuscript for publication.

Payment information
National Institute of Public Health NIH – National Research Institute, 24 Chocimska street, 00-791 Warsaw, Poland
Payment from Poland: Bank Gospodarstwa Krajowego nr: 93 1130 1017 0080 1180 2020 0001
Payment from other countries: IBAN PL 93 1130 1017 0080 1180 2020 0001, SWIFT: GOSKPLPW
Title of the payment: Fee for publication of the article: article number, name of the first Author, title of the article.
The cost of bank charges is covered by the Authors.
The invoice is issued by the publisher: National Institute of Public Health NIH - National Research Institute, after the fees have been transferred on the publisher's bank account.

RULES FOR THE ACCEPTANCE OF THE MANUSCRIPTS

Editorial policy and basic information

Only high scientific quality articles complying with the scope of the journal will be considered for the publication.

Copyright. All the copyrights to the articles published in the ***Roczniki Państwowego Zakładu Higieny*** are reserved for the publisher: National Institute of Public Health NIH - National Research Institute. It means that after article is published the authors transfer the copyright to the publisher and cannot publish this article or its parts elsewhere for commercial purposes without the written permission from the publisher.

License Creative Commons. Submitting the manuscript to Editorial Office means that the Authors declares that they have read the full text of the Creative Commons Attribution – Non Commercial 3.0 Poland license (CC-BY-NC) available at: http://creativecommons.org/licenses/by-nc/3.0/pl/deed.en.

Ethics. Clinical articles should comply with the generally accepted ethical standards and the Helsinki Declaration. For animal experiments reported in the articles the author(s) must obtain the acceptance by the relevant local Ethics Commission.

Conflict of interest. Conflict of interest exists if authors or their institutions have financial or personal relationships with other people or organizations that could inappropriately influence their actions. Such relationships should be disclosed to the publisher. All authors should provide a signed statement of their conflict of interest as a part of the author's statement form.

Transparency. Openness of information on any party contributing to preparation of a publication (content related, financial, etc. input) is proof of ethical attitude of a researcher and of high editorial standards.

"Ghostwriting"* and *"guest authorship" are indications of scientific dishonesty and all cases will be exposed and adequate institutions will be informed. "Ghostwriting" is a situation where a person contributes significantly to a publication and is not disclosed as one of the authors or named in the acknowledgments and "guest authorship" is a situation where an author's contribution is insignificant or nonexistent and he is still listed as author/ co-author of a publication.
The editor requires from the authors of the articles that they reveal the contribution of individual authors to the manuscript, i.e. who is the author of the concept and study design, data/material collection, study/analysis, performance, statistical analysis, interpretation of the results, manuscript development etc. Authors of the manuscript should provide "Authors' statement" form signed by the all authors. Also the information concerning the sources of financial support to the study presented in the submitted manuscript should be provided.

Ethical standards and procedures

The journal applies the principles of publication ethics in accordance with the Committee on Publication Ethics (COPE) guidelines https://publicationethics.org/

Peer review procedure for manuscripts

1. The manuscripts submitted to the ***Roczniki Państwowego Zakładu Higieny*** undergo preliminary evaluation in the editorial office to determine whether the topic is within the scope of the journal and to evaluate the adherence to the journal format, as well as to the rules of acceptance. In cases when the above prerequisites are not met the manuscript is not qualified, and the corresponding author is informed.
2. Following the positive preliminary evaluation the paper is directed for peer review to at least two independent and recognized experts representing the scientific experience in the field covered by the manuscript and affiliated in the different institution than the institution(s) where the authors have been affiliated. The reviewers must ensure independence and lack of conflict of interests.
3. During the entire reviewing the double-blind review process is maintained.
4. The review in the written form is forwarded to the corresponding author with the accompanying statement suggesting status of the paper as: "accepted", "accepted after revisions" or "not accepted". The **Review form** is available on the journal's website: http://wydawnictwa.pzh.gov.pl/roczniki_pzh/for-reviewers.
5. The list of reviewers is published once a year in the fourth issue of each volume and is available on the journal's website: http://wydawnictwa.pzh.gov.pl/roczniki_pzh/reviewers

Submission of manuscripts

For the publication the following attachments must be submitted:

1. **Cover letter**, in which the corresponding author applies for the publication of the paper in the *Roczniki Państwowego Zakładu Higieny*. The cover letter, signed by the corresponding author, should contain the name of the institution where he is affiliated, address, phone and fax numbers, e-mail. The corresponding author is requested to suggest two or three potential reviewers (including their affiliations and e-mails) from institutions other than the author's one.
2. **Author(s)' statement**, signed by all authors, stating that the manuscript complies with the general rules set for the scientific articles and was not published and/or submitted for publication elsewhere and will not be send for publication to the other journal, and there is no infringement of property rights to any interested third parties. In the case of experiments on animals the approval of the relevant ethics commission is required. The clinical studies must be accompanied by the written statement, signed by the authors confirming that the research was conducted in accordance with the Helsinki Declaration. The contribution of the individual authors to preparation of a publication should be specified. All authors are required to sign the statement of their conflict of interest as a part of the author's statement form. **Author's statement form** is available on the journal's website: http://wydawnictwa.pzh.gov.pl/roczniki_pzh/media/Authors_statement_v.2.pdf
3. **Manuscript**. Text of the manuscript, tables and figures (in the separate files) should be sent by the e-mail: roczniki.pzh@pzh.gov.pl.

Arrangement of manuscripts

Manuscripts submitted for publication should be typed by the editor Microsoft Word using Times New Roman 12 font and 1.5 space between lines on A4 paper size.

Title page. It should contain the title of the paper in English and the name(s) and surname(s) of the author(s), the complete name(s) of the institution(s) where the work was performed, address of the corresponding author's institution, phone and/or fax numbers and the e-mail inserted at the bottom of the title page. If appropriate, the number and the title of the project under which the study has been carried out should also be given on this page as a footnote.

Abstract. It should contain 250-300 words and consist of the following sections: Background, Objective, Material and methods, Results, Discussion and Conclusions. The abstract of a review should not be divided into sections.

Key words. Should be placed after the abstract. 3-6 words or short phrases according to the MeSH (*Medical Subject Headings Index Medicus*) catalogue available at www.nlm.nih.gov/mesh/meshhome.html

Polish authors are asked to attach the abstract and keywords also in Polish language.

Text. The text of the manuscript should be divided into the following sections: Introduction, Material and methods, Results, Discussion, Conclusions, Acknowledgements, References.

Introduction. It should contain the scientific rationale and the aim of the study or in the case of a review the purpose of the article. Only references related to the paper should be cited.

Material and methods. This section should provide detailed information on the subject of the study, methods, chemicals, apparatus and techniques used in sufficiently exhaustive way to enable readers to repeat the experiments or observations. For generally known methods references should be given together with name of the methods or statistical analysis used in the study. For new or substantially modified methods detailed descriptions are to be added. In the case of experimental studies on laboratory animals, the information should be provided on the approval by a local Ethics Commission, or in the case of clinical studies that they have been performed with the ethical standards according to the Helsinki Declaration.

Results. These should be presented in a logical sequence in the text, the same applies to the tables and figures. The data from the tables and figures should not be repeated in the text, where only the most important observations from the studies are to be summarized. The place where the tables, figures or photographs should appear in the text should be indicated.

Discussion. Emphasize the new and important aspects of the results and a comprehensive interpretation of the results obtained against the background of results obtained by other authors. Quotations should be restricted to those with immediate relevance to the author's findings.

Conclusions. They should be stated precisely in points or descriptively and should be logically connected with the aims stated in the introduction. Statements and conclusions not derived from own observations should be avoided. If a hypothesis is proposed it must be stated clearly.

Acknowledgements. These should be placed directly after the Conclusion section**.** One or more statements should specify: (1) persons who contributed substantially to the study but cannot be regarded as authorship, such as technical assistants, statisticians, data collectors etc. Their assistance should be acknowledged for the sake of transparency. It must be clear that they are not responsible for the final version of the article. You must ensure you have the consent of all the persons named in the acknowledgements; (2) all sources of financial and material support, which should specify the nature of the support. The recommended form is: "This work was supported by: (name of the organization, project number etc.)"; (3) relationships that may pose the conflict of interest.

References. References should be presented according to the Vancouver referencing style. Citation of sameone else's publication in the text should be indicated by the use of a number (Arabic numerals in square brackets). References should be cited in the order in which they first appear in the text. If the same publication is cited again, the same number should be given. The journal title should be abbreviated in accordance with MEDLINE. Each position in the list of references should start from the new line and contain: consecutive number, author's (authors') surname(s) and initials of name(s) (written in italic), full title of the paper, official journal title abbreviation, year, volume, the first and the last page number of the paper. If the article or book has a DOI number ('Digital Object Identifier'), it should be included in the references. The titles of the cited papers in other language than English should be also translated into English [and given in square brackets]. The information on the original language should be given in the brackets after the page numbers, for example: (in Polish). The examples of reference style are given below:

Article in a periodical:

1. *Snopczyński T.*, *Struciński P.*, *Góralczyk K.*, *Czaja K.*, *Hernik A.*, *Korcz W.*, *Kucharska A.*, *Ludwicki J.K.*: Zastosowanie metody QuEChERS w połączeniu z chromatografią gazową z detektorem wychwytu elektronów (GC-ECD) w analizie pozostałości pestycydów w żywności [Application of the QuEChERS method coupled with gas chromatography with electron capture detection (GC-ECD) in analysis of pesticide residues in food]. Rocz Panstw Zakl Hig 2011;62(2):145-151 (in Polish).
2. *Lopes M.*C.*, Giudici K.V.*, *Marchioni D.M.*, *Fisberg R.M.*, *Martini L.A.*: Relationships between n-3 polyunsaturated fatty acid intake, serum 25 hydroxyvitamin D, food consumption, and nutritional status among adolescents. Nutr Res 2015;35(8):681-688.

3. *Shridhar G.*, *Rajendra N.*, *Murigendra H.*, *Shridevi P.*, *Prasad M.*, *Mujeeb M.A.*, *Arun S.*, *Neeraj D.*, *Vikas S.*, *Suneel D.*, *Vijay K.*: Modern diet and its impact on human health. J Nutr Food Sci 2015;5:6 doi:10.4172/2155-9600.1000430.
4. *Wu M.*, *Yu G.*, *Cao Z.*, *Wu D.*, *Liu K.*, *Deng S.*, *Huang J.*, *Wang B.*, *Wang Y.*: Characterization and human exposure assessment of organophosphate flame retardants in indoor dust from several microenvironments of Beijing, China. Chemosphere 2015, doi:10.1016/j.chemosphere.2015.12.111.

Books and chapter in a book:

5. *Riley D.M., Fishbeck P.S.*: History of methylene chloride in consumer products. In: *Salem H.*, *Olajos E.J.* (eds.). Toxicology in Risk Assessment. London, Taylor & Francis, 2000.

Legislative acts:

6. Commission Regulation (EC) No 1881/2006 of 19 December 2006 setting maximum levels for certain contaminants in foodstuffs. Off J EU L 364, 20.12.2006.

Internet source:

7. The Rapid Alert System for Food and Feed (RASFF) Portal. Available
8. https://webgate.ec.europa.eu/rasff-window/portal (accessed 18.10.2010)

Tables. These should be printed on separate sheets and numbered using Arabic numerals. The title should be place directly above each table. Tables should always be cited in the text in consecutive numerical order. Each column in tables should have a brief heading, more extensive explanation should be given below the table, if necessary. The number of tables should be limited to indispensable for the documentation of results.

Figures and photographs. These **s**hould be numbered in Arabic numerals according to the sequence of their appearance in the text. Figures should be made by computer technique and the titles should be placed below the figures. Photographs must be of high quality, digital format is preferred.

Tables, figures or photographs should be cited in the text in consecutive numerical order in the following way: (Table 1), (Figure 1), (Photo 1).

Abbreviations, symbols, units. Generally known and used abbreviations may be left unexplained, others must be explained at the first use in the text. Metric SI units are recommended, however also other generally used units are accepted.

General information

The editor reserves the right for introducing the editorial corrections in the manuscript which will not influence the scientific contents of the article without prior informing the author.

SUBSCRIPTION

The subscription information is available at:

Library of the National Institute of Public Health NIH - National Research Institute
24 Chocimska street, 00-791 Warsaw, Poland

Phone: +48 22 54 21 262 or +48 22 54 21 264
e-mail: biblioteka@pzh.gov.pl

Roczniki Państwowego Zakładu Higieny - Annals of the National Institute of Hygiene may be ordered through the Library of the National Institute of Public Health NIH - National Research Institute mailing address: biblioteka@pzh.gov.pl

Payment

The payment should be made after invoice receipt to the following account:
Bank Gospodarstwa Krajowego nr: 93 1130 1017 0080 1180 2020 0001
National Institute of Public Health NIH - National Research Institute
24 Chocimska street, 00-791 Warsaw, Poland

Annual subscription in Poland in year 2022 (Vol. 73, 4 issues): 120,00 PLN

Indeks 37468

ROCZNIKI PAŃSTWOWEGO ZAKŁADU HIGIENY
[ANNALS OF THE NATIONAL INSTITUTE OF HYGIENE]

Volume 73 **2022** **Number 3**