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ROCZNIKI PAŃSTWOWEGO ZAKŁADU HIGIENY
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OD REDAKTORA NACZELNEGO

Szanowni Państwo,

Pragnę poinformować, że aktualny drugi numer Roczników Państwowego Zakładu Higieny (Annals of National Institute of Hygiene) jest jednocześnie pierwszym, przygotowanym w całości przez nowy Zespół Redakcyjny pod moim kierownictwem, jako redaktor naczelnej. Jest on również pierwszym numerem przygotowanym z wykorzystaniem elektronicznego systemu redakcyjnego, który mam nadzieję ułatwi, zarówno Autorom, jak i Redakcji, procedowanie manuskryptów.

Serdecznie dziękuję Pani dr Kazimierze Ćwiek-Ludwickiej, długoletniej redaktor naczelnej Roczników PZH, za życzliwe podzielenie się swoim doświadczeniem w redagowaniu czasopisma oraz pomoc i wsparcie we wdrażaniu nowego systemu redakcyjnego.

Jednocześnie pragnę zapewnić, że profil czasopisma nie ulega zmianie, nadal będą publikowane prace z obszaru bezpieczeństwa żywności i żywienia, badań nad składnikami żywności i ich rolą w profilaktyce chorób żywieniowozależnych, oceną sposobu żywienia i nawyków żywieniowych oraz zdrowiem publicznym.

Zachęcam Państwa do zapoznania się z artykułami opublikowanymi w aktualnym numerze czasopisma, w którym znajdują się m.in. prace dotyczące badań nad składnikami żywności i ich rolą w profilaktyce chorób żywieniowozależnych (*Mrázová J. et al. The effect of regular chokeberry juice consumption on anthropometric and lipid parameters in women with overweight or obesity*), bezpieczeństwem żywności (*Chaiwong K. et al. Pesticide exposure and blood cholinesterase levels among adolescents from farming families in northern Thailand*), oceną wiedzy żywieniowej (*Wyka J. et al. Assessment of knowledge and nutritional status of students before and after nutritional education*) i wykorzystaniem nowoczesnych metod do oceny stanu odżywienia (*Gažarová M. et al. The use of portable abdominal bioimpedance analyzer Yscope in the assessment of abdominal obesity*).

Zapraszam Państwa do publikowania w Rocznikach PZH.

Z poważaniem,

dr hab. Hanna Mojska, prof. NIZP PZH – PIB
Redaktor naczelna
Roczników Państwowego Zakładu Higieny

EDITORIAL INTRODUCTION

Ladies and Gentlemen,

I have a pleasure to inform you that the current second issue of the journal Roczniki Państwowego Zakładu Higieny (Annals of the National Institute of Hygiene) is also the first one prepared entirely by the new Editorial Board under my leadership as Editor-in-Chief. It is also the first issue prepared using an electronic Editorial System, which I hope will make it easier for both Authors and the editing staff to process manuscripts.

I would like to thank Dr. Kazimiera Ćwiek-Ludwicka, the long-time Editor-in-Chief of the Roczniki Państwowego Zakładu Higieny, for kindly sharing her experience in editing the journal and for her help and support in implementing the new Editorial System.

At the same time, I would like to assure you that the profile of the journal does not change, it will continue to publish manuscripts in the area of food safety and nutrition, research on nutrients and other active food ingredients and their role in the prevention of nutrition-related diseases, assessment of diet and eating habits, and public health.

I encourage you to read the published articles in the current issue of the journal, which includes, among others: works on research on food ingredients and their role in the prevention of nutrition-related diseases (*Mrázová J. et al. The effect of regular chokeberry juice consumption on anthropometric and lipid parameters in women with overweight or obesity*), food safety (*Chaiwong K. et al. Pesticide exposure and blood cholinesterase levels among adolescents from farming families in northern Thailand*), assessment of nutritional knowledge (*Wyka J. et al. Assessment of knowledge and nutritional status of students before and after nutritional education*) and the use of modern methods to assess nutritional status (*Gažarová M. et al. The use of portable abdominal bioimpedance analyzer Yscope in the assessment of abdominal obesity*).

I would like to invite you to publish in the Roczniki Państwowego Zakładu Higieny (Annals of the National Institute of Hygiene).

Kind regards,

Assoc. Prof. Hanna Mojska, PhD
Editor-in-Chief
Roczniki Państwowego Zakładu Higieny

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ORIGINAL ARTICLE

ANTIMICROBIAL ACTIVITIES OF ESSENTIAL OILS OF PLANTS SPECIES FROM MOROCCO AGAINST SOME MICROBIAL STRAINS

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ABSTRACT

Background. Essential oils have important antibacterial activities and can successfully replace antibiotics, which show their inefficiency, especially against fungi and multi-resistant bacteria.
Objective. The main purpose of our research was to investigate the antibacterial and antifungal activity of essential oils from fifteen plants harvested in the Taroudant region.
Material and Methods. In this work, the essential oils were extracted by hydrodistillation using a Clevenger-type apparatus. The method of disc diffusion in agar medium (aromatogram) is the one used to evaluate the activity of these essential oils against four pathogenic bacteria (*Staphylococcus aureus, Escherichia coli, Bacillus* sp., and *Enterococcus cloacae*) and two yeasts (*Candida albicans* and *Cryptococcus neoformans*).
Results. Our findings, show that all of the plants' leaves yielded extremely aromatic essential oils that differed in look and color. Furthermore, the 93.33% of the fifteen essential oils that were evaluated proved to be effective against at least one kind of bacteria or fungus. This suggests that the proportion of essential oils with no antibacterial action was rather low, at around 7%. Our data also showed that the freshness or dryness of the plant at the time of harvest could affect the extraction rate of essential oils. This screening showed us that these essential oils present inhibitory activities towards the studied Gram+ bacteria, as well as a resistance against Gram-, in particular *Enterococcus cloacae*.
Conclusion. These essential oils can therefore be used in the prevention and treatment of certain infectious diseases and to fight against bacteria that are multi-resistant to the usual antibiotics.

Keywords: *antibacterial activity, antifungal activity, Bacillus, Cryptococcus, Candida, Staphylococcus, essential oils*

INTRODUCTION

Plants have long held significant significance in human daily existence [1]. Traditional medicinal plants, widely utilized due to their affordability, accessibility, and lack of evidence of resistance or inefficacy to whole plant extracts, have been integral to human healthcare practices [2]. This traditional medicinal wisdom has been consistently upheld within households and transmitted through generations over time. Moreover, Man has always been inspired by nature, using its resources, especially plants for his medical and food needs. This has led scientists, over the centuries, to develop their knowledge of medicinal plants. Indeed, the remedies of good reputation have prevailed in spite of the development of modern medicine, which has marginalized the recourse to natural medical techniques [3]. Medicinal and aromatic plants (MAPs) play a significant role in the food and cosmetic industries, but they are also crucial for pharmacological research and the development of drugs. Plant components are used as building blocks for the synthesis of medications and as therapeutic agents, serving as models for compounds with

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pharmacological activity [4]. Nowadays, the use of plants as a means of treatment is still very important for many rural and urban Moroccans citizens [5]. In developing countries, bacterial infections take a heavy toll, causing millions of deaths each year [6]. As well as fungi, they are the cause of many diseases in animals and plants. However, The effectiveness of medicines like antibiotics, considered the universal solution to dangerous infections decreases, due to increasing resistance of bacteria [7]. With its diverse geographical contrasts, Morocco provides a wide spectrum of bioclimates, facilitating the establishment of a diverse flora. Scientific studies estimate that there are about 4500 species and subspecies and a diversity of phylogenetic resources in MAPs [8]. In this context Morocco has long been a provider of MAPs to the global market. This practice involves taking use of both cultivated and wild species. For the food herb trade, a number of items (more than 70) are transported as dried plants. More than twenty species are utilized to produce aromatic extracts, or essential oils (EOs), mostly for the perfume and cosmetics industries. These extracts are also used to prepare sanitary goods and flavor formulations [9]. However, the area of southwest Morocco, especially the region of Taroudant, is known for its richness and diversity of flora including MAPs, which account for almost one-third of the total flora of the country [10]. Given that the region of Taroudant is a region of rural life and given its wealth of aromatic and medicinal plants, traditional medicine is much practiced and often uses aromatic and medicinal plants. These plants are not valued for their biological potential. Moreover, the discovery of antibiotics and synthetic antifungals has caused the decline of herbal medicine and relegated it to a secondary rank. In addition to their food and cosmetic properties, MAPs contain natural substances with antibacterial and antifungal properties.

Based on the above, the aim of our research was to evaluate the antibacterial and antifungal activity of essential oils extracted from plants in the Taroudant region against selected microbial strains. We assessed the efficacy of these oils using two key parameters: minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC).

MATERIAL AND METHODS

Biological materials

Fifteen plants were collected manually in their natural habitat in the region of Taroudant, Morocco, and transported in paper and plastic bags. Some of these plants were collected in the region of Ouzioua (latitude: 30.7207°, longitude: -8.0349, altitude: 1352 meters); others were collected in the region of Ouled Berhil (latitude: 30.646397, longitude:-8.479927). The identification of the studied medicinal and aromatic plants is carried out according to Bellakhdar [11] and is reported in (Table 1). The pictures of the studied medicinal and aromatic plants are reported in (Figure 1).

Figure 1. Pictures of the 15 medicinal and aromatic plants harvested

Table 1. Common and scientific name of the plants studied

Common name	Plant specie
Sage	*Salvia officinalis*
Rosemary	*Rosmarinus officinalis* L.
Vermifuge Anserine	*Chenopodium ambrosioides* L.
Marjoram with shells	*Origanum majorana* L.
Round-leaved mint	*Mentha suaveolens*
Rose geranium	*Pelargonium graveolens*
Absinthe	*Artemisia absinthium*
Tooth-leaved lavender	*Lavandula dentata* L.
Thyme	*Thymus vulgaris*
Pennyroyal mint	*Mentha pulegium* L.
White mugwort	*Artemisia herba-alba*
Cypress	*Cupressus atlantica*
Green mint	*Mentha spicata*
Basil	*Ocimum basilicum*
Lemongrass	*Cymbopogon citratus*

Essential oil extraction

For each plant, the EOs extraction was made from a fresh sample and a dried sample. For drying, the plants were air-dried, protected from light and humidity, or even oven-dried at 45°C. The samples were finely ground to create small particles, which facilitated the extraction process. Each sample weighing 100 g underwent extraction through water distillation using a Clevenger apparatus for three hours, in accordance with the guidelines outlined in the European Pharmacopoeia [12]. The weight of the EOs collected post-sodium sulfate dehydration was meticulously measured, and the resultant EOs was stored in darkness at 4°C until required. The EOs yield was calculated based on weight percentage (w/w). This procedure was repeated three times for each plant. Subsequently, the average yield of EOs from three repetitions for each site was reported as the mean ± standard deviation. The essential oils were stored in dark-colored bottles in a refrigerator (+4°C) to shield them from light and heat.

Microorganisms and media

Concerning the evaluation of the biological activities of these plant extracts, Four bacterial and two fungal species were included in the antimicrobial test strains; these were obtained from different culture collections, such as the National Cultures Collection of Microorganisms at the Pasteur Institute in Paris, France; the Collection of Pasteur Institute (CIP); and the Fungi Culture Collection (FCC) of the former. *Candida albicans* CIP884.65 and *Cryptococcus neoformans* ATCC11576 were the strains of fungi, while *Bacillus* sp. CIP104717, *Escherichia coli* CIP54127, *Staphylococcus aureus* CIP209 (ATCC25923), and *Enterobacter cloacae* ATCC13047 were the strains of bacteria. These strains were cultured and maintained on Luria Bertani agar medium for bacteria and Sabouraud's agar medium for yeast [13].

Agar disc-diffusion assay

The antimicrobial activity screening of the essential oils utilized the agar disk-diffusion method [14], employing Muller Hinton Agar (MHA) medium [Difco] for antibacterial assessment and yeast morphological agar (YMA) medium [Difco] for antifungal evaluation. Bacterial and yeast inoculums were prepared by suspending colonies from 24-hour cultures on Luria Bertani and Sabouraud's agar medium, respectively, in Galerie API NaCl 0.85% medium (3 ml) from Biomerieux. The cell density was determined using a Biomerieux ATB 1550 densitometer and adjusted to 104 CFU/ml for yeast and 106 CFU/ml for bacteria. Each cellulose disk (6 mm in diameter) saturated with 20 µL of EOs was applied to the test media, which were previously inoculated with each test strain. The agar plates were kept at 4°C for at least 2 hours to allow the diffusion of essential oils, and then incubated at 37°C for bacteria or 28°C for fungus. The inhibition zones were measured after 24 h of incubation for bacteria and 48 h for fungus. Standard disks (30 µg) of rifampicin and chloramphenicol served as antibacterial positive controls, while Fluconazole and Econazole served as antifungal positive controls. All tests were performed in triplicate. The antimicrobial activity was determined in millimeters using a ruler measuring the diameter of the inhibition zone, and the result was the average of the three tests. The scale of antimicrobial activity estimation is given by Mutai et al. [15] they classified the inhibition zones into five classes (Table 2).

Table 2. Estimation scale of antimicrobial activity [15]

Diameter (D) of inhibitory zone	Activity
D ≥30 mm	Very strongly inhibitory
21 mm≤ D ≤29 mm	Strongly inhibitory
16 mm≤ D ≤20 mm	Moderately inhibitory
11 mm≤ D ≤16 mm	Slightly inhibitory
D <10 mm	Non-inhibitory

RESULTS AND DISCUSSION

Medicinal plants are an important part of traditional medicine in many parts of the world, including our study region, where people have traditionally relied on plant extracts to treat acute illnesses such as bacterial and fungal infections. For our study, we chose plants

that are locally cultivated and have been used for generations by the community to treat infections.

Given the points mentioned above, the fifteen medicinal and aromatic plants studied belong to six families. Nine species, *Salvia officinalis, Rosmarinus officinalis* L.*, Origanum majorana* L.*, Mentha Suaveolens, Lavandula dentata* L.*, Thymus vulgaris, Mentha pulegium* L.*, Mentha spicata* and *Ocimum basilicum* belong to the *Lamiaceae* family; two species, *Artemisia absinthium* and *Artemisia herba-alba,* belong to the *Asteraceae* family; and only one species belongs to each of the families of *Amaranthaceae, Geraniaceae, Cupressaceae,* and *Poaceae*, which are respectively *Chenopodium ambrosioides* L.*, Pelargonium graveolens, Cupressus atlantica,* and *Cymbopogon citrates.*

The EOs from the leaves of the fifteen plants collected by hydrodistillation all have strong and persistent smells, but their color and appearance are variable among species. The results of the EOs yields of the plants studied according to the fresh or dry phase of the plant are summarized in (Table 3). We observed that the EOs yields of the plants in our study ranged from 0.1% to 3.73%. *Thymus vulgaris* presents the highest yield (3.73%), followed by *Arthemisia herba-alba* (3.59%). *Cupressus atlantica* has the lowest yield.

The results of the assay show that yield depends on the physiological state of the plant. For the same parameters, *Chenopodium ambrosioides* L. yields more essential oil when fresh, while *Rosmarinus officinalis* L. and *Mentha suaveolens* yield more when dried. In order to give more value to our work, a comparison of the yields of essential oils obtained in our study with those obtained in previous research works showed that in the case of *Rosmarinus officinalis* L. (yield = 0.5%), whereas Wang et al. [16] obtained a yield of 0.76%. This difference (of 0.26%) may be due to the various factors that come into play; among these factors we can cite: the nature of the soil, the harvest period, and the drying time. For *Cupressus atlantica,* the yield of essential oils that we obtained by hydrodistillation is 0.1%. The latter is significantly lower than that obtained by steam distillation (0.41%) in the study of Amara and Boughérara [17]. However, the findings of Mouden et al. [18], suggest that this difference in yield is due to the extraction technique. The distillation time influences not only the yield but also the composition of the extract [19]. The comparison of the yield of *Salvia officinalis* (0.15%) with that of other research works showed that this value is lower than the results obtained by Benkherara et al. [20] on the same species, whose yields of EOs obtained are between 1 and 2.5%. However, the yield of *Thymus vulgaris* (3.73%) is higher than the yield found by El-Akhal [21] (1%) of the same species harvested during the period March–June 2010 from different stations in central Morocco. This variation may be due to environmental and climatic factors and the extraction techniques used. The outcomes of the antibacterial and antifungal activities of the EOs against pathogenic bacteria and fungi are outlined in Tables 4 and 5, respectively. In addition, the Antibacterial and antifungal tests showed that of the 15 essential oils tested, 14 essential oils exhibited antimicrobial activity against at least one target bacteria or fungus. Either (93.33%) of the essential oils tested showed antibacterial and antifungal activity, while only (6.66%) of the essential oils tested had no

Table 3. Essential oil yields of the plants studied

Plants	Yield (%)	
	Fresh leaves	Dry leaves
Salvia officinalis	0.15	-
Rosmarinus officinalis L.	0.32	0.5
Chenopodium ambrosioides L.	0.5	0.26
Origanum majorana	-	2
Mentha suaveolens	0.8	0.9
Pelargonium graveolens	-	0.33
Artemisia absinthiul L.	0.6	-
Lavandula dentata L.	-	2
Thymus vulgaris	-	3.73
Mentha pulegium L.	2	-
Arthemisia herba-alba	-	2.4
Cupressus atlantica	0.1	-
Mentha spicata	-	0.6
Ocimum basilicum	0.3	-
Cymbopogon citratus	0.6	-

Table 4. *In vitro* antimicrobial activity of essential oils

Essential oil	Diameter of the inhibition zones in mm			
	Gram positive bacteria		Gram negative bacteria	
	Bacillus sp. *CIP104717*	*Staphylococcus aureus* CIP209	*Enterobacter cloacae* ATCC13047	*Escherichia coli* CIP54127
Salvia officinalis	0	9	9.66	12
Rosmarinus officinalis L.	0	10	0	8
Chenopodium ambrosioides L.	0	0	0	0
Origanum majorana	0	0	0	0
Mentha suaveolens	12.66	15.5	13.33	11.66
Pelargonium graveolens	13	0	0	15.5
Artemisia absinthiul L.	0	0	0	0
Lavandula dentata L.	0	7	11	12
Thymus vulgaris	0	27	27	37
Mentha pulegium L.	7	0	0	8.33
Arthemisia herba-alba	0	0	0	0
Cupressus atlantica	0	0	0	0
Mentha spicata	0	0	0	0
Ocimum basilicum	8	17.5	15	15.5
Cymbopogon citratus	26.5	19	0	13.33
Rifampicin (30 mg)	41.66	33	13,33	41.66
Chloramphénicol (30 mg)	18.66	30.66	13	38

effect on the microbial strains studied. This is the EOs extracted from *Arthemisia herba-alba*. In addition, the essential oils extracted from seven plants, i.e. 33.33% (*Mentha suaveolens, Artemisia absinthiul* L.*, Lavandula dentata* L.*, Thymus vulgaris, Mentha pulegium* L.*, Ocimum basilicum* and *Cymbopogon citratus*) are active both against the bacteria and yeasts studied. Among these essential oils, only three (*Mentha suaveolens, Lavandula dentata* L. and *Ocimum basilicum*) have the ability to inhibit all the strains studied. While the essential oils of the other plants tested (*Salvia officinalis, Rosmarinus officinalis* L.*, Chenopodium ambrosioides* L.*, Origanum majorana, Pelargonium graveolens, Cupressus atlantica* and *Mentha spicata*) only inhibited the bacteria. In this context, several studies have shown that essential oil from *Salvia officinalis* can exhibit activity against multi-resistant bacteria; the work of Benkherara et al. [20] showed that *Salvia officinalis* exhibits inhibitory activity even against the most resistant strains, such as *Pseudomonas aeruginosa*.

The analysis of the findings presented in Table 5 show that *Enterococcus cloacae* is resistant to most essential oils, a remarkable sensitivity of *Staphylococcus aureus* has been observed. The same results are obtained by Benkherara et al. [20], which confirms the sensitivity of *Staphylococcus aureus* and the resistance of *Enterococcus cloacae*. The findings of Bounihi's study [22] show that EOS have activity against all bacterial strains. Indeed, *Staphylococcus aureus* is the most sensitive, while *Pseudomonas aeruginosa* and *Esherichia coli* are the most resistant compared to the oils tested. Our results are in agreement with the literature, according to which gram-positive bacteria show the greatest sensitivity towards essential oils. Gram-positive bacteria are less protected against antibacterial agents because they lack a layer of peptidoglycan, which prevents the passage of essential oils through the cell wall. In this context, Multiple studies have demonstrated the antibacterial properties of EOs, particularly against bacteria that are multi-resistant [23, 24]. Terpenes, aromatic molecules, and the oxygenated compounds generated from them, such as terpenic alcohols, aldehydes, and ketones, are the active ingredients in essential oils [24, 25, 26]. The breakdown of bacterial membranes and walls by the phenolic chemicals in EOs is probably the source of its antibacterial effect.

The EOs of *Mentha suaveolens* and *Ocimum basilicum* are active against the four bacterial strains. *Thymus vulgaris* and *Cymbopogon citratus* show significant inhibition diameters in all three strains. According to the scale of estimation of antimicrobial activity given by Mutai et al. [15] *Mentha suaveolens* is slightly inhibitory of all strains, while *Ocimum basilicum* is slightly inhibitory of Gram-negative bacteria and exhibits moderate inhibition against *S. aureaus* and considered non-inhibitory against

Table 5. *In vitro* antifungal activity of essential oils

Essential oil	Diameter of the inhibition zones in mm	
	Candida albicans CIP884.65	*Cryptococcus neoformans* ATCC11576
Mentha suaveolens	30	45
Thymus vulgaris	37	24
Ocimum basilicum	16	14.67
Cymbopogon citratus	35	13.5
Fluconazole 30 µg	30	18.5
Econazole 30 µg	30	23.33

Bacillus sp. According to the same scale, *Thymus vulgaris* is highly inhibitory against *S. aureus* and *En. cloacae* and shows very strong inhibition against *E. coli*. The strong activity of this essential oil is probably due to its richness in phenolic compounds such as eugenol, thymol, and carvacrol, which possess strong antibacterial activity due to the acidity of their hydroxyl substituents [23]. The broad spectrum of EOs inhibition of different Gram+, Gram-, and even multi-resistant bacteria is due to the biodiversity of chemical compounds in essential oils. In this sense, a study of *Klebsiella pneumoniae*, which is resistant to all standard antibiotics with the exception of amoxicillin-clavulanate, was shown to be sensitive to the majority of the essential oils studied. These results confirm our hypothesis that essential oils can be used as an antibacterial and antifungal alternative for strains resistant to standard antibiotics. Furthermore, the antifungal activity was evaluated in vitro by measuring the diameters of the zones of inhibition. The results are shown in the Table 5.

Based on the results of this table, we find that *Candida albicans* and *Cryptococcus neoformans* are sensitive to the four essential oils. We can also point out the high sensitivity of *Candida albicans* compared to that of *Cryptococus neoformans.* Based on the scale given by Mutai [15]. EOs from *Mentha suaveolens*, *Thymus vulgaris,* and *Cymbopogon citratus* exert a very strong inhibition against *Candida albicans*. On the other hand, *Ocimum basilicum* presents moderate inhibition. Concerning *Cryptococcus neoformans*, it is very strongly inhibited by *Mentha suaveolens* with a diameter of 45 mm (more than the controls). Indeed, the effectiveness of these essential oils against the fungi studied is directly linked to their chemical compositions as oxygenated terpenes. These results are consistent with data published in 2020 by Ben Salha [27] indicating that alcohols and polar terpene compounds act as potent antifungal agents and delay the biodegradation process.

CONCLUSION

In this work, the cold percolation technique was used to assess the antibacterial activities of fifteen traditional medicinal herbs from the Taroudant region. Among the plants studied, the results showed potential antibacterial properties. First, we sampled fifteen plants, and for each of them, we extracted by hydrodistillation essential oils that have different yields and vary between 0.1% and 3.73%, whose aim is to evaluate the activity of these essential oils against pathogenic agents. This screening has shown us that these essential oils have inhibitory activities against the Gram+ bacteria studied, as well as resistance against Gram-, in particular *Enterococcus cloacae*. These essential oils can therefore be used in the prevention and treatment of certain infectious diseases and to fight against bacteria that are multi-resistant to the usual antibiotics. In addition, these essential oils can be used as biopesticides, especially in the post-harvest treatment of fruits intended for export.

Disclosure conflict of interest

The authors declare that they have no conflicts of interest concerning this article.

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ORIGINAL ARTICLE

EVALUATION OF PHENOLIC CONTENT IN SELECTED RED FRUIT JUICES

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ABSTRACT

Background. Red fruits are characterised by a particularly high content of bioactive compounds, e.g. anthocyanins, tannins, pectins, vitamins and minerals. Dietary supply of proper amounts of antioxidants is essential to reduce oxidative stress, and thus is an important element in the prevention of lifestyle diseases.

Objective. The aim of the study was to evaluate and compare the content of polyphenols in selected red fruit juices (chokeberry, elderberry, pomegranate, cranberry), as well as to assess the impact of storage time on the content of these compounds in the analysed samples.

Material and Methods. The research material consisted of 17 juices (100%): 3 chokeberry juices, 4 elderberry juices, 5 pomegranate juices and 5 cranberry juices, which differed in terms of the manufacturer, type, price range, country of origin and production method. The total polyphenol content was measured by spectrophotometry using the Folin-Ciocalteu reagent. The procedure was based on a modified method described by Waterhouse. Active acidity (pH) was measured with the potentiometric method using a pH-meter and the sucrose content was measured using a refractometer.

Results. The highest mean content of polyphenolic compounds was found in chokeberry and elderberry juices. Juice storage time did not reduce the mean content of polyphenolic compounds. The highest sucrose content was found in chokeberry juices and the lowest in cranberry juice.

Conclusions. Chokeberry and elderberry juices had the highest content of polyphenols among the tested products. Juices stored after opening in accordance with the manufacturer's instructions (at 4°C) do not lose their nutritional properties.

Keywords: *polyphenols, fruit juices, chokeberry, elderberry, pomegranate, cranberry*

STRESZCZENIE

Wprowadzenie. Czerwone owoce charakteryzują się szczególnie wysoką zawartością związków bioaktywnych m.in. antocyjanów, garbników, pektyn, witamin oraz składników mineralnych. Dostarczenie wraz z codzienną dietą odpowiedniej ilości przeciwutleniaczy jest niezbędne w celu redukcji stresu oksydacyjnego, a tym samym stanowi istotny element w prewencji chorób cywilizacyjnych.

Cel badań. Celem prowadzonych badań była ocena i porównanie zawartości związków polifenolowych w wybranych sokach z czerwonych owoców (aronia, czarny bez, granat, żurawina), a także zbadanie wpływu przechowywania na zawartość ww. związków w badanym materiale. Ocenie podlegały także inne parametry fizykochemiczne: kwasowość aktywna (pH), zawartość sacharozy oraz zawartość suchej masy.

Materiał i Metody. Materiał badawczy stanowiło 17 soków (100%): 3 soki z aronii, 4 soki z czarnego bzu, 5 soków z granatu, 5 soków z żurawiny, zróżnicowanych pod względem: producenta, rodzaju, przedziału cenowego, kraju pochodzenia oraz sposobu produkcji. Oznaczenie całkowitej zawartości polifenoli było prowadzone metodą spektrofotometryczną z wykorzystaniem odczynnika Folin-Ciocalteu`a. Procedura opierała się na zmodyfikowanej metodzie opisanej przez Waterhouse`a. Kwasowość aktywną (pH) oznaczono metodą potencjometryczną przy pomocy pH-metru, a zawartość sacharozy oznaczono przy użyciu refraktometru.

Wyniki. Największą średnią zawartość związków polifenolowych oznaczono w soku z aronii i czarnego bzu. Przechowywanie soków nie wpłynęło na zmniejszenie średniej zawartości związków polifenolowych. Zawartość sacharozy w badanych produktach była największa w sokach z aronii, a najmniejsza w soku z żurawiny.

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Wnioski. Soki z aronii i czarnego bzu charakteryzowały się największą zawartością polifenoli wśród badanych produktów. Soki przechowywane po otwarciu zgodnie z zaleceniami producenta (w temperaturze 4°C) nie tracą swoich właściwości odżywczych.

Słowa kluczowe: *polifenole, soki owocowe, aronia, czarny bez, granat, żurawina*

INTRODUCTION

Fruit and vegetables are products rich in health-supporting ingredients. However, the group of red or dark-coloured fruits, which are characterised by a high content of, among other things, polyphenolic compounds, including anthocyanins, tannins, pectins, carotenoids, vitamins and minerals, is particularly noteworthy [15]. Dietary supply of proper amounts of antioxidants is essential to reduce oxidative stress, and thus is an important element in the prevention of lifestyle diseases [16].

Chokeberry (*Aronia melanocarpa*) is a plant whose fruits are particularly rich in polyphenolic compounds, including flavonoids, anthocyanins, flavan-3-ols and phenolic acids. The content of phenolic compounds depends on the cultivar, degree of maturity, growing environment, post-harvest storage conditions and processing techniques [2, 16]. Chokeberry is particularly recommended in the prevention of cardiovascular diseases due to its anti-atherosclerotic, antihypertensive and anti-platelet properties. Polyphenolic compounds found in its fruits reduce blood cholesterol (LDL) and triglycerides [5]. Furthermore, chokeberry fruits show gastroprotective, hepatoprotective and antiproliferative effects, and are also used in the prevention and treatment of diabetes and other lifestyle diseases [13, 4]. Due to their tart taste, they are most often consumed in processed form (jams, tea or juices). The latter are increasingly popular among consumers due to their wide assortment, including sugar-free, preservative or dye-free products, which is increasingly important for the wide range of consumers [33].

Elderberry (*Sambucus nigra* L.) is a shrub or a small tree belonging to Adoxaceae, commonly known as the moschatel family. Fruit and flowers are most often used for medicinal, cosmetic and food purposes. The plant was used in traditional medicine for respiratory diseases, common colds, flu, burns, swelling, wounds, joint sprains and dislocations, skin problems, insect bites and stings, rheumatic problems, dental pain, nephrological disorders, gastrointestinal and eye diseases. Elderberry infusions, extracts, and syrups are currently recommended for colds, rhinitis, and as a diaphoretic, diuretic, laxative and anti-inflammatory agent. The use of elderberry in these conditions is associated with its antiviral and antibacterial action, as well as its ability to boost the immune system [31]. The health-supporting properties of elderberry are related primarily to its high content of antioxidants, anthocyanins, flavonols (quercetin, kaempferol) and phenolic acids in particular [6]. These compounds neutralize oxidative stress, which produces beneficial effects in the form of cardiovascular support, reduced glycaemia, immune system stimulation, anti-cancer effects, as well as increased activity of antioxidant plasma enzymes, including glutathione, and reduced uric acid levels [31]. Elderberry fruits are most often used for juices, jams, marmalades, jellies, desserts, candies, syrups, teas, wines and as colourants for ice cream, yoghurts, candies and cakes. Raw fruits are poisonous, but toxic substances are decomposed by heat treatment [17].

In terms of botanical classification, large-fruited cranberry (*Vaccinium macrocarpon* L.) belongs to the Ericaceae family. Its fruits are spherical red berries with thin, smooth skin. Cranberries are a source of valuable ingredients for the body. They contain vitamins (A, C, E), minerals (potassium, sodium, selenium), as well as lutein and β-carotene. Polyphenols, i.e. flavonoids, phenolic acids and stilbenes, are the most important group of compounds contained in cranberry fruits [21]. Cranberry is very popular among consumers not only due to its taste, but also because of its multiple health-supporting properties. It is most often available in the form of fresh and dried fruit, but it is also used to produce juices, sauces, preserves and jellies [34].

Its beneficial properties include, among others, anti-cancer, anti-inflammatory and antimicrobial effects. Studies have shown that cranberry reduces LDL cholesterol and glucose levels in patients with metabolic syndrome. This is due to the presence of proanthocyanins, flavonoids and a high content of vitamin C, all of which have strong antioxidant properties [25, 29]. Active substances contained in cranberry, such as citric acid and benzoic acid, help prevent urolithiasis and support the treatment of bladder inflammation. Furthermore, the high content of ascorbic acid acidifies the urine, which hinders the growth of bacteria, and the proanthocyanidins prevent uropathogenic *E. coli* from adhering to the urinary epithelial mucosa, therefore cranberries are recommended in the prevention of genitourinary conditions [10].

Pomegranate (*Punica granatum* L.) was known for its health-supporting properties already in antiquity, when the juice made of this fruit was recommended against diarrhoea, caries, ulcers, and pharyngitis. Pomegranate and its products are a very rich source of

vitamins A, C, E, and B, as well as minerals, proteins, folic acid, fibre, beta-carotene and polyphenols, in particular ellagitannins (punicalagin), anthocyanins and hydrolysable tannins [1]. The latter components contribute to multiple health-supporting properties of pomegranate. These include, among others, cardioprotective, blood pressure lowering and LDL lowering action. The active substances present in the pomegranate help neutralise oxidative stress and alleviate inflammation [30, 36]. There is also scientific evidence for antimicrobial, antifungal and antiviral effects of this fruit. However, the anti-proliferative and anti-cancer effects of pomegranate (prostate, colon, oral and breast cancer) are particularly noteworthy [1].

Regular consumption of food products rich in, among others, polyphenolic compounds contributes to maintaining proper health, as well as helps prevent and treat many diseases. It is important that the consumed products have a low degree of processing to minimise the loss of beneficial components. 100% fruit juices widely available all year round, regardless of the season, are an example of this type of product.

The aim of the study was to estimate and compare the content of polyphenols in selected red fruit juices (chokeberry, elderberry, pomegranate, cranberry), as well as to assess the impact of storage time on the content of these compounds in the tested samples. Other physical and chemical parameters, such as active acidity (pH), sucrose content and dry matter content, were also assessed.

MATERIALS AND METHODS

The study included 17 juices (100%) from the following red fruits: chokeberry (n=4 products), elderberry (n=3 products), pomegranate (n=5 products) and cranberry (n=5 products); a total of 68 samples were tested. Juices was differed in terms of the manufacturer, type, price range, country of origin (Poland, European Union countries or outside the EU – Turkey, Canada), and production method (directly squeezed from fruit or obtained from concentrated juice). Each of the products of a given type of juice was represented by various manufacturers. Four juices of each manufacturer from different batches were analysed to obtain the most representative results.

The tested juices were purchased in generally accessible grocery stores, health food stores and pharmacies. The selected juices did not contain added sugar or preservatives and were packed in dark glass (except for pomegranate juices). A detailed description of the tested juices is shown in Figure 1.

Each of the juices was assessed for the content of polyphenolic compounds immediately after opening, as well as for the impact of storage on the content of the above-mentioned compounds in the tested material. Other physical and chemical parameters, such as active acidity (pH), sucrose content and dry matter content, were also assessed (Figure 2). Measurements were performed on the day of the opening of the product, 24 and 48 hours after opening, and then 1, 2, 3 weeks after opening. After opening, the juices were stored according to the manufacturer's instructions, i.e. in a refrigerator at 4°C.

Total polyphenol content

The total polyphenol content was measured by spectrophotometry using the Folin-Ciocalteu reagent. The procedure was based on a modified method described by Waterhouse [37]. Before the analysis, the

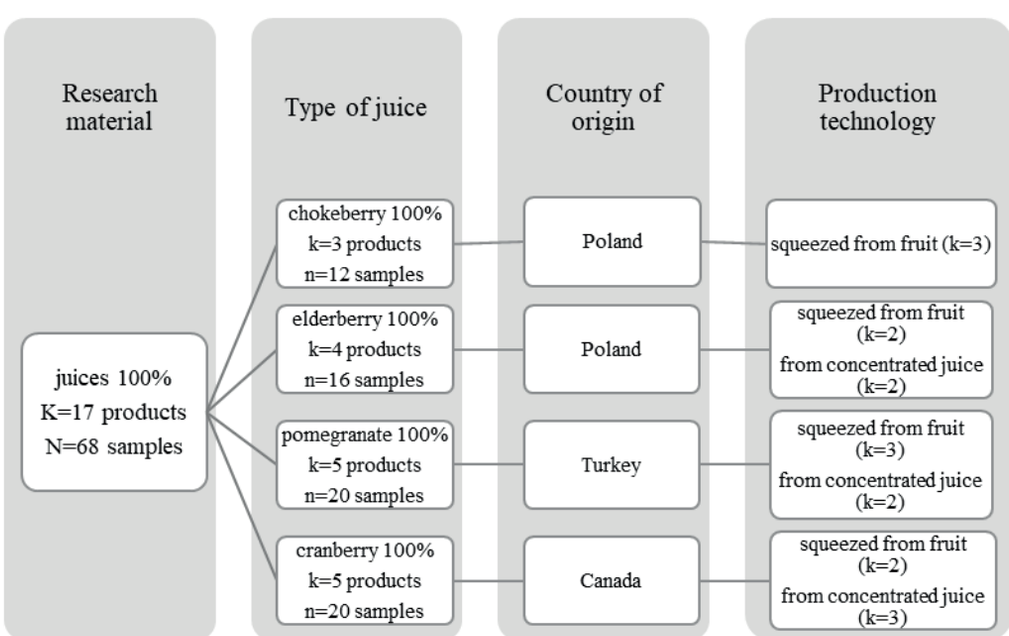

Figure 1. Characteristics of the research material

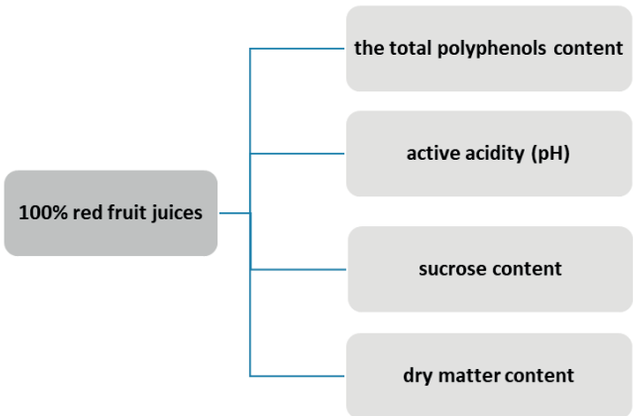

Figure 2. Physicochemical parameters determined in the tested 100% red fruit juices

juices were thoroughly stirred, samples were collected and diluted with demineralised water (10 x dilution for chokeberry and elderberry juices, 10 x dilution for pomegranate and cranberry juices), and then filtered through a soft quantitative filter. To determine the total polyphenol content, 0.1 mL of a diluted and filtered juice sample was transferred into a 10 mL flask, and then 5 mL of distilled water was added. Next, 0.5 mL of Folin-Ciocalteu's reagent was added and mixed. Finally, 1.5 mL of 20% sodium carbonate (Na_2CO_3) was added, and the sample was made up to the mark with distilled water and thoroughly mixed. The samples prepared in this way were placed in an incubator (Incucell/V111, Memmert) at 40°C for 30 minutes. After the pre-set time, the samples were removed from the incubator and transferred successively to glass cuvettes with an optical path length of 10 mm. The absorbance of the samples was then measured using a UV/VIS spectrophotometer (HALO SB-10 Spectrophotometer, Biogenet) at a wavelength of 765 nm against blank. The measurements were performed in quadruple. In parallel with the tested samples, the absorbance was measured for a series of gallic acid standards (0-500 µg/mL). Each standard was measured in triplicate. Based on the obtained results, a curve y=0.0009x+0.0015 was plotted; R2=0.9995, which was used to estimate the total content of polyphenols in the tested samples. The result was expressed in gallic acid equivalents (mg GAE/100 mL of the product).

Active acidity (pH)

Active acidity (pH) was measured with the potentiometric method using a pH-meter (pH-meter CPC-505, Elmetron). Before starting the measurements, the device was calibrated in buffering solutions (pH values: 4, 7 and 9). The measurements were performed at room temperature.

Sucrose content

The sucrose content was measured using a refractometer (HI 96801 portable digital sucrose refractometer, HANNA Instruments), with the result given in Brix (°Bx) corresponding to % sucrose (g/100mL of product). Before the measurements, the device was calibrated using distilled water. The measurements were performed at room temperature.

Dry mass

Dry matter content was measured with moisture analyser (MB23, Ohaus). Samples of 3 g of each juice were collected, spread on tissue paper and placed in a moisture analyser at 105°C. The results were then read: water content (%), dry matter content (%) and dry matter content (g). The measurements were performed in triplets for each juice.

Statistical analysis

MS Excel 2013 and Statistica v.13.3 (StatSoft Polska) software were used for statistical data processing. Measurable data are presented as mean and standard deviation – X±S, along with the median and interquartile range expressed by the lower and upper quartile – M ($Q_{0.25}$-$Q_{0.75}$). The Shapiro-Wilk test was used to assess the normality of the distribution. Differences in the distributions in two unrelated groups, due to the asymmetry, were assessed using the Mann-Whitney U test. The significance of the differences in distributions for multiple related measurements (separate time units) was assessed with the Friedman ANOVA test. Spearman rank correlation coefficient with its significance test was used for the assessment of the dependence of measurable variables. Significance level was set at p=0.05.

RESULTS

The content of polyphenols

When analysing the content of polyphenols (expressed in gallic acid equivalents [mg GAE/100 mL]) in individual types of juice, their significant differentiation over time was observed in the case of chokeberry, pomegranate and cranberry juice. In the case of chokeberry juice, there was a decrease in the mean polyphenol content the day after opening, followed by a significant increase in its levels. However, it should be noted that the minimum content of polyphenols immediately after opening the juice (on the first day) was the highest compared to subsequent measurements. The average content of polyphenols in elderberry juice remained stable, except for the second day after opening the juice, when it increased at the border of statistical significance (p=0.05). On the other hand, the analysis of pomegranate juices showed a significant variation over time, with a significant decrease in the mean content of gallic acid over 3 weeks: from 382.2 mg GAE/100mL to 349.2 mg GAE/100mL (p<0.0001).

In the case of cranberry juice, the average polyphenol content remained constant, except for the measurement performed one week after opening the juices. This value significantly increased (by about 15%) compared to measurements at other time points ($p<0.0001$) (Table 1). There was no analysis of differences in the content of polyphenols in different juices of the same fruit. However, an additional statistical analysis was performed between individual types of juices (chokeberry, elderberry, pomegranate and cranberry juice) and their average gallic acid content values on the opening day ($p=0.0001$), after 24 h ($p=0.001$), after 48 h ($p=0.001$), after 1 week ($p=0.0001$), after 2 weeks ($p=0.0001$) and after 3 weeks ($p=0.0001$).

The technology of juice production (directly squeezed from fruit or produced from concentrated juice) was also taken into account in the analyses. It was only in the case of cranberry juices that significant differences in the mean content of polyphenols were observed in the period from the opening day to two weeks after opening. Higher polyphenol levels were found in products made from concentrated juice (Table 2).

From the juices analysed in the present study, the highest mean content of polyphenols was found in chokeberry juices (491.2-663.15 mg GAE/100 mL), and the lowest in cranberry juices (79.38-185.49 mg GAE/100 mL). Elderberry juices contained from 401.16 to 571.30 mg GAE/100 mL, and pomegranate juices from 253.36 to 420.60 mg GAE/100 mL. In the case of chokeberry and elderberry juice, it was observed that juices from organic crops (bio farming) contain on average 100 to 200 mg GAE/100mL more polyphenols compared to juices from conventional sources. No such correlation was found for pomegranate and cranberry juices.

Active acidity (pH)

In addition to the total content of polyphenols, we also measured the active acidity (pH) of the juices – starting from the baseline (opening) and continuing for the 3-week storage period. Cranberry juices had the lowest pH (from 1.82 to 1.94), whereas elderberry juices had the highest pH (from 3.51 to 4.28). Active acidity ranged from 3.2 to 3.54 for chokeberry juices, and from 2.39 to 3.19 for pomegranate juices. No significant pH changes were observed for elderberry and chokeberry juices, considering the measurement on the day of opening the juice and one week afterwards. The increase in pH occurred in the second week after opening and continued until the end of the experiment. In pomegranate juices, the pH decreased in the first week after opening, and then it remained at the baseline level until week 3 after opening.

Water and dry matter content

The tested juices were also assessed for water and dry matter content. The highest dry matter content was found in chokeberry (14.78–20.35%) and elderberry juices (12.33–16.58%). Dry matter content ranged from 11.73 to 14.7% for pomegranate juice, and from 5.75 to 6.75% for cranberry juice. A significantly lower water content was found for pomegranate and elderberry products obtained from direct pressing, which was also associated with a significantly higher dry matter content (Table 3).

Sucrose content

The highest sucrose content was found in chokeberry juices (14.05–17.1 °Brix). The other juices had a sucrose content of 14–16.1 °Brix (pomegranate), 7.03-10.15 °Brix (black elderberry), and 7.23–8.05 °Brix (cranberry). We observed a significant positive

Table 1. Average content of gallic acid in the tested juices depending on the time from opening by the type of juice

Gallic acid content [mg GAE/100 mL]							
	Time from opening						p-value
	Opening day	24 hours	48 hours	1 week	2 weeks	3 weeks	
Chokeberry n=12	623.5±89 640 (542.2-691.7)	569±104 590 (477.2-654.4)	578.6±77.9 616.7 (493.9-642.8)	600±76 626.1 (511.1-666.1)	595.8±77.1 634.4 (518.3-642.8)	610.4±96.8 666.7 (485.6-681.7)	p=0.005
Elderberry n=16	513.1±88.7 539.4 (448.9-570.6)	501±86.1 533.9 (441.7-556.7)	572.6±58.3 559.4 (531.1-615.6)	509.9±96.1 557.2 (441.1-566.7)	490.7±81.8 512.2 (392.8-565)	520.9±88.6 553.9 (471.1-581.1)	p=0.05
Pomegranate n=20	364.9±75.3 382.2 (283.6-426.1)	371.9±77.6 404.7 (307.2-429.2)	326.1±78.3 343.6 (246.7-361.4)	338.6±82.1 363.3 (260.8-420)	342.5±63 356.7 (283.9-373.6)	320±60.7 349.2 (243.1-369.4)	p<0.0001
Cranberry n=20	123.2±48.5 104.4 (81.4-175.3)	123.7±41.1 107.2 (89.7-158.9)	114.6±42.5 93.6 (80-163.6)	132.3±44.6 121.9 (90.3-175)	119.8±39.6 105.8 (82.2-161.7)	129.8±79.3 105.3 (82.8-153.3)	p<0.0001

Measurable data are presented as mean and standard deviation (X±S), along with the median and interquartile range expressed by the lower and upper quartile – M ($Q_{0.25}$-$Q_{0.75}$); p-value – Friedman ANOVA results with repeated measures

Table 2. Average content of gallic acid depending on the production technology by the type of juice and opening time

Gallic acid content [mg GAE/ 100 mL]	Production technology	Time from opening					
		Opening day	24 hours	48 hours	1 week	2 weeks	3 weeks
Chokeberry n=12	squeezed from fruit	623.5±89 640 (542.2-691.7)	569±104 590 (477.2-654.4)	578.6±77.9 616.7 (493.9-642.8)	600±76 626.1 (511.1-666.1)	595.8±77.1 634.4 (518.3-642.8)	610.4±96.8 666.7 (485.6-681.7)
Elderberry n=16	squeezed from fruit	461.7±96.4 457.8 (371.7-551.7)	457.2±104.9 441.7 (362.2-555.6)	549.4±49.4 538.9 (523.3-563.3)	458.1±113.8 457.2 (352.8-564.4)	461±88.2 456.7 (373.3-544.4)	488.2±119 498.3 (377.2-597.8)
	from concentrated juice	564.6±39.2 562.8 (530.6-601.7)	544.9±21.6 540 (533.9-556.7)	595.8±60.1 576.1 (559.4-654.4)	561.8±25.8 558.3 (543.9-580)	520.4±67.3 541.7 (495.6-567.8)	553.6±14.7 553.9 (542.2-564.4)
	p-value	p=0.046	p=0.13	p=0.08	p=0.25	p=0.21	p=0.96
Pomegranate n=20	squeezed from fruit	378.8±77.7 411.7 (286.7-431.4)	361.5±80 404.7 (255.6-420.8)	314.3±56.5 343.6 (241.7-358.9)	335.2±89.6 372.8 (240.6-420)	333.4±45.4 356.7 (296.9-362.5)	319.4±59.5 349.2 (243.1-361.1)
	from concentrated juice	343.9±71.2 334.2 283.6-382.2)	387.5±76.4 384.7 (315-461.4)	343.8±105 327.8 (248.1-430.8)	343.8±75.2 325.8 (281.1-407.5)	356.1±84.8 358.9 (276.9-435)	321±66.7 323.1 (262.2-382.5)
	p-value	p=0.33	p=0.23	p=0.23	p=0.62	p=0.56	p=0.64
Cranberry n=20	squeezed from fruit	88.8±19.1 91.7 (68.9-104.4)	95.5±13.1 96.4 (84.2-107.2)	82.8±13.2 81.7 (72.5-93.6)	103.1±18.4 98.1 (86.4-121.9)	93.1±14.7 95 (78.3-105.8)	93.7±15.2 89.7 (81.7-105.3)
	from concentrated juice	146.1±49 171.9 (88.9-181.4)	142.5±43 151.9 (92.5-177.8)	135.8±42.3 151.4 (82.2-173.1)	151.9±46.7 172.5 (92.5-181.1)	137.5±41.3 160.3 (85.3-165.6)	153.8±95.6 151.4 (84.4-160.3)
	p-value	p=0.03	p=0.02	p=0.02	p=0.03	p=0.04	p=0.06

Measurable data are presented as mean and standard deviation (X±S), along with the median and interquartile range expressed by the lower and upper quartile – M ($Q_{0.25}$-$Q_{0.75}$); p-value – U Mann-Whitney test result

effect of the sucrose level on gallic acid content in elderberry, pomegranate and cranberry juices. In the case of chokeberry juices, the correlation was moderate, but not statistically significant (Table 4).

DISCUSSION

The antioxidant capacity of fruit juices was also assessed by Nowak et al. [26], who investigated both self-prepared juices and those produced on an industrial scale. Chokeberry, apple, blackberry and blackcurrant juices came from Polish plantations, whereas sea buckthorn and pomegranate juices were imported from Georgia, and orange juices from Spain. The juices were produced using traditional or organic methods. The pH values ranged between 3.03 and 3.74. Sea buckthorn juice had the lowest pH, whereas the highest pH was found for apple nectar. The total content of polyphenols in chokeberry juice was about 550 mg GAE/100 mL, which corresponded to our findings. Other authors confirm a similar total polyphenol content (approximately 500-600 mg GAE/100 mL) in chokeberry juices [15, 9]. The content of polyphenols in individual parts of this plant can range from 3.73 g/100 g dry matter in juice, through 7.85 g/100 g dry matter in fruits to 10.58 g/100 g dry matter in pomace [38]. Other authors report that the total polyphenol content in chokeberry juice can range from 690 to 2560 mg GAE/100 g fresh matter. The content of polyphenols in the juice obtained from this fruit is 2 to 8 times higher than that in blackberry, blueberry, raspberry or red currant juices [14].

Oszmiański and Lachowicz [28] assessed, among other things, antioxidant activity, the content of bioactive compounds, water and dry matter in juices from crushed and uncrushed chokeberry fruits. The dry matter content was 16.87% for uncrushed fruit and 15.46% for crushed fruit. In our study, chokeberry juices had a dry matter content of 15–19%.

Tolić et al. [33] assessed 22 products made of chokeberry (juices, powders, capsules, fruit teas, dried fruits) for their physicochemical properties, phenolic content and antioxidant properties. The active acidity of the tested juices ranged from 3.54 to 3.94; dry matter content from 13.42 to 21.54%, and total polyphenol content from 300.2 to 663.9 mg GAE/100 mL.

Table 3. Water and dry matter content in the tested juices

Type of juice	Production technology	Water content [%]	Water content [g]	Dry matter content [%]	Dry matter content [g]
Chokeberry n=12	overall	82±3.3 82.3 (80.8-84.6)	2.5±0.1 2.5 (2.4-2.5)	18.1±3.3 17.7 (15.4-19.4)	0.5±0.1 0.5 (0.5-0.6)
	squeezed from fruit	82±3.3 82.3 (80.8-84.6)	2.5±0.1 2.5 (2.4-2.5)	18.1±3.3 17.7 (15.4-19.4)	0.5±0.1 0.5 (0.5-0.6)
Elderberry n=16	overall	85.6±3.2 85 (83.4-88)	2.6±0.1 2.5 (2.5-2.6)	14.4±3.3 15.1 (12-16.7)	0.4±0.1 0.5 (0.4-0.5)
	squeezed from fruit	83.8±2.6 83.8 (82.2-84.6)	2.5±0.1 2.5 (2.46-2.54)	16.2±2.6 16.3 (15.4-17.9)	0.5±0.1 0.5 (0.46-0.55)
	from concentrated juice	87.5±2.9 87 (85.6-89.8)	2.6±0.1 2.6 (2.6-2.7)	12.6±2.9 13 (10.3-14.4)	0.4±0.1 0.4 (0.3-0.4)
	p-value	p=0.04	p=0.04	p=0.04	p=0.04
Pomegranate n=20	overall	86.6±1.2 86 (85.6-87.8)	2.7±0 2.7 (2.7-2.8)	13.4±1.3 14 (12.2-14.4)	0.3±0 0.3 (0.2-0.3)
	squeezed from fruit	85.7±0.3 85.7 (85.6-86)	2.7±0 2.7 (2.71-2.72)	14.3±0.6 14.4 (14-14.4)	0.3±0 0.3 (0.28-0.29)
	from concentrated juice	88±0.8 88.2 (87.5-88.4)	2.8±0 2.8 (2.8-2.8)	12.1±0.8 11.9 (11.6-12.5)	0.2±0 0.2 (0.2-0.3)
	p-value	p=0.0002	p=0.0002	p=0.0002	p=0.0002
Cranberry n=20	overall	94±0.4 94 (94-94.3)	2.9±0 2.9 (2.9-2.9)	6±0.4 6 (5.7-6)	0.1±0 0.1 (0.1-0.1)
	squeezed from fruit	94.2±0.3 94 (94-94.5)	2.9±0.01 2.88 (2.88-2.89)	5.8±0.3 6 (5.5-6)	0.1±0.01 0.1 (0.11-0.12)
	from concentrated juice	93.9±0.5 94 (93.5-94.1)	2.9±0.01 2.9 (2.87-2.88)	6.2±0.5 6(5.9-6.5)	0.1±0.01 0.1 (0.12-0.13)
	p-value	p=0.26	p=0.16	p=0.26	p=0.16

Measurable data are presented as mean and standard deviation (X±S), along with the median and interquartile range expressed by the lower and upper quartile – M ($Q_{0.25}$-$Q_{0.75}$); p-value – U Mann-Whitney test result

Table 4. Average content of gallic acid depending on the average content of sucrose on the opening day of the tested products by to the type of juice

Type of juice	Measurement on the opening day		Spearman rank correlation coefficient p-value
	gallic acid content [mg GAE/100 mL]	sucrose content [°Brix]	
Chokeberry n=12	623.5±89 640 (542.2-691.7)	15.8±1.4 16.3 (14.2-17.1)	0.5 p=0.1
Elderberry n=16	513.1±88.7 539.4 (448.9-570.6)	9.1±1.3 9.6 (8.2-10)	0.66 p=0.005
Pomegranate n=20	364.9±75.3 382.2 (283.6-426.1)	15.2±0.7 15.1 (14.9-15.7)	0.62 p=0.003
Cranberry n=20	123.2±48.5 104.4 (81.4-175.3)	7.8±0.3 7.8 (7.7-8)	0.47 p=0.03

Measurable data are presented as mean and standard deviation (X±S), along with the median and interquartile range expressed by the lower and upper quartile – M (Q0.25-Q0.75); p-value – the result of the significance test of the R Spearman correlation coefficient

These findings are similar to those obtained in our study. Similar pH values were also reported by other authors. Bolling et al. [3], reported that the pH of chokeberry juice ranged from 3.15 to 3.45 pH, depending on the harvest week. The authors also measured the content of polyphenols in chokeberry juices, which ranged from 400 to 500 mg GAE/100 mL. The value for refractometric extract in the analysed

juices fluctuated depending on the harvest week (from 10.5 °Brix in week 1, through 7.8 in week 2 and increasing up to week 7 to 14.3 °Brix). Jeszka-Skowron et al. [11] evaluated the antioxidant activity of methanol/water extracts of dried goji berries, dried cranberries and raisins. Food products came from organic and conventional cultivation in various parts of the world (China, USA, Poland, Canada, Turkey, Iran). The highest content of polyphenols was found in dried goji fruits (a mean of 11.5 mg GAE/g DM), while it was approximately 2.5 mg GAE/g dry matter for dried cranberries, and about 1.9 mg GAE/g dry matter for raisins. No correlation was found between the higher antioxidant activity of the fruit and its origin from organic farms.

Vu et al. [35] assessed the effects of heat treatment and exposure to light and oxygen on the stability of bioactive compounds in cranberry, and analysed whether cranberry extracts and juices could effectively inhibit the proliferation of colorectal cancer cells. It was shown that compared to thermal treatment, freezing is a better method to inhibit the drop in polyphenol levels in cranberries. The total phenolic content in three juices (raw, filtered and concentrated) was also assessed. It was on average from 1375 μg GAE/mL (pH 7) to 1681 μg GAE/mL of juice for unfiltered juices (pH 2.5), and from 1255 μg GAE/mL to 1595 μg GAE/mL of juice for filtered juices. The polyphenol content in concentrated juices was 2 to up to 6 times higher (from 2655 μg GAE/mL [pH 7] to 8648 μg GAE/mL [pH 2.5]). The above studies support the potential use of cranberry extracts or juices to aid cancer prevention and/or treatment.

Results similar to those presented in this paper were obtained by Nowak et al. [27], who conducted a comparative analysis of phenolic compounds in organic chokeberry, elderberry, cranberry and pomegranate juices. The juices came from organic Polish (chokeberry and elderberry) and foreign (cranberry – Canada; pomegranate – Turkey) crop systems. The lowest and the highest pH was reported for cranberry (2.78) and elderberry (4.21), respectively, which corresponds to our findings. Pomegranate juice had a pH of 3.53, which was higher than in juices used in our research. Chokeberry juices, on the other hand, had a pH of 3.63–3.88, which corresponds to our findings. All analysed juices were a rich source of phenolic compounds. Chokeberry juices were characterised by the highest total polyphenols (up to 790 mg GAE/100 mL). Lower values were reported for pomegranate and elderberry juices: 356 mg GAE/100 mL and 324 mg GAE/100 mL, respectively. The lowest content of polyphenols (163 mg GAE/100 mL) was found in cranberry juice. The total polyphenol content in the tested juices was also very similar to the results obtained in our research.

Narwojsz et al. [24] compared, among other things, polyphenol content in cranberry fruits differing in terms of variety and place of cultivation. The mean total content of polyphenols in the tested material ranged from 163 to 315 mg GAE/100 g fresh matter.

Sidor and Gramza-Michałowska [31] performed a review of research on the antioxidant properties of elderberry. The fruits of this plant contain an average of 371 mg to 583 mg GAE/100 g fresh matter. Pliszka [23] showed that the mean content of polyphenols in elderberry fruits ranged from about 600 to 1000 mg GAE per 100 g of fresh matter. Johnson et al. [12] measured the total content of polyphenols and anthocyanins in 3 genotypically different elderberries (different geographic origins). The estimations were performed immediately after harvest and during the period of 3, 6, 9 months of storage of frozen fruit. There were differences in polyphenol content between fruits from plants of different genotypes: 570 mg GAE/100 mL for Adams II, 800 mg GAE/100 mL for Bob Gordon and 680 mg GAE/100 mL for Wyldewood. The greatest drop in the content of polyphenols was observed after a 3-month storage period (by about 50-200 mg GAE/100 mL of juice). Nine months after opening, the content of polyphenols in fruit juices of individual varieties was 450, 600 and 490 mg GAE/100 mL, respectively, which was not less than 72% of the baseline polyphenol content in the tested material.

In our study, the content of polyphenols in pomegranate juices was on the average level, i.e. from 278.1 to 420.6 mg GAE/100 mL. The results reported by other authors indicate a very large variation in the content of polyphenols in the juice of this fruit. Mahdavi R. et al. [20] showed that the mean total polyphenol content was 421.42 mg GAE/100 mL in fresh pomegranate juices and 381.73 mg GAE/100 mL in commercial pomegranate juices. Other researchers reported a total polyphenol content of 146.94 mg GAE/100 g (Fu et al. [8]) or 115.77 mg GAE/100 g (Kościuk et al. [18]). Esposto et al. [7] assessed polyphenol content and antioxidant activity, and performed a sensory analysis in pomegranate juices from conventional and organic crops, as well as from concentrate-based and freshly squeezed juices. The average content of polyphenols was from 1379.9 to 3748.8 mg GAE/L in concentrate juices, and from 1632.0 to 2736 GAE/L in freshly squeezed juices. The varied content of polyphenols in pomegranate juices is not only due to the production method or type of cultivation, but it also depends on the type of fruit and the place of cultivation. One study compared the content of polyphenols in juices made of "Wonderful" cultivars from Spain, where the average polyphenol

content ranged widely from 1562 to 4500 mg GAE/L (Mena et al. [22]). Turkish pomegranate juices contained between 2,602 and 10,086 mg GAE/L (Tezcan et al. [32]). Labbe et al. [19] compared the content of polyphenols in pomegranate juices at different stages of fruit ripeness and between different cultivars. Their findings indicate that the fruit species plays a decisive role in the content of polyphenols in juices.

CONCLUSIONS

Chokeberry and elderberry juices had the highest content of polyphenols among the tested products. Differences between juices from the same type of fruit may result from different places of origin, growing conditions (exposure to sunshine), degree of ripeness at harvest, species variety, as well as the technology for juice production and storage. The average content of polyphenols in all tested juices remained at a similar level from the moment of opening to the end of the 3-week storage period. Juices stored after opening in accordance with the manufacturer's instructions (at 4°C) do not lose their nutritional properties. All analysed juices were acidic, with the lowest pH in cranberry juices and the highest pH in elderberry juices. The acidity of the juices persisted from the moment of opening to the end of the 3-week storage period. Chokeberry and elderberry juices had the highest dry matter content, while cranberry juice had the lowest dry matter content. The highest sucrose content was found for chokeberry and pomegranate juices, and the lowest in cranberry juices.

Disclosure conflict of interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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ORIGINAL ARTICLE

APPLICATION OF HALOINHALATIONS AT THE SANATORIUM-RESORT STAGE OF REHABILITATION OF PATIENTS AFTER COVID-19

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ABSTRACT

Background. After suffering from COVID-19, a large number of patients need respiratory rehabilitation. One of the methods of rehabilitation is inhalation with salt aerosols.

Objective. Our work aimed to study the effectiveness of inhalations of a dry aerosol of salt precipitated from the mineral water of the "Teplitsa multidisciplinary sanatorium", Transcarpathian region of Ukraine.

Material and Methods. 30 male patients were examined after suffering from COVID-19. We formed two groups of patients, control and main, 15 people each. Patients in the control group received inhalation with a dry aerosol of table salt of the "Aero-M-sol". In contrast, patients in the main group received a course of inhalations with a dry aerosol of salt precipitated from the mineral water.

Results and discussion. Under the influence of the rehabilitation complex in both groups, there is a performance improvement but significant changes are observed only in patients of the main group. The indicator Forced Vital Capacity1 increased to the greatest extent, which after rehabilitation is significantly higher than in the control group ($p<0.05$). As a result, the Tiffeneau index significantly increases in the main group compared to the control group, reaching normal values. The main effect is associated with a decrease in obstructive complications of the respiratory tract as a result of a decrease in inflammation. The use of iodine-bromine brines (as in our case) for inhalation in the treatment of respiratory diseases has been proven to be effective, with systemic effects in the form of decreased IgE and increased IgA in the blood serum having been noticed.

Conclusions. The use of haloinhalations with MW salts in the rehabilitation of patients after suffering from COVID-19 disease significantly improves the clinical condition of convalescents.

Keywords: *COVID-19, rehabilitation, inhalation, mineral waters*

INTRODUCTION

After suffering from the coronavirus disease COVID-19, many patients develop respiratory dysfunction associated with restrictive lung changes and inflammatory obstructive changes in the bronchi. Also, bronchial hyperreactivity is primarely associated with reduced inorganic substances in the airways [1].

These violations necessitate directed respiratory rehabilitation [2, 3]. One of the physiotherapeutic techniques that increases the effectiveness of the basic treatment of respiratory pathologies is halotherapy, inhalation with a dry salt aerosol [4, 5, 6].

Halotherapy has been found to positively affect patients suffering from chronic respiratory diseases, improving mucociliary clearance and lung function in common chronic respiratory diseases, as well as quality of life.

There are currently no formal guidelines for using halotherapy in the form of salt chambers (halo chambers) or dry powder inhalers, but there is evidence for its use as a possible adjuvant therapy [7].

Based on these data, some researchers propose to include halotherapy in the rehabilitation complex for patients who have suffered from COVID-19 [8].

Usually, for halotherapy, dry dispersed NaCl aerosol is used. The features of its action are the improvement of the condition of the ciliated epithelium, the normalization of bronchial patency, the progress of the drainage function of the bronchi, the reduction of edema of the bronchial mucosa, the pronounced desensitizing effect of the immunosuppressive action (decrease in eosinophilia, circulating immune

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complexes). The role of histamine in the activation of bronchoconstriction has been shown by many [9, 10]. In this regard, the results of studies showing that histamine mediates the respiratory tract reaction to isotonic NaCl solution are exciting [11, 12].

When studying the absorption of dry NaCl aerosol in the respiratory organs, it was found that the degree of retention of particles of the same dispersion is higher in dry aerosol. In this regard, the use of a highly dispersed aerosol makes it possible to use low doses and reduce the likelihood of unwanted side reactions [13, 14]. Possible short-term side effects after halotherapy are dizziness, headache, cough, nasal discharge, and a slight increase in body temperature.

Recently, there has been an increasing interest in using various mineral waters (MW) for inhalation, especially in the form of evaporated salts [15, 16].

When inhaled, the salt composition of mineral water, enters the cavity of the upper respiratory tract and lungs in the form of a fine aerosol. It directly affects the mucous membrane of the respiratory tract. As a result of MW salt dispersion, its contact with mucous membranes increases, the absorption of chemical components is accelerated, and the condition of the external respiration improves. In the aerosol coagulation zone in the trachea and bronchi, the motor activity of the ciliated epithelium increases, and the viscosity of the bronchial secretion decreases, which increases mucociliary clearance and airway clearance in patients with obstructive disorders of the respiratory system, including those caused by coronavirus infection [17].

The extensive absorption surface of the mucous membrane of the respiratory tract during haloinhalation causes not only a direct effect of the biologically active components of MW on the upper and lower respiratory tract and lungs, but also indirectly on the entire body [18].

Features of the action of different types of MW are associated with the presence of ions of various elements and gas composition, which determines their differentiated use.

The work aims to study the effectiveness of halotherapy with mineral water salts in rehabilitating patients with COVID-19.

MATERIALS AND METHODS

30 male patients were examined after suffering from COVID-19. The average period of convalescence after suffering a disease was (2.6±1.6) months. Rehabilitation was carried out in the «Multiprofile sanatorium Teplitsa» of the Transcarpathian region of Ukraine.

Inclusion criteria: confirmed clinical diagnosis of COVID-19 in history, radiographically or tomographically confirmed pneumonia of different localization, age from 50 to 60 years, normal body weight (BMI – 20-25 kg/m²).

Exclusion criteria: history of tuberculosis or other chronic infectious and non-infectious respiratory diseases, confirmed presence of COPD, presence of neoplasms, time from clinical recovery from COVID-19 of more than 4 months, overweight, obesity (BMI>25 kg/m²).

We formed 2 groups of patients.

Group 1 (control) – 15 people. The mean age was 58.7±3.3 years. Patients in this group received a basic rehabilitation complex. It included massage, physical rehabilitation in the form of group sessions of therapeutic physical exercises, and ultrasound exposure to the chest area (for a course of 10 procedures). A course of inhalation with a dry aerosol of environmentally friendly table salt was added to the basic rehabilitation complex. The chemical composition of this salt was as follows:

- Sodium chloride – 97.7%
- Calcium ion – 0.60%
- Magnesium ion – 0.10%
- Sulfate ion – 1.30%
- Iron oxide – 0.10%
- Sodium sulfate – 0.20%.

The dispersity of the aerosol was at least 80% of particles no larger than 5 microns in size; the procedure duration is 5 minutes, daily for the course No. 10 inhalations.

Group 2 (main) – 15 people. The mean age was 57.7±3.1 years. To this group of patients, a course of inhalations with a dry aerosol of salt evaporated from the MW of the Teplitsa deposit (Multipurpose sanatorium Teplitsa, Transcarpathian region, Ukraine) was added to the basic medical rehabilitation complex for this group of patients. The chemical composition of this salt was as follows:

- Sodium and potassium – 12.83 g/l,
- Chloride – 20.15 g/l,
- Bromine – 7.20 mg/l,
- Iodine – 0.75 mg/l,
- Metasilicic acid – 7.41 mg/l.

Inhalations with dry aerosols were dispensed by the Haloinhalator Galoneb apparatus (GISA-01), Ukraine. The dispersion of aerosols was not less than 80% of the particles, no larger than 5 microns; the procedure duration was 5 minutes daily for a course of No. 10 inhalations.

All patients underwent a comprehensive examination before the start of the rehabilitation course and after its completion. The complex of examinations included clinical methods, functional studies, complete blood count, and biochemical blood tests (total protein content, bilirubin and its fractions, creatinine, glucose and C-reactive protein) by standard laboratory methods.

When examining the function of external respiration using a computer spirograph «Cardio-Spiro», Ukraine, the vital capacity of the lungs (VC), the forced vital capacity of the lungs (FVC), forced expiratory volume per one second (FEV1), and the Tiffeneau index were evaluated. The due VC values were calculated according to Anthony's formula.

To assess physical performance, Six-Minute Walk Test (6MWT) was used [19].

Statistical processing of the obtained data was carried out using the XLSTAT 2021 program. To assess the significance of differences between the samples, the φ* – criterion, Fisher's angular transformation, was used. The Student's method for related samples was used o assess the significance of differences in groups before and after rehabilitation; The Student's method for unrelated samples was used to determine intergroup differences. Differences $p<0.05$ were considered significant.

The study was a prospective, explorative and observational trial. This trial was performed in accordance with the Declaration of Helsinki; it was approved by the Commission on Bioethics of the "Ukrainian Research Institute of Medical Rehabilitation and Resort Therapy of the Ministry of Health of Ukraine", No. 8 of 15.08.2022.

All patients provided written informed consent before inclusion in the study.

RESULTS AND DISCUSSION

Anthropometric parameters of the examined patients are presented in Table 1. The average BMI in patients of both groups was at the upper limit of normal.

Before treatment, patients in both groups complained of fatigue, cough, shortness of breath, headache, and decreased physical activity (Table 2). As seen from Table 2, as a result of treatment in both groups, there was a decrease in the frequency of clinical symptoms. However, if significant changes were recorded only in the control group in four indicators, then in the main group, the frequency of all indicators significantly decreased. In addition, the frequency of manifestation of five symptoms out of six observed after the end of treatment was significantly lower in the main group than in the control group. The only indicator for which there was no significant difference between groups was headache.

The spirographic parameters of the subjects are presented in Table 3. Before the start of rehabilitation, the vital capacity of the lungs in patients of both groups was at the lower limit of the norm (concerning the predicted one). The values of the forced vital capacity and especially the forced vital capacity for 1 second, are significantly below the norm ERS. This is also evidenced by the extremely low value of Tiffeneau index – 57.1% for both groups, with a norm of no less than 75.0% [20]. The value of this index clearly indicates the presence of obstructive respiratory failure in patients. This condition may be associated with spasms or swelling of the bronchial mucosa, which makes it difficult for free air circulation.

Under the influence of the rehabilitation complex in both groups, there is a performance improvement. However, significant changes are observed only

Table 1. Anthropometric parameters, M±m

Indicators	Control group n=15	Main group n=15	p
Height, m	1.71±0.73	1.72±0.67	>0.05
Weight, kg	83±4.8	85±4.3	>0.05
BMI, kg/m²	24.3±2.1	24.7±1.9	>0.05

p – reliability between indicators in the main and control groups after the course of treatment

Table 2. Changes of clinical signs before and after rehabilitation, n, (%)

Clinical manifestations	Control group n=15		Main group n=15		p
	Before treatment	After treatment	Before treatment	After treatment	
Cough	13 (87)	8 (53)*	14 (93)	4 (27)*	<0.05
Dyspnea	11 (73)	7 (47)	12 (80)	5 (33)*	<0.05
Fatigue	12 (80)	7 (47)*	12 (80)	4 (27)*	<0.05
Headache	7 (47)	4 (27)*	9 (60)	4 (27)*	>0.05
Sleep disturbance	6 (40)	4 (27)	6 (40)	0*	<0.05
Irritation	6 (40)	2 (13)*	6 (40)	0*	<0.05

* – reliability of changes between indicators in the group before and after the course of treatment ($p<0.05$);
p – reliability between indicators in the main and control groups after the course of treatment ($p<0.05$)

in patients of the main group. The indicator FEV1 increased to the greatest extent, which after rehabilitation is significantly higher than in the control group ($p<0.05$). As a result, the Tiffeneau index significantly increases in the main group compared to the control group, reaching normal values.

As a consequence of improved respiratory performance, there was an increase in the distance that patients walked in the Six-Minute Walk Test (Table 4). The initial values of 6-minutes walking distance (6MWD) were reduced in both groups, which indicates a decrease in the patients' physical fitness. The recorded 6MWD values are typical for patients with interstitial lung diseases (250-275 m). After treatment, statistically significant changes were recorded in both groups. However, the increase in distance in the main group is much more powerful and significantly higher than in the control group.

It should be noted that the value of pulse oximetry in both groups before the start of rehabilitation was within the normal range and slightly increased after (Table 5).

The study of peripheral blood confirms the oximetry data. As seen from Table 6, even before the start of rehabilitation, the patients had no pathological changes in the red blood - the number of erythrocytes and the hemoglobin content were close to the upper limit of the physiological norm. An increase in these characteristics during the rehabilitation period is noted in patients of both groups but is not statistically significant.

As for the indicators of white blood, the percentage of leukocytes before the start of rehabilitation did not exceed the reference values in both groups and subsequently decreased slightly. The ratio of neutrophils and lymphocytes in both groups changed in the same direction but to a different extent. There was a decrease in the percentage contribution of neutrophils, especially stab. Their reduction was significant in both groups; however, in the main group, it was statistically significantly more potent than in the control group ($p<0.05$). There was also a decrease in the contribution of segmented neutrophils in both groups, but it was insignificant.

On the contrary, the percentage of lymphocytes increased, and in the main group, it was statistically significant. As a result, there was a decrease in the Krebs index, which was significantly lower in the main group ($p<0.05$). This phenomenon can be interpreted as a decrease in the level of inflammation preserved after suffering from COVID-19 [21, 22, 23].

Also, in the main group, the number of eosinophils significantly increases, which can be explained by an

Table 3. Changes of spirography indicators before and after rehabilitation, M±m

Indicators	Control group n=15		Main group n=15		p
	Before treatment	After treatment	Before treatment	After treatment	
VC, l	2.8±0.2	3.1±0.3	2.8±0.1	3.3±0.2*	>0.05
VC\VC predicted, %	75.7±4.9	83.8±5.3	75.7±4.8	89.2±5.5*	>0.05
FVC, l	2.6±0.2	2.8±0.3	2.5±0.2	3.1±0.2*	>0.05
FEV1, l/s	1.6±0.1	1.9±0.2	1.6±0.2	2.5±0.2*	<0.05
Tiffeneau index, %	57.1±4.7	61.3±4.9	57.1±4.8	75.8±5.1*	<0.05

* – reliability of changes between indicators in the group before and after the course of treatment ($p<0.05$);
p – reliability between indicators in the main and control groups after the course of treatment ($p<0.05$)

Table 4. Changes of Six-Minute Walk Test, M±m

Indicator	Control group n=15		Main group n=15		p
	Before treatment	After treatment	Before treatment	After treatment	
6MWD, m	275±14.0	326.0±10.4*	288±10.7	362.7±11.6*	<0.05

* – reliability of changes between indicators in the group before and after the course of treatment ($p<0.05$);
p – reliability between indicators in the main and control groups after the course of treatment ($p<0.05$)

Table 5. Changes of pulse oximetry, M±m

Indicator	Control group n=15		Main group n=15		p
	Before treatment	After treatment	Before treatment	After treatment	
SpO_2, %	96.9±0.5	97.8±0.2	96.7±0.3	98.1±0.2	>0.05

p – reliability between indicators in the main and control groups after the course of treatment

Table 6. Changes of indicators of peripheral blood, M±m

Indicators	Control group n=15		Main group n=15		p
	Before treatment	After treatment	Before treatment	After treatment	
Erythrocytes, 10^{12}/l	4.7±0.2	4.8±0.4	4.7±0.1	4.9±0.1	>0.05
Hemoglobin, g/l	140.5±4.0	141.0±4.0	139.7±5.5	141.2±3.2	>0.05
Leukocytes, 10^9/l	7.3±0.7	6.9±0.6	7.1±0.5	6.7±0.6	>0.05
Band neutrophils, %	4.9±0.6	3.6±0.5*	5.1±0.6	2.7±0.5*	<0.05
Segmented neutrophils, %	58.0±6.0	56.2±4.2	57.0±3.0	54.1±2.4	>0.05
Lymphocytes, %	27.8±3.2	31.5±2.2	28.4±3.1	35.0±2.0*	>0.05
Krebs index, c.o.	2.25±0.7	1.88±0.6*	2.21±0.8	1.63±0.6*	<0.05
Eosinophils, %	2.1±0.8	3.6±0.7	2.2±0.7	3.9±0.8*	>0.05
Monocytes, %	5.6±1.2	5.9±1.0	5.3±0.6	5.1±1.1	>0.05
Basophils, %	0±	0±	0±	0±	>0.05
Platelets, 10^9/l	271.2± 41.7	276.2±40.8	288.2±24.9	291.5±17.9	>0.05

* – reliability of changes between indicators in the group before and after the course of treatment ($p<0.05$);
p – reliability between indicators in the main and control groups after the course of treatment ($p<0.05$)

Table 7. Changes of metabolic indicators, M±m

Indicators	Control group n=15		Main group n=15	
	Before treatment	After treatment	Before treatment	After treatment
Total bilirubin, μmol/l	11.7±2.1	11.2±1.9	12.2±1.9	10.8±1.5
Direct (bound) bilirubin, μmol/l	4.0±0.7	3.9±0.5	4.2±0.5	3.8±0.4
Indirect bilirubin (free), mmol/l	7.7±1.4	7.4±1.4	7.9±1.4	7.0±1.1
Creatinine, μmol/l	79.1±6.7	75.3±5.3	79.9±3.6	71.8±2.9
Total protein, g/l	72.5±5.9	71.2±4.2	71.1±5.2	71.2±4.6
C-reactive protein, mg/l	2.2±0.8	0.9±0.2	2.1±0.7	0.5±0.1*
Fibrinogen, g/l	3.8±0.4	3.5±0.4	4.1±0.6	3.5±0.7
Glucose, mmol/l	5.5±0.1	5.5±0.1	5.9±0.8	6.1±1.2

* – reliability of changes between indicators in the group before and after the course of treatment ($p<0.05$)

allergic-like reaction to the active components of the mineral salt.

The level of monocytes was at the upper limit in both groups of patients and practically did not change. Since the function of these cells is associated with specific phagocytosis (mainly with the elimination of the remains of destroyed cells), it can be assumed that the examined patients continue to utilize cells affected by coronavirus infection.

The values of metabolic parameters in both groups were within the normal range and did not significantly change during the rehabilitation period. The only exception is the C-reactive protein, the value of which significantly decreased in the main group, which indicates the attenuation of residual inflammatory manifestations after COVID-19 (Table 7).

Thus, we see that the main respiratory complications after COVID-19 are associated with airway obstruction. One of the most common manifestations of respiratory tract diseases is allergic reactions, namely: runny nose, itching, cough, shortness of breath, and swelling of the mucous membranes. Respiratory tract infections, particularly those caused by COVID-19, are complicated by immune sensitization involving the lung immune response. Experimental studies have shown that halotherapy reduces the infiltration of inflammatory cells into the lungs and airway mucosa. It also relieved oxidative stress in the lung tissue. Halotherapy relieves chronic obstructive pulmonary disease by alleviating NLRP3 inflammasome-mediated pyroptosis [24].

Our study has several limitations. The main ones are:

- the small sample size,
- the sex and age of the patients,
- the time since the illness,
- the chosen regimen for the procedures.

Changing these parameters will provide additional information on the impact of halotherapy with mineral water salts on the rehabilitation of patients after COVID-19.

CONCLUSIONS

The use of haloinhalations with MW salts in the rehabilitation of patients after suffering from COVID-19 disease significantly improves the clinical condition of convalescents. The main effect is associated with a decrease in obstructive complications of the respiratory tract as a result of a decrease in inflammation.

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Disclosure conflict of interest

The authors declare that they have no conflicts of interest concerning this article.

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ORIGINAL ARTICLE

FREQUENCY OF CONSUMPTION OF SELECTED FOODS BY PEOPLE WITH ACNE VULGARIS

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ABSTRACT

Background. Acne is an inflammatory disease affecting adolescents during puberty, but also adults. Determinants of acne may include genetic predisposition as well as diet. The Western diet is rich in processed products with low nutrient density, resulting in a lack of supply of many essential minerals that are needed for the body to function properly.
Objective. The aim of this study was to assess the dietary behaviour of people with acne vulgaris and respondents' self-assessment of the severity of acne lesions after consumption of selected products.
Material and Methods. The study was carried out by means of an online, self-administered questionnaire, a link to which was inserted in group of people struggling with acne on a social network.
Results. More than half of the respondents said that acne was present in their parents. Almost 91% of the women surveyed said that acne lesions were exacerbated before menstruation. Respondents were most often treated by a dermatologist. The most common foods consumed by the respondents were milk and milk products and wheat bread. The least frequently consumed products were: sultanas, cornflakes, alcohol and fizzy drinks. Statistical analysis showed a strong strength of association between consumption of white rice, fast food, omega-6-rich oils on the severity of acne lesions, while sultana consumption was very strong correlated. In addition, the study showed an almost certain correlation regarding the consumption of white flour pasta on acne lesions.
Conclusions. Statistical analysis showed a relationship between the consumption of white rice, white flour pasta, fast food products, sultanas, oils rich in omega-6 fats and the exacerbation of acne lesions.

Key words: *acne vulgaris, diet, eating behaviour*

STRESZCZENIE

Wprowadzenie. Trądzik jest chorobą o charakterze zapalnym, dotykająca młodzież w okresie dojrzewania, ale także osoby dorosłe. Do czynników, warunkujących wystąpienie trądziku mogą należeć predyspozycje genetyczne, a także dieta. Dieta zachodnia bogata jest w produkty przetworzone o niskiej gęstości odżywczej, co skutkuje tym, że nie dostarcza ona wielu niezbędnych składników mineralnych, które są potrzebne do prawidłowego funkcjonowania organizmu.
Cel. Celem pracy była ocena zachowań żywieniowych osób z trądzikiem pospolitym i samooceną ankietowanych nasilenia zmian trądzikowych po spożyciu wybranych produktów.
Materiał i Metody. Badanie przeprowadzono za pomocą internetowej, autorskiej ankiety, do której link został wstawiony w grupie osób borykających się z trądzikiem na portalu społecznościowym.
Wyniki. Ponad połowa ankietowanych stwierdziła, że trądzik występował u ich rodziców. Prawie 91% badanych kobiet stwierdziło, że zmiany trądzikowe nasilają się przed miesiączką. Ankietowani najczęściej leczyli się u dermatologa. Najczęstszą grupą spożywaną przez ankietowanych były: mleko i jego przetwory oraz pieczywo pszenne. Najrzadziej spożywanymi produktami były: rodzynki, płatki kukurydziane, alkohol, napoje gazowane. Analiza statystyczna wykazała silny związek między spożyciem białego ryżu, fast food, olejów bogatych w kwasy omega-6 na nasilenie zmian trądzikowych, podczas gdy spożycie sułtanki było bardzo silnie skorelowane. Ponadto badanie wykazało prawie pewną korelację między spożyciem makaronu z białej mąki a zmianami trądzikowymi.
Wnioski. Analiza statystyczna wykazała związek między spożyciem białego ryżu; makaronu z białej mąki; produktów typu fast food; rodzynek; olejów bogatych w tłuszcze omega-6 a zaostrzeniem zmian trądzikowych.

Słowa kluczowe: *trądzik pospolity, dieta, zachowania żywieniowe*

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INTRODUCTION

Acne vulgaris is a chronic inflammatory skin disease that affects approximately 85% of people aged 12-25 years, and can persist into adulthood [6]. Negative effects of acne include the possibility of depression, anxiety, or insomnia [5]. Genetic factors, *Propionibacterium acnes*, androgenic activity, increased sebum production, keratinisation of the skin, as well as certain medicinal substances and cosmetics are cited as its main causes [4, 6]. The main hormones responsible for the development of acne are androgens, insulin and IGF-1 (insulin-like growth factor-1) [2].

The Western diet is also thought to influence the development of inflammatory skin lesions among other acne. In the past, groups such as the indigenous Canadian Inuit, the Japanese people of Okinawa, the Aché of Paraguay, the South African Zulus and the Kitavan Islanders of Papua New Guinea did not suffer from skin diseases. When they were introduced to Western dietary habits, processed foods, dairy products and refined sugars, cases of acne began to be reported in them [1]. The Western dietary model is deficient in many essential vitamins and nutrients, minerals [3].

Vitamins such as A, E, K, C, B vitamins and minerals such as selenium, zinc, copper and iron, as well as essential fatty acids (EFAs) are needed to maintain a healthy complexion [3]. Therefore, in order to treat acne lesions, a properly selected treatment, in combination with skin care and a correctly balanced nutrition, seems to be extremely important [4].

Studies show that a high glycaemic index contributes to hyperinsulinaemia, which affects androgen hormone secretion through IGF-1 stimulation, resulting in increased seborrhoea [12]. These factors contribute to a decrease in insulin-like growth factor binding protein IGFBP-3 that controls epidermal proliferation, as well as alterations in retinoid pathways [11].

Milk is a product that contains bioactive compounds such as insulin-like growth factor IGF-1, hormone precursors, transforming growth factor beta (TGF-β) in its composition [12]. Protein isolates in protein supplements have a higher insulin index than milk, due to the defatting of the product, isolation and increased concentration of selected essential amino acids. Insulin/IGF-1 levels are increased by whey protein, with a concomitant reduction in transcription factor elevated FoxO1 gene expression [1, 12].

Essential fatty acids (EFAs) have a positive effect on the complexion, reducing inflammation and inhibition of bacterial growth [4]. It has been proven that omega-3 fatty acids reduce IGF-1 levels [1]. The ratio of omega 3 and omega 6 fatty acids should be 1:3-1:5. However, disrupting this ratio and increasing the supply of omega-6 can lead to inflammatory skin conditions, including the development of acne vulgaris [4].

The microbiota is related to the skin, which may mean that when the skin or microbiota is disturbed, increased activity of substance P and upregulation of P-containing nerve substances are observed. Substance P has been observed in acne and intestinal dysbiosis, and substance P can cause an increase in interleukin-6 levels and an increase in tumour necrosis factor-α which also contribute to acne formation [1]. Supplementation with *Lactobacillus rhamnosus* GG is thought to improve the complexion and reduce acne lesions due to a reduction in IGF-1 levels [1].

The aim of this study was to assess the dietary behaviour of people with acne vulgaris and the respondents' self-assessment of the severity of acne lesions after consumption of selected products.

MATERIAL AND METHODS

The study was conducted using an anonymous, online, self-administered questionnaire, a link to which was posted in groups of people struggling with acne vulgaris on a social networking site. The author's questionnaire consisted of 16 questions and included single and multiple choice. The questionnaire consisted of a metric, questions about the prevalence of *acne vulgaris* and a dietary section. The exclusion criterion for the study was people who did not suffer from acne vulgaris. The sampling was voluntary, so that respondents decided for themselves whether they wanted to take part in the survey. Due to the nature of the implementation of the survey, fully anonymous, the approval of the bioethics committee was not required.

Sixty people (55 women and five men) took part in the study. The respondents were aged between 13 and 35 years. The average age of the people surveyed was 23.5 years. The survey was conducted between October 2022 and January 2023. Results were processed using Excel and Statistica 13. The strength of the relationship was determined using *Gamma* correlation. Statistical significance was determined at $p<0.05$. *Gamma* correlation strengths were determined using the classification according to J. Guilford.

RESULTS

Among the group of respondents (n=60), the largest group consisted of people with secondary education (48.3%) and tertiary education (45%) and those studying (38.3%) or studying and simultaneously working (40%). The least, only 1.7% of respondents had a vocational education, while secondary education was held by 5% of respondents. Those only working accounted for 18.3% of those

surveyed. At the time of filling in the questionnaire, 3.3% were unemployed. The majority of respondents were urban residents (68.3%), while 31.7% were rural residents.

More than half (51.7%) of the respondents answered that their relatives (father, mother) had acne, while 21.7% declared that their parents did not have acne. The remainder of the respondents (26.7%) have no knowledge of this subject.

The most common location of acne among the respondents was the cheeks, forehead, jaw, and back. The question was multiple choice (Table 1).

Table 1. Location of acne

Location of acne	n	%
Cheeks	53	88.3
Mandible	41	68.3
Forehead	45	75
Nose	24	40
Cleavage	17	28.3
Back	32	53.3
Chin	2	3.3
Buttocks	2	3.3
Arms	2	3.3
Legs	2	3.3
Other (arms, abdomen, neck, head)	1	1.7

The majority of respondents – 75% did not complain of other illnesses, while the most frequently reported diseases were hypothyroidism (8.3%), polycystic ovary syndrome (5%) and atopic dermatitis (5%). The question was multiple choice.

Most (35%) respondents had been treated for acne lesions by a specialist, but had discontinued such treatment, while 33.3% were currently undergoing treatment. The remaining percentage (31.7%) of respondents responded negatively to this question. More than half of the respondents who were treated (58.3%) answered that they treated their lesions at a dermatologist, the second most treated among respondents was a gynaecologist (16.7%), followed by an endocrinologist (8.3%), a dietician (3.3%) and a general practitioner (1.7%). The question was multiple choice.

Almost all of the women surveyed (90.9%) responded that they noticed an increase in acne lesions before menstruation.

Respondents in the survey were asked about the use of any diet. 81.7% of the respondents were not on a diet, while the most frequently mentioned diets among respondents were plant-based diets: vegan (5%), vegetarian (3.3%). There were also responses mentioning a low glycaemic index diet (1.7%), as well as a carbohydrate-restricted diet (3.3%) and a dairy-free diet (1.7%).

More than half of the respondents (56.7%) consumed 4-5 meals per day. Three meals a day were consumed by 38.30% of the respondents. The smallest number of respondents (1.70%) consumed 1-2 meals per day, while more than 5 meals per day were taken by 3.30% of respondents.

Table 2 examines the correlation of the effect of frequency of consumption of each food on self-assessment of skin condition (whether, and to what extent, there is an exacerbation of acne lesions according to them) after consumption of the food in question. Table 2 shows only those who have declared that they consume the product in question.

Respondents to the questionnaire were asked how often they consumed each product. They had the following answers to choose from: several times a day, once a day, several times a week, once a week, 1-3 times a month, I do not consume.

In the next question, respondents were asked to indicate in their evaluation how the consumption of a particular product affects their skin condition. Respondents were asked to rate on a 5-point scale, where 0 meant no difference, 1-slightly, 3-average, 5-very aggravated.

Those who answered 'I do not consume' in the question were not included in the statistical analysis. A *Gamma* coefficient was used to examine the correlation between the frequency of consumption of each individual product and its impact in the respondent's self-assessment on acne lesions (Table 2).

Responses that related to consumption and also to the severity of acne lesions were combined for statistical analysis as follows: one or mildly exacerbates (0-1 scale); moderately exacerbates (scale 2-3); severely exacerbates (scale 4-5) (Table 2). The largest number of people surveyed consumed products such as white rice (n=56) and white flour pasta (n=55). In contrast, the fewest people decried the consumption of sultanas (n=25). On the other hand, white bread (n=29) and milk and dairy products (n=27) were consumed daily by the largest number of respondents (Table 2).

There was a correlation between the consumption of: white rice ($p=0.02$); white flour pasta ($p=0.007$); fast food products ($p=0.002$); sultanas ($p=0.045$); oils rich in omega-6 fats ($p=0.02$) and the exacerbation of acne lesions (Table 2). The finding of a statistical relationship ($p<0.05$) between the consumption of: white rice, white flour pasta, fast food products, sultanas, oils rich in omega-6 fats and the exacerbation of acne lesions suggests that there is some association between the consumption of these products and the appearance of acne or its exacerbation. In practical terms, this means that diet can have a significant impact on skin health, and the consumption of the products mentioned may

Table 2. Exacerbation of acne lesions after consumption of food products

Food products	Frequency of consumption	None or only slightly exacerbates	Moderately exacerbates	Severely exacerbates	Summary of those who have consumed (n)	p-value	*Gamma* correlation coefficient
Milk and milk products	Once or several times a day	17 (62.96%)	7 (25.93%)	3 (11.11%)	53	0.74	0.05
	Once or several times a week	16 (69.57%)	6 (26.09%)	1 (4.35%)			
	1-3 times a month	1 (33.30%)	0 (0%)	2 (66.70%)			
Wheat bread	Once or several times a day	23 (79.31%)	4 (13.79%)	2 (6.90%)	54	0.87	-0.04
	Once or several times a week	17 (80.95%)	4 (19.05%)	0 (0%)			
	1-3 times a month	3 (75%)	1 (25%)	0 (0%)			
Groats	Once or several times a day	0 (0%)	0 (0%)	1 (100%)	53	0.42	-0.23
	Once or several times a week	24 (96%)	1 (4%)	0 (0%)			
	1-3 times a month	25 (92.59%)	2 (7.41%)	0 (0%)			
White rice	Once or several times a day	0 (0%)	0 (0%)	1 (100%)	56	0.02	0.64
	Once or several times a week	28 (96.55%)	1 (3.45%)	0 (0%)			
	1-3 times a month	24 (92.31%)	2 (7.69%)	0 (0%)			
White flour pasta	Once or several times a day	1 (50%)	0 (0%)	1 (50%)	55	0.007	0.91
	Once or several times a week	37 (88.10%)	5 (11.90%)	0 (0%)			
	1-3 times a month	11 (100%)	0 (0%)	0 (0%)			
Cornflakes	Once or several times a day	0 (0%)	0 (0%)	1 (100%)	27	0.47	0.27
	Once or several times a week	5 (100%)	0 (0%)	0 (0%)			
	1-3 times a month	18 (85.71%)	3 (14.29%)	0 (0%)			
Fast food	Once or several times a day	0 (0%)	0 (0%)	1 (100%)	53	0.002	0.50
	Once or several times a week	3 (15.79%)	9 (47.37%)	7 (36.84%)			
	1-3 times a month	15 (45.45%)	11 (33.33%)	7 (21.21%)			
Sweeteners	Once or several times a day	5 (35.71%)	3 (21.43%)	6 (42.86%)	46	0.16	-0.02
	Once or several times a week	5 (21.74%)	13 (56.52%)	5 (21.74%)			
	1-3 times a month	4 (44.4%)	5 (55.6%)	0 (0%)			

Sweets	Once or several times a day	2 (20%)	2 (20%)	6 (60%)	55	0.29	0.16
	Once or several times a week	7 (21.21%)	15 (45.45%)	11 (33.33%)			
	1-3 times a month	3 (25%)	5 (41.67%)	4 (33.33%)			
Salty snacks	Once or several times a day	0 (0%)	1 (25%)	3 (75%)	51	0.59	0.08
	Once or several times a week	11 (45.83%)	9 (37.50%)	4 (16.67%)			
	1-3 times a month	8 (34.78%)	9 (39.13%)	6 (26.09%)			
Fizzy drinks	Once or several times a day	1 (33.33%)	0 (0%)	2 (66.67%)	37	0.64	0.09
	Once or several times a week	7 (43.75%)	6 (37.5%)	3 (18.75%)			
	1-3 times a month	9 (50%)	6 (33.33%)	3 (16.67%)			
Alcohol	Once or several times a day	0 (0%)	0 (0%)	1 (100%)	30	0.14	0.32
	Once or several times a week	8 (57.14%)	3 (21.43%)	3 (21.43%)			
	1-3 times a month	7 (46.67%)	3 (20%)	5 (33.33%)			
Hot spices	Once or several times a day	2 (33.33%)	2 (33.33%)	2 (33.33%)	42	0.31	0.17
	Once or several times a week	11 (52.38%)	5 (23.81%)	5 (23.81%)			
	1-3 times a month	6 (40%)	6 (40%)	3 (20%)			
Ripe banana	Once or several times a day	5 (83.33%)	0 (0%)	1 (16.67%)	52	0.86	0.04
	Once or several times a week	27 (87.1%)	4 (12.9%)	0 (0%)			
	1-3 times a month	13 (86.67%)	2 (13.33%)	0 (0%)			
Fatty meat	Once or several times a day	0 (0%)	0 (0%)	2 (100%)	39	0.08	-0.38
	Once or several times a week	19 (79.17%)	3 (12.50%)	2 (8.33%)			
	1-3 times a month	10 (76.92%)	3 (23.08%)	0 (0%)			
Sultanas	Once or several times a day	0 (0%)	0 (0%)	1 (100%)	25	0.045	0.71
	Once or several times a week	4 (100%)	0 (0%)	0 (0%)			
	1-3 times a month	19 (95%)	1 (5%)	0 (0%)			
Oils rich in omega-6 fats	Once or several times a day	7 (77.78%)	1 (11.11%)	1 (11.11%)	42	0.02	0.64
	Once or several times a week	13 (100%)	0 (0%)	0 (0%)			
	1-3 times a month	17 (85%)	2 (10%)	1 (5%)			

increase the risk of acne occurrence or worsening of acne symptoms. Therefore, people struggling with acne may benefit from reducing or eliminating these foods from their diet to help control symptoms.

There was no association between intake of: milk and milk products (p=0.74); wheat bread (p=0.87); groats (p=0.42); cornflakes (p=0.47); sweeteners (p=0.16); sweets (p=0.29); salty snacks (p=0.59); fizzy drinks (p=0.64); alcohol (p=0.14); hot spices (p=0.31); ripe banana (p=0.86); fatty meat (p=0.08) and exacerbation of acne lesions (Table 2). The finding that there was no correlation (p>0.05) between the consumption of the listed products and the exacerbation of acne lesions means that a diet containing these products is unlikely to have a significant effect on the occurrence of acne or its exacerbation. In practice, this means that consumption of milk and milk products, wheat bread, groats, cornflakes, sweeteners, sweets, salty snacks, fizzy drinks, alcohol, hot spices, ripe banana, fatty meat should not be responsible for worsening the skin condition in most people.

The strength of the association of statistical characteristics determining the consumption of: milk and dairy products; wheat bread; sweeteners; salty snacks; ripe banana; fizzy drinks with the exacerbation of acne lesions is negligible, suggesting that there is minimal or insignificant association between the consumption of these products and acne exacerbation (Table 2). In practical terms, this means that the consumption of milk and dairy products; wheat bread; sweeteners; salty snacks; ripe banana; fizzy drinks is unlikely to have a significant effect on the exacerbation of acne lesions in the subjects.

The strength of the association of statistical characteristics determining the consumption of groats, cornflakes, sweets with the exacerbation of acne lesions is weak, suggesting that there is a very weak association between the consumption of these products and acne exacerbation (Table 2). In practice, this means that the consumption of groats, cornflakes and sweets is unlikely to have a significant effect on the exacerbation of acne lesions.

The strength of the association of statistical characteristics determining the consumption of: alcohol; fatty meat and acne lesion exacerbation is medium, suggesting that there is an average association between the consumption of these products and acne exacerbation. Which may mean that among the subjects, it may influence, in part, the condition of the complexion (Table 2).

The strength of the association of statistical characteristics determining the consumption of: white rice; fast food; oils rich in omega-6 fats and exacerbation of acne lesions is strong (Table 2). In practice, this means that consumption of: white rice; fast food; oils rich in omega-6 fats may be a significant risk factor for exacerbation of acne lesions in the study subjects. People with acne or who are concerned about the health of their skin may benefit from reducing their intake of these products to reduce acne symptoms, as found in a study in which less frequent consumption of these products resulted in less severe acne lesions.

The strength of the association of the statistical characteristics determining the consumption of sultanas and exacerbation of acne lesions is very strong (Table 2).

The strength of the association of the statistical characteristics determining the consumption of white flour pasta is almost certain, meaning that there is a very strong relationship. In practice, this means that the consumption of white flour pasta is very consistently and clearly related to the exacerbation of acne lesions in the subjects of the study.

Analyzing the data from the Table 2, it can be concluded that less frequent consumption of white flour pasta and omega-6-rich oils does not reduce the severity of acne lesions. For the products: milk and milk products, sweets, salty snacks, fizzy drinks, alcohol, hot spices that were most frequently selected with the highest lesion exacerbation, no reduction in lesion reduction was generally observed in those who consumed less frequently (Table 2).

DISCUSSION

According to the most recent English-language literature available in the Pubmed database, it is concluded that a high glucose intake and a high glycaemic index diet, promotes the development of acne vulgaris, while dairy intake shows a positive correlation among populations living in Europe, Australia and the United States [10].

In the study, more than half of the respondents answered affirmatively that acne was present in their parents, similar results were obtained in a 2015 study by Jakubczyk et al. [7] in which 51% surveyed adolescents aged 12 to 25 years from Szczecin confirmed the presence of acne in their parents. It is estimated that there is more than a 50% probability of acne occurrence in offspring whose parents both suffered from acne [7]. Among the adolescents studied, acne was most common on the face (85%), back (36%), and arms (22%) [7].

Similar to the study by Jakubczyk et al. [7], in our study, acne lesions most frequently affected respondents on the face. Similar results were obtained by Rokowska-Waluch et al. [13] in a 2009 study with 100 participants aged 11-55 years, where respondents also indicated the face (93%), back (54%), shoulders (39%), and décolleté (24%) most frequently [13] .

In our study, almost all women, noticed an increase in lesions before menstruation. In the study

by Jakubczyk et al. [7], more than half of the women noted an increase in lesions, which also indicates a hormonal basis for the occurrence of acne.

In the study by Jakubczyk et al. [7], 71% of the surveyed adolescents noticed an exacerbation of changes after eating chocolate, 56% after chips and fast food, while 44% responded that they experienced an exacerbation of symptoms after eating spicy food. In our own study, respondents also identified sweets (chocolate, cocoa, chocolate bars, biscuits, cakes, ice cream, shake), fast food, salty snacks, fizzy drinks, hot spices and sweeteners as the products that most exacerbated their acne lesions. Also in the study by Rokowska-Waluch et al. [13], sweets were the main product that the subjects thought had an impact on acne lesions.

In a study by Akpinar Kara and Ozdemir [8] involving 53 acne patients and 53 non-acne patients, acne exacerbation was found to be exacerbated with carbohydrate consumption (p=0.007), whereas the authors did not associate exacerbation with fat consumption (p=0.69). In their own study, respondents rated milk as having an average effect on lesion exacerbation and did not notice any difference after oil consumption.

Also, in a study by LaRosa et al. [9] involving 225 participants (120 people with acne, 105 people without acne lesions), there were no statistical differences in the consumption of full-fat dairy (p=0.95) and reduced-fat dairy (p=0.36) between the study group and the control group. The authors of the same study showed no significant differences in mean glycaemic index values (5.09% acne subjects vs. 4.73% control group; p=0.7) or glycaemic load (73.37% vs. 50.69%; p=0.18) between the study groups [9]. The study authors concluded that their study shows that it is low-fat milk and skimmed milk (p=0.01) that are positively associated with moderate acne incidence, while full-fat milk has no statistically significant effect on acne (p=0.75) [9]. On the other hand, a study by Smith et al. [14] found that after a 12-week intervention, in groups of 16 and 15 participants, subjects who were on a low-glycaemic load diet showed an improvement in acne lesions (59%) compared to the control group (38%) (p=0.046).

For vegan and vegetarian diets with a reduced dairy supply, researchers Stewart and Bazergy [15] found in their study that adherence to a plant-based diet did not significantly differ between those with acne (n=8; 1.7%) and the control group (n=9; 3.6%) (p=0.13). In contrast, the study did not include a description of the meals and thus the glycaemic load and glycaemic index are unknown.

CONCLUSIONS

Statistical analysis showed a relationship between the consumption of white rice (p=0.02); white flour pasta (p=0.007); fast food products (p=0.002); sultanas (p=0.045); oils rich in omega-6 fats (p=0.02) and the exacerbation of acne lesions.

It was observed that the consumption of products that are statistically significant and are in addition characteristics are highly correlated, less frequent consumption influences the reduction in severity, or lack of severity, of acne lesions after consumption of these products.

Respondents rated sweets, fast food, salty snacks, fizzy drinks, spicy condiments and sweeteners as exacerbating their acne lesions.

Those who have indicated that a particular product has exacerbated their lesions the most should observe whether reducing or eliminating such a product will help them improve their complexion.

It is important to raise patient awareness of the impact of diet on skin condition as an additional support for treatment. For patients with acne vulgaris, it is recommended to limit the following products: milk and milk products, hot spices, processed or high glycaemic index products.

More scientific studies need to be carried out in order to make clear recommendations.

Conflict of interest

The authors declare no conflict of interest.

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ORIGINAL ARTICLE

THE USE OF PORTABLE ABDOMINAL BIOIMPEDANCE ANALYZER YSCOPE IN THE ASSESSMENT OF ABDOMINAL OBESITY

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ABSTRACT

Background. Obesity, especially abdominal obesity, is strongly correlated with metabolic and other health risks. Diagnosis and assessment of obesity is important in clinical and routine preventive practice. From the point of view of risk, it is necessary to distinguish not only the area of fat tissue accumulation, but also its type.

Objective. The aim of the study was to use a new portable abdominal bioimpedance analyzer, which is intended for the area of abdominal adipose tissue, as part of the evaluation of the body structure of a selected group of volunteers with a focus on the differentiation of subcutaneous and visceral adipose tissue and to assess its usefulness in practice.

Material and Methods. Body composition was analyzed using a portable abdominal bioimpedance analyzer Yscope (PA-BIA) in combination with a bioimpedance device InBody 970 (high-frequency bioelectrical impedance/HF-BIA). Eighty-three volunteers at the age of 24.92±7.24 years with representation of both sexes participated in the study.

Results. Abdominal fat did not differ significantly between the sexes, women reached an average value of 2.01±1.14 kg, men 2.22±1.60 kg ($p>0.05$). Gender differentiation was manifested in the case of visceral fat ($p<0.01$) and visceral fat area ($p<0.01$), the values of which were lower in women than in men. In the case of subcutaneous fat, we found the opposite trend of values in relation to gender, where lower values were achieved by men, but there were no significant differences ($p>0.05$). Visceral fat was most correlated with abdominal fat ($r=0.86$) and waist circumference ($r=0.85$), subcutaneous fat had the strongest positive correlations with abdominal fat ($r=0.93$) and with body fat mass ($r=0.93$).

Conclusions. PA-BIA in combination with HF-BIA makes it possible to determine the representation of subcutaneous and visceral fat in the abdominal area, which the conventional MFS-BIA method does not allow. When evaluating body composition, significant gender differentiation is confirmed, which is an important factor affecting different health risks related to gender and the representation of different types of fat tissue localized and accumulated in different parts of the body.

Keywords: *fat, abdominal, visceral, subcutaneous, bioimpedance, Yscope, InBody*

INTRODUCTION

Adipose tissue has been considered an energy reservoir for decades, but nowadays it is considered a complex organ that, although it still fulfills the function of an energy source, is also metabolically active and interacts with systemic and local inflammation [23]. The health risk is represented by adipose tissue dysfunction in the form of adipocyte hypertrophy, low level of free fatty acid intake, reduced triglyceride synthesis, impaired adipogenesis, resistance to the inhibitory effect of insulin on lipolysis, adipose tissue fibrosis, secretion of pro-inflammatory cytokines and others [19, 27, 28, 46]. There is no doubt that not only total adiposity, but also abdominal adiposity is strongly associated with metabolic disorders and cardiovascular risk factors [7, 8, 33, 40].

Abdominal obesity is a condition in which fat accumulates excessively in the abdominal area. It is associated with diseases such as dyslipidemia, diabetes mellitus, atherosclerosis, and hypertension [1, 21, 33, 38]. Abdominal fat can be divided into subcutaneous and visceral fat according to its location. Abdominal visceral adipose tissue represents the largest proportion of visceral fat in the body. It consists of fat deposits in the retroperitoneal, omental and mesenteric spaces [23]. We can currently estimate the prevalence of visceral adiposity at a total of more than 20%. Excessive visceral adiposity is observed not only in the obese, but also in those with

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overweight or even normal body weight [8]. Visceral fat is considered a key factor in the pathogenesis of insulin resistance, chronic inflammation [11, 48] and type 2 diabetes [4]. Excessive visceral adipose tissue produces inflammatory mediators, producing three times more interleukin-6 compared to subcutaneous fat [29]. Increased IL-6 levels contribute to an increase in C-reactive protein, another inflammatory marker [10]. Excessive accumulation of visceral adipose tissue induces low-grade systemic inflammation [2, 5, 47]. The health risks of subcutaneous fat are still unclear and controversial. Compared to visceral adipose tissue, subcutaneous tissue is less cellular, has a smaller proportion of large adipocytes, is less vascular, and contains a smaller number of inflammatory and immune cells [15]. Several studies have suggested a protective effect of subcutaneous tissue on glucose metabolism [12, 14]. However, several studies suggest a positive relationship between the amount of subcutaneous fat and metabolic and atherogenic risks [9, 22]. Even excessive accumulation of subcutaneous adipose tissue can cause insulin resistance, so any adiposity, whether subcutaneous or visceral, should be considered and evaluated [34].

Abdominal obesity can be determined based on anthropometric data such as waist circumference, the ratio of waist circumference to hip circumference or using the ratio of waist circumference to height. However, these variables do not differentiate between subcutaneous and visceral fat, and are characterized by a high degree of imprecision [41]. Therefore, it is necessary to determine abdominal fat using technologies that are most suitable for this diagnosis. Among all methods, computed tomography (CT) is considered the gold standard [24]. In addition to this technique, magnetic resonance imaging (MRI) and dual-energy X-ray absorptiometry (DXA) are also used. However, they are very expensive, not readily available, and expose the patient to the risk of radiation exposure [17, 32]. In contrast, bioelectrical impedance (BIA) is non-invasive, inexpensive and without the risk of exposure to radiation [35, 50]. The principle of the method consists in the passage of an electric current through parts of the body that create resistance and cause a delay in conduction through the membranes, which causes reactance [30]. BIA uses the difference in electrical conductivity according to the biological characteristics of the tissue [18]. BIA is able to determine the representation of fat and fat-free mass, as well as body water. Among other things, the multi-frequency segmental BIA method (MFS-BIA) is also capable of segmental body composition analysis using electrodes that are in contact with the limbs [26]. Currently, non-invasive MFS-BIA is mainly used to estimate visceral adipose tissue [20, 36]. For visceral fat, this technique has been shown to have a significant correlation with values determined by CT, MRI or DXA [37]. This correlation is moderate, therefore creating an opportunity for improvement of the BIA technique. Scharfetter et al. [42] developed a technique to quantify abdominal subcutaneous fat with electrical impedance in the waist region. Ryo et al. [39] introduced the determination of VFA using local BIA. In 2020, a portable abdominal impedance analyzer (Yscope R; InBody Corporation, Seoul, South Korea) was developed for these purposes, which works on the principle of transverse abdominal impedance and waist circumference measurements. It can quantify the amount and area of subcutaneous and visceral fat. Yoon et al. [50] showed that the use of the MFS-BIA technique in combination with a portable abdominal BIA device improved the correlation with CT measurements.

The aim of the study was to carry out anthropometric measurements using the InBody 970 bioimpedance device (high-frequency bioelectrical impedance/HF-BIA) together with the Yscope (portable abdominal bioimpedance analyzer/PA-BIA) device, which is specified for the area of abdominal fat tissue, and to assess its relevance and usefulness in common practice.

MATERIALS AND METHODS

Study design

The study was conducted from October to December 2023. The total number of subjects settled at eighty-three, of which 55 were women and 28 were men. The participants were informed about the measurement procedure and possible risks in the case of an electrical device implanted in the body on the heart or in the case of pregnancy. Before the measurement, participants were asked to exclude and refrain from drinking large amounts of water, not to consume alcohol 24 hours before testing, to avoid food with a high sugar, salt or fat content for 12 hours before testing, to refrain from intense physical activity for at least 12 hours beforehand. In addition to informed written consent, all participants also signed consent to the processing of personal data. The study was conducted with the approval of the Ethics Committee of the Specialized Hospital of St. Zoerardus Zobor in Nitra, Slovakia (protocol no. 20230512/2) according to the guidelines of the Declaration of Helsinki.

Body composition was analyzed using the InBody 970 (HF-BIA; InBody Corporation, Seoul, South Korea), which measured the impedance of five body segments at 1, 5, 50, 250, and 500 kHz and 1, 2, and 3 MHz (higher frequencies allow constant current to pass and minimize error) [13, 49], and Yscope (PA-BIA; InBody Corporation, Seoul, South Korea) with sine waves of 50 and 250 kHz. When measuring

with the InBody 970, the measured subject stood barefoot on the platform electrodes and held both hand electrodes. The Yscope measurement was performed on the right side of the abdomen after wiping the skin with a wet tissue. After each measurement, the contact surfaces were cleaned with an alcohol swab. Visceral and subcutaneous fat area was estimated using axial and transverse impedance values [50].

For the purpose of assessing body composition, most parameters, with the exception of height and age, were determined directly by bioimpedance analysis. When evaluating individual parameters and indicators and their mutual correlation, we relied on classifications of risk values. According to BMI, obesity was defined as BMI ≥30 kg·m^{-2}, underweight as BMI <18.5 kg·m^{-2}, healthy weight between 18.5 and 25 kg·m^{-2} and overweight between 25 and 30 kg·m^{-2}. Obesity was also defined in our study as waist circumference ≥88 cm and ≥102 cm for women and men, %FM ≥28% for women and ≥20% for men. According to the WHR, we defined obesity at values higher than 1.0 in men and 0.85 in women. WHR values of 0.94 in men and 0.8 in women defined the cutoff value of low health risk related to abdominal obesity. The optimal value of the visceral fat area is less than 100 cm^2.

Statistical analysis

We used Microsoft Office Excel 2016 (Los Angeles, CA, USA) in combination with XLSTAT (version 2019.3.1) for data processing. We performed statistical analysis using the computer software STATISTICA 13 (TIBCO Software, Inc., Palo Alto, CA, USA) and MedCalc software (MedCalc® Statistical Software Ltd, Ostend, Belgium, version 22.021). The normality of the variable distribution was checked by the Shapiro-Wilk test. We used the paired t-test if the data were normally distributed, if the distribution was not normal, the Wilcoxon signed rank test was used. We performed descriptive analysis using mean ± standard deviation. For the monitored parameters, we present the 95% CI (confidence interval). To evaluate the relationship between variables, we used Spearman›s correlation analysis and expressed it graphically with color scales through correlograms. The level of statistical significance was set as $p < 0.05$.

RESULTS AND DISCUSSION

The study was conducted on a sample of eighty-three young subjects with an average age of 24.92±7.24 years. The female gender was represented by 55 women aged 25.67±8.66 years, the male by 28 individuals aged 23.43±2.43 ($p>0.05$). The research group consisted of individuals with an average BMI of 24.10±3.76 kg·m^{-2}, which categorizes them as a group with a normal body weight. Waist circumference was 82.01±11.31 cm, WHR 0.84±0.07, body fat mass 18.08±7.69 kg, while it turned out that the most fat mass was located in the trunk, then in the arms and least in the legs. The proportion of fat in body weight was 25.65±8.65%. The total abdominal fat was 2.08±1.31 kg, of which visceral fat was 0.71±0.55 kg and subcutaneous fat 1.37±0.86 kg. The average value of the area of visceral adipose tissue was 73.8±52.91 cm^2, which is within the reference range, and the area of subcutaneous adipose tissue was 139.44±85.98 cm^2. The results clearly show that subcutaneous fat has a significant advantage compared to visceral fat. The individual parameters are summarized in more detail in Table 1.

However, we found some differences in sexual differentiation (Table 2). The average value of BMI in women was 23.28±3.37 kg·m^{-2}, which categorizes them as a group with normal body weight, but in the male group the average value of BMI was 25.69±4.01 kg·m^{-2} ($p<0.01$), which categorizes them as an overweight group. However, as it turned out subsequently in connection with the representation of fat and muscle components, the increased BMI values were caused by a higher proportion of muscular mass, which is an expected condition for the male sex. Waist circumference was 79.85±10.44 cm in the female group, 86.25±11.95 cm in the male group ($p<0.05$), in both cases these were optimal values. Women had an average value of body fat mass higher than men in the order of 19.40±6.91 kg versus 15.50±8.60 kg ($p<0.05$). However, gender differentiation was not significantly demonstrated in the representation of segmental body fat ($p>0.05$), even though women had higher values than men. In both sexes, it was shown that the most fat mass was located in the torso, followed by the arms and the least in the legs. The proportion of fat in body weight was 29.55±6.41% in women, which places women in the category with an increased and risky amount of fat. The proportion of fat in body weight was significantly lower in men than in women, namely 17.98±7.28% ($p<0.001$), which is within the optimal values. Abdominal fat did not differ significantly between the sexes, women reached an average value of 2.01±1.14 kg, men 2.22±1.60 kg ($p>0.05$). However, gender differentiation was evident in visceral fat ($p<0.01$) and visceral fat area ($p<0.01$). Visceral fat in women was 0.58±0.47 kg, in men 0.98±0.59 kg. Similarly, the area of visceral fat was lower in women (62.34±48.83 cm^2) than in men (96.30±54.24 cm^2). Visceral fat representation did not reach risk values. Abdominal fat is also made up of subcutaneous fat. In this case, we registered the opposite trend of values in relation to gender, where higher values were achieved by women, but there were no significant differences ($p>0.05$). Subcutaneous fat in women had an average value of 1.44±0.73 kg, the area of subcutaneous fat was 149.83±74.55 cm^2. Subcutaneous fat in men had an

Table 1. Basic descriptive characteristics of study group

Parameters (n=83)	Mean	SD	95% CI
Age, years	24.92	7.24	23.334 - 26.497
Basal Metabolic Rate (BMR, kcal)	1505	288	1442 - 1567
InBody Score (points)	76.65	8.46	74.804 - 78.497
Weight (kg)	70.61	15.28	67.272 - 73.947
Height (cm)	170.58	9.30	168.547 - 172.609
Body Mass Index (BMI, kg·m^{-2})	24.10	3.76	23.275 - 24.915
Waist Circumference (WC, cm)	82.01	11.31	79.539 - 84.480
Hip Circumference (HC, cm)	97.04	6.62	95.596 - 98.488
Waist-Hip Ratio (WHR)	0.84	0.07	0.827 - 0.859
Body Cell Mass (BCM, kg)	34.41	8.92	32.465 - 36.361
Soft Lean Mass (SLM, kg)	49.48	12.61	46.727 - 52.234
Lean Mass of Left Arm (%)	105.84	13.42	102.904 - 108.766
Lean Mass of Right Arm (%)	106.52	12.63	103.761 - 109.275
Lean Mass of Left Leg (%)	103.56	6.84	102.066 - 105.055
Lean Mass of Right Leg (%)	103.96	6.76	102.485 - 105.435
Lean Mass of Trunk (%)	103.87	7.45	102.243 - 105.495
Fat Free Mass (FFM, kg)	52.53	13.33	49.617 - 55.438
Skeletal Muscle Mass (SMM, kg)	29.34	8.11	27.566 - 31.108
Total Body Water (TBW, L)	38.47	9.76	36.336 - 40.597
Extracellular Water (ECW, L)	14.44	3.55	13.662 - 15.213
Intracellular Water (ICW, L)	24.03	6.22	22.670 - 25.387
TBW/FFM (%)	73.22	0.16	73.189 - 73.257
ECW/TBW	0.38	0.01	0.374 - 0.377
Body Fat Mass (BFM, kg)	18.08	7.69	16.402 - 19.762
BFM of Left Arm (%)	134.67	90.91	114.815 - 154.518
BFM of Right Arm (%)	133.83	89.85	114.213 - 153.452
BFM of Left Leg (%)	119.04	41.29	110.025 - 128.056
BFM of Right Leg (%)	119.73	41.62	110.644 - 128.819
BFM of Trunk (%)	180.44	83.90	162.123 - 198.763
Percent Body Fat (PBF, %)	25.65	8.65	23.761 - 27.538
Arm Circumference (AC, cm)	31.24	3.77	30.413 - 32.057
Arm Muscle Circumference (AMC, cm)	27.40	3.50	26.636 - 28.166
AC minus AMC (cm)	3.83	1.33	3.544 - 4.124
Abdominal Fat (AF, kg)	2.08	1.31	1.797 - 2.368
Visceral Fat of abdomen (VF, kg)	0.71	0.55	0.594 - 0.833
Subcutaneous Fat (SF, kg)	1.37	0.86	1.180 - 1.557
Visceral Fat Area (VFA, cm^2)	73.80	52.91	62.244 - 85.352
Subcutaneous Fat Area (SFA, cm^2)	139.44	85.98	120.669 - 158.218
VFA/SFA	0.57	0.43	0.473 - 0.662

SD – standard deviation; CI – confidence interval

average value of 1.24±1.08 kg, the area of subcutaneous fat was 119.05±103.37 cm^2. More detailed results based on gender differentiation are presented in Table 2.

Based on the correlation analysis, we further found that visceral fat was most correlated with abdominal fat (r=0.86), waist circumference (r=0.85), arm circumference (r=0.79), the proportion of fat in the trunk (r=0.71), the circumference of the arm muscle (r=0.70), the proportion of fat in the right and left leg (r=0.61/r=0.60), the proportion of fat in the right and left arm (r=0.57/r=0.56) and the same with body fat mass (r=0.56). Correlation dependences of visceral fat area

Table 2. Descriptive characteristics of the group adjusted by gender

Parameters	Female n=55		Male n=28		p-value
	Mean	SD	Mean	SD	
Age, years	25.67	8.66	23.43	2.43	ns
Basal Metabolic Rate (BMR, kcal)	1337.04	112.18	1833.79	238.69	<0.001
InBody Score (points)	73.82	5.71	82.21	10.17	<0.001
Weight (kg)	64.17	10.33	83.26	15.67	<0.001
Height (cm)	165.97	6.92	179.63	6.25	<0.001
Body Mass Index (BMI, kg·m^{-2})	23.28	3.37	25.69	4.01	<0.01
Waist Circumference (WC, cm)	79.85	10.44	86.25	11.95	<0.05
Hip Circumference (HC, cm)	94.80	5.26	101.45	6.89	<0.001
Waist-Hip Ratio (WHR)	0.84	0.07	0.85	0.07	ns
Body Cell Mass (BCM, kg)	29.17	3.39	44.72	7.24	<0.001
Soft Lean Mass (SLM, kg)	42.12	4.87	63.94	10.41	<0.001
Lean Mass of Left Arm (%)	102.05	10.26	113.28	15.80	<0.001
Lean Mass of Right Arm (%)	103.25	9.75	112.94	15.15	<0.001
Lean Mass of Left Leg (%)	103.17	7.10	104.33	6.36	ns
Lean Mass of Right Leg (%)	103.53	6.90	104.80	6.49	ns
Lean Mass of Trunk (%)	101.84	5.70	107.86	8.86	<0.001
Fat Free Mass (FFM, kg)	44.77	5.20	67.77	11.05	<0.001
Skeletal Muscle Mass (SMM, kg)	24.57	3.08	38.71	6.58	<0.001
Total Body Water (TBW, L)	32.78	3.78	49.63	8.09	<0.001
Extracellular Water (ECW, L)	12.41	1.44	18.41	3.07	<0.001
Intracellular Water (ICW, L)	20.37	2.36	31.22	5.05	<0.001
TBW/FFM (%)	73.21	0.14	73.25	0.19	ns
ECW/TBW	0.38	0.01	0.37	0.01	<0.001
Body Fat Mass (BFM, kg)	19.40	6.91	15.50	8.60	<0.05
BFM of Left Arm (%)	138.42	65.75	127.30	127.95	ns
BFM of Right Arm (%)	137.01	65.63	127.60	125.87	ns
BFM of Left Leg (%)	120.41	35.73	116.35	51.11	ns
BFM of Right Leg (%)	120.91	35.86	117.41	51.77	ns
BFM of Trunk (%)	177.73	68.04	185.77	109.89	ns
Percent Body Fat (PBF, %)	29.55	6.41	17.98	7.28	<0.001
Arm Circumference (AC, cm)	29.82	2.94	34.01	3.69	<0.001
Arm Muscle Circumference (AMC, cm)	25.59	2.05	30.95	3.02	<0.001
AC minus AMC (cm)	4.23	1.06	3.06	1.47	<0.001
Abdominal Fat (AF, kg)	2.01	1.14	2.22	1.60	ns
Visceral Fat of abdomen (VF, kg)	0.58	0.47	0.98	0.59	<0.01
Subcutaneous Fat (SF, kg)	1.44	0.73	1.24	1.08	ns
Visceral Fat Area (VFA, cm^2)	62.34	48.83	96.30	54.24	<0.01
Subcutaneous Fat Area (SFA, cm^2)	149.83	74.55	119.05	103.37	ns
VFA/SFA	0.37	0.20	0.96	0.50	<0.001

SD – standard deviation; p – significance; ns – non-significant

almost followed the trend of visceral fat dependences. VFA was most correlated with waist circumference (r=0.91), followed by abdominal fat (r=0.81), arm circumference (r=0.81), body fat percentage (r=0.76), muscle circumference arm (r=0.70), the proportion of fat in the right and left leg (r=0.64), with body fat mass (r=0.61) and the proportion of fat in the right and left arm (r=0.61/r=0.60) (Table 3).

Table 3. Correlation analysis of VF, VFA, SF and SFA in relation to anthropometric parameters

Parameters	Visceral Fat		Subcutaneous Fat		VFA		SFA	
	r	p	r	p	r	p	r	p
Fat Free Mass	0.559	***	0.046	ns	0.531	***	-0.008	ns
Skeletal Muscle Mass	0.558	***	0.041	ns	0.527	***	-0.012	ns
Body Fat Mass	0.56	***	0.928	***	0.613	***	0.936	***
BFM % of Left Arm	0.556	***	0.904	***	0.598	***	0.917	***
BFM % of Right Arm	0.568	***	0.904	***	0.608	***	0.917	***
BFM % of Left Leg	0.602	***	0.874	***	0.636	***	0.889	***
BFM % of Right Leg	0.611	***	0.873	***	0.644	***	0.888	***
BFM % of Trunk	0.707	***	0.884	***	0.761	***	0.888	***
Percentage of Body Fat	0.242	*	0.779	***	0.302	**	0.814	***
Arm Circumference	0.791	***	0.467	***	0.808	***	0.447	***
Arm Muscle Circumference	0.699	***	0.226	*	0.696	***	0.197	ns
Waist Circumference	0.845	***	0.678	***	0.914	***	0.655	***
Abdominal Fat	0.86	***	0.933	***	0.812	***	0.92	***
TBW (Total Body Water)	0.558	***	0.046	ns	0.533	***	-0.008	ns
TBW/FFM	-0.119	ns	-0.287	**	-0.102	ns	-0.281	*
Intracellular Water	0.556	***	0.041	ns	0.526	***	-0.012	ns
Extracellular Water	0.566	***	0.065	ns	0.548	***	0.009	ns
Basal Metabolic Rate	0.557	***	0.044	ns	0.531	***	-0.01	ns
Recommended Calorie Intake	0.119	ns	-0.316	**	0.058	ns	-0.376	***

r – Spearman correlation coefficient; p – significance; * – statistical significance at the level $p<0.05$; ** – statistical significance at the level $p<0.01$; *** – statistical significance at the level $p<0.001$; ns – non-significant

The correlations between subcutaneous fat tissue and subcutaneous fat area in the abdominal area are different than in the case of visceral fat tissue (Table 3). The strongest positive correlations were found between subcutaneous fat and abdominal fat (r=0.93), with body fat mass (r=0.93), the proportion of fat in the arms (r=0.90), with the proportion of fat in the trunk (r=0.88), the proportion of fat in the legs (r=0.87), the proportion of body fat (r=0.78), waist circumference (r=0.68) and arm circumference (r=0.47). With few exceptions, subcutaneous fat area replicated the correlations of subcutaneous fat amount. The strongest correlations were the area of subcutaneous fat with body fat mass (r=0.94), with abdominal fat (r=0.92), with the proportion of fat in the arms (r=0.92), with the proportion of fat in the trunk (r=0.89), with the proportion of fat in the legs (r=0.89), with the proportion of body fat (r=0.81), waist circumference (r=0.66) and arm circumference (r=0.45).

Correlation analysis also showed that while visceral fat and visceral fat area had moderately strong positive dependence with fat-free mass (r=0.56/r=0.53), skeletal muscle mass (r=0.56/r=0.53), total body water (r=0.56/r=0.53), ICW (r=0.56/r=0.53), ECW (r=0.57/r=0.55) and BMR (r=0.56/r=0.53), in the case of subcutaneous fat and subcutaneous fat area the correlations were weak, in some cases negative (FFM r=0.05/r=-0.01; SMM r=0.04/r=-0.01; TBW r=0.05/r=-0.01; ICW r=0.04/r=-0.01; ECW r=0.07/r=0.01 and BMR r=0.04/r=-0.01, respectively) (Tables 3 and 4). Subcutaneous fat and subcutaneous fat area showed weaker correlation associations also in relation to body weight (r=0.43 and r= 0.39, respectively) (Table 4). Visceral fat and visceral fat area had stronger associations (r=0.77 and r=0.78, respectively). In relation to BMI, stronger correlations were again confirmed for visceral fat and visceral fat area (r=0.80 and r=0.84, respectively) than subcutaneous fat and subcutaneous fat area (r=0.62 and r=0.62, respectively).

Two studies found that visceral adipose tissue was strongly correlated with body fat, suggesting a positive relationship of visceral fat to overall adiposity [2, 25]. In our study, its correlation with body fat mass, but not with PBF, was confirmed. Snell Bergeon et al. [43] reported that subcutaneous adipose tissue was more strongly correlated with BMI and waist circumference than visceral adipose tissue, but not with WHR. However, our results did not confirm this and showed a stronger correlation in the case of visceral fat.

Carroll et al. [6] found that African Americans and women had significantly lower values of visceral adipose tissue than whites and Hispanics. The amount of subcutaneous adipose tissue, on the other hand, was higher in African Americans. Mundi et al. [31]

Table 4. Correlogram of mutual associations between individual anthropometric parameters

	Weight	BMI	WHR	SLM	FFM	SMM	BFM	PBF	AC	AMC	WC	AF	VF	SF	VFA	SFA
Weight		<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	0.9677	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
BMI	0.828		<0.001	<0.001	<0.001	<0.001	<0.001	<0.01	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
WHR	0.522	0.57		<0.01	<0.01	<0.01	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
SLM	0.862	0.55	0.287		<0.001	<0.001	0.7193	<0.001	<0.001	<0.001	<0.001	<0.05	<0.001	0.6922	<0.001	0.9314
FFM	0.864	0.551	0.289	1		<0.001	0.7425	<0.001	<0.001	<0.001	<0.001	<0.05	<0.001	0.6771	<0.001	0.9463
SMM	0.858	0.55	0.286	0.999	0.999		0.6708	<0.001	<0.001	<0.001	<0.001	<0.05	<0.001	0.7111	<0.001	0.9148
BFM	0.397	0.637	0.614	-0.04	-0.037	-0.047		<0.001	<0.001	0.1306	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
PBF	-0.005	0.321	0.419	-0.449	-0.446	-0.455	0.881		0.5295	0.0541	<0.01	<0.001	<0.05	<0.001	<0.01	<0.001
AC	0.929	0.925	0.536	0.757	0.756	0.758	0.437	0.07		<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
AMC	0.909	0.784	0.407	0.879	0.877	0.882	0.167	-0.212	0.948		<0.001	<0.001	<0.001	<0.05	<0.001	0.0741
WC	0.801	0.823	0.895	0.549	0.55	0.548	0.657	0.34	0.808	0.68		<0.001	<0.001	<0.001	<0.001	<0.001
AF	0.63	0.768	0.747	0.274	0.276	0.272	0.856	0.615	0.661	0.457	0.835		<0.001	<0.001	<0.001	<0.001
VF	0.771	0.801	0.697	0.558	0.559	0.558	0.56	0.242	0.791	0.699	0.845	0.86		<0.001	<0.001	<0.001
SF	0.431	0.622	0.64	0.044	0.046	0.041	0.928	0.779	0.467	0.226	0.678	0.933	0.637		<0.001	<0.001
VFA	0.782	0.836	0.789	0.529	0.531	0.527	0.613	0.302	0.808	0.696	0.914	0.812	0.939	0.59		<0.001
SFA	0.389	0.616	0.626	-0.01	-0.008	-0.012	0.936	0.814	0.447	0.197	0.655	0.92	0.619	0.995	0.575	

BMI – Body Mass Index; WHR – Waist-to-Hip Ratio; SLM – Soft Lean Mass; FFM – Fat-free Mass; SMM – Skeletal Muscle Mass; BFM – Body Fat Mass; PBF – Percentage of Body Fat; AC – Arm Circumference; AMC – Arm Muscle Circumference; WC – Waist Circumference; AF – Abdominal Fat; VF – Visceral Fat; SF – Subcutaneous Fat; VFA – Visceral Fat Area; SFA – Subcutaneous Fat Area

found in the case of non-diabetic Caucasians visceral adipose tissue values of 40 cm^2 for women and 74 cm^2 for men, subcutaneous adipose tissue values were 169 cm^2 and 133 cm^2 for women and men, respectively.

As part of gender differentiation, males have a significantly larger area of visceral adipose tissue, while females tend to have slightly more subcutaneous adipose tissue, especially in the lower abdomen [9, 16, 24]. Our results confirm these findings. The largest areas of visceral adipose tissue are present in the upper abdomen, while the area of subcutaneous adipose tissue is higher in lower anatomical locations [9, 16].

Results from the Jackson Heart Study of 2477 African Americans showed that visceral and subcutaneous adipose tissue in the abdominal region were associated with adverse cardiometabolic risk factors (including diabetes), and that the strength of the effect of visceral adipose tissue was greater in women than subcutaneous adipose tissue [22]. Framingham researchers found that both types of adipose tissue, visceral and subcutaneous, were significantly associated with hypertension, dyslipidemia, impaired fasting glucose, and metabolic syndrome. However, visceral fat area had a significantly stronger association compared to subcutaneous fat [9]. The Jackson Heart Study of 2799 African Americans found a direct association between subcutaneous adipose tissue and adiponectin that persisted after controlling for BMI and WC in men, while significance was borderline in women [3]. There are differences between subcutaneous and visceral adipose tissue in the abdominal region, at the anatomical, cellular, molecular, physiological and other levels [15].

Yoon et al. [50] found that the application of PA-BIA in combination with MFS-BIA significantly improves the accuracy of abdominal visceral fat measurements. In their study, it was shown that in concordance with CT results, PA-BIA technology together with MFS-BIA was more accurate than MFS-BIA alone in determining VFA. Results did not differ either in the overall population or in subgroups adjusted for sex, age, and BMI. The use of PA-BIA allows, among other things, to determine the values of the subcutaneous fat area, which the conventional MFS-BIA method does not allow.

However, in humans, the development of obesity does not only lead to an increase in adipose tissue reserves in classical locations, such as subcutaneous and visceral adipose tissues, but also around specific organs, such as the heart (pericardial), blood vessels (perivascular) and kidneys (renal) or internal organs such as skeletal muscle (intramuscular) and liver (intrahepatic), which have all been described as sites for ectopic fat deposition [44, 45]. Excessive regional deposits of adipose tissue can alter organ function through mechanical compression or through secreted cytokines and chemokines [45] and thus pose significant health risks. Therefore, further studies and the development of more accurate but easily available diagnostic technologies determining not only visceral and subcutaneous fat tissue, but also ectopic ones are needed.

CONCLUSIONS

Obesity or excessive accumulation of fat beyond the body's physiological needs is one of the serious diseases in which, in addition to evaluating the location of fat storage, it is also very important to distinguish what type of adipose tissue it is. While subcutaneous fat is not significantly associated with serious health complications, visceral adipose tissue located around internal organs is associated with the development of several serious metabolic disorders. Many methods are currently used to determine the amount of fat in the body. Recently, a bioimpedance device designed for the assessment of abdominal obesity was developed, with the possibility of estimating not only visceral, but also subcutaneous fat. The use of such technologies in the diagnosis of obesity, especially abdominal obesity, is of great importance in practice, especially due to their non-invasiveness, affordability and relative safety. The assessment of body composition confirms significant gender differentiation, which is an important factor affecting different health risks related to gender and the representation of different types of fat tissue localized and accumulated in different parts of the body.

Conflict of interest

The authors declare no conflict of interest.

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ORIGINAL ARTICLE

THE EFFECT OF REGULAR CHOKEBERRY JUICE CONSUMPTION ON ANTHROPOMETRIC AND LIPID PARAMETERS IN WOMEN WITH OVERWEIGHT OR OBESITY

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ABSTRACT

Background. *Aronia melanocarpa* is nowadays valued for its high content of biologically active substances, the main group of which are polyphenols, which include anthocyanins, flavonols, flavanols, proanthocyanidins and phenolic acids. From the available sources, we can conclude that extracts and juices from black chokeberry have a great potential in human nutrition and influence on their health.

Objective. The research was to evaluate the effect of regular consumption of 100% organic chokeberry juice on selected anthropometric and lipid parameters of overweight or obese women.

Material and Methods. A clinical study consisted of 19 women with overweight and obesity, age from 44 to 63. The probands consumed 50 ml of chokeberry juice daily for 8 weeks as part of their regular diet. Body composition and biochemical indicators were monitored before consumption, after 4 and 8 weeks of nutritional intervention. Body composition was determined using multifrequency bioelectrical impedance analysis (MFBIA) – InBody 720. Biochemical analyzes of blood serum were performed using standard methods in an accredited laboratory using automatic biochemical analyzer a BioMajesty JCA-BM6010/C.

Results. The monitored group of probands is characterized by menopausale and postmenopausale women, overweight or obese women with hypercholesterolemia without pharmacological treatment. Statistically significant differences ($p<0.05$) were observed when evaluating the amount of body fat (BFM) of the probands before the start of consumption and after the consumption of chokeberry juice. We noted a statistically significant reduction especially in the assessment of visceral fat (VFA) ($p<0.001$). There were no fundamentally significant changes in the lipid profile of women in this intervention study. With short-term consumption of chokeberry juice (after 4 weeks), we recorded an average reduction in total cholesterol and LDL-cholesterol, but without statistical significant. We also focused on the evaluation of the inflammatory marker CRP and noted a significant beneficial reduction of CRP ($p<0.05$).

Conclusions. In the research, we evaluated the effect of 8 weeks consumption of 100% chokeberry juice on selected anthropometric parameters, focusing on changes in visceral fat and total fat in overweight and obese women. In conclusion, we can state that the regular consumption of chokeberry juice has a beneficial effect on fat tissue in women of reproductive age, which can reduce the risk of cardiovascular diseases.

Keywords: *black chokeberry, body composition, lipid parameters, inflammatory marker*

INTRODUCTION

Aronia melanocarpa (Michx.) Elliot, black chokeberry, belongs to the rose family (*Rosaceae*) and the apple subfamily (*Pomodieae*). It comes from the eastern parts of North America and eastern Canada. Aronia reached Europe through Russia in the 19th century, where it was cultivated in Siberia and later spread throughout Russia and other European countries [19]. Aronia, thanks to its resistance to cold, can be grown in mild climatic conditions, but it can withstand temperatures below -35°C [6]. In the past, the nutritional value of aronia was not sufficiently appreciated, but the constantly emerging evidence of its health-promoting properties is increasing the interest in this crop. Fresh and unprocessed chokeberry berries are often not consumed due to their bitter taste, therefore chokeberry fruits are used in the production of juices, nectars, syrups, jams, fruit desserts, jellies, wines and various liqueurs, but also as a food coloring [15, 28]. The composition and quality of individual components of chokeberry fruit depends on many factors, e.g. on the variety, maturity, environment, climatic conditions, soil, harvesting

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method, etc. Aronia became known thanks to its high biological activity, which is ensured by polyphenols. Berries are one of the richest sources of polyphenols, which include anthocyanins, flavonols, flavanols, proanthocyanidins and phenolic acids. Furthermore, aronia is considered an excellent source of minerals such as potassium, calcium, phosphorus, magnesium, sodium, iron and zinc [22]. *Aronia melanocarpa* has the highest antioxidant activity among all berry varieties, which is related to the high content of polyphenolic compounds, especially procyanidins and anthocyanins [11]. The content of total phenolic substances in chokeberry fruits ranges from 0.69 to 2.56 $mg \cdot g^{-1}$ of fresh weight and from 3.44 to 7.85 $mg \cdot g^{-1}$ of dry weight. The mechanisms of *in vivo* antioxidant activity of chokeberry phenolic substances include scavenging of free radicals, suppression of reactive oxygen species (ROS) formation, prooxidant inhibition and restoration of antioxidant enzymes, and probably restoration of cellular signaling responsible for regulating the level of antioxidant compounds and enzymes [26].

Obesity is reaching epidemic proportions and has therefore become a major global public health problem. The condition is the result of excessive accumulation of adipocytes, which store and regulate energy in the form of triacylglycerols or free fatty acids. The pathogenesis of obesity-related complications is thought to involve abnormal adipokine production by adipocytes, although the exact mechanism has not been established. Oxidative stress and obesity are thought to be linked, and oxidative stress has also been found in obese individuals. In a study conducted by Kim et al. [7], an inhibitory activity on adipocyte differentiation was proven by chokeberry extract, which contained substances such as chlorogenic acid, methyl-3-O-caffeoylquinic acid, dihydroxycinnamoylcyclopenta-2,3-diol, cyanidin-3-glucoside, cyanidin-3-xyloside. The most abundant anthocyanin in chokeberry, cyanidin-3-O-galactoside, significantly reduced the weight of rats in studies by Jiao et al. [5], also reduced lipid parameters, levels of triacylglycerols, total cholesterol, LDL cholesterol, and on the contrary increased levels of HDL cholesterol.

The lipid-lowering effect of flavonoids has been demonstrated in many animal and human studies [8]. Aronia juice has been shown to have beneficial effects on total cholesterol and lipid levels [27]. Significant anti-inflammatory effects of chokeberry have been demonstrated in various *in vivo* and *in vitro* studies. Oral administration of chokeberry extract inhibits the production of prostaglandin E2 and reduces levels of nitric oxide, production of interleukin-6 and tumor necrosis factor (TNFα) in macrophages and microglia, increases production of interleukin 10. These anti-inflammatory effects were primarily caused by specific polyphenols (cyanidin-3-arabinoside and quercetin), which are represented as a minor component of total chokeberry polyphenols [4].

The aim of the research was to evaluate the effect of regular consumption of 100% organic chokeberry juice on selected anthropometric and lipid parameters of overweight or obese women.

MATERIALS AND METHODS

The research was carried out at the Institute of Nutrition and Genomics of the Faculty of Agrobiology and Food Resources of Slovak University of Agriculture in Nitra (SUA), with the focus on monitoring the effect of regular consumption of 100% organic chokeberry juice (product of a company from Slovakia) on selected anthropometric and biochemical parameters of the probands. The clinical study involved 30 women and we selected 19 women with overweight and obesity, age from 44 to 63 (mean age 51.21±4.63 years). The probands consumed 50 ml of chokeberry juice daily for 8 weeks and they were instructed to maintain their normal eating habits during the study, to refrain from consuming any drugs and dietary supplements and not to modify their physical activity. We used the 24-h dietary records, where respondents provides information about the type and quantity of all food and beverages consumed during the period. We evaluated the data using Planeat. This software is designed for a complete analysis of food, meals, and recipes based on the composition of the raw ingredients. Body composition and biochemical indicators were monitored before consumption, after 4 and 8 weeks of nutritional intervention. Participants were their own controls and the changes in their parameters were evaluated. The study was approved by the Ethics Committee at the Specialized Hospital of St. Zoerardus in Nitra, Slovak Republic (protocol number 3/101921/2021) and study was conducted between March and May 2022. The monitored group of probands consisted of volunteers without health problems and pathological changes in the basic biochemical parameters in the blood. The condition of participation in the research was the informed consent of the participants to the conditions of the study and the planned examinations. Body composition (body weight – BW, body fat mass – BFM, visceral fat area – VFA, skeletal muscle mass – SMM, fat free mass – FFM, body mass index – BMI, waist to hip ratio – WHR) was determined using multifrequency bioelectrical impedance analysis (MFBIA) – InBody 720 (Biospace Co. Ltd., Seoul, Korea).

Biochemical analyzes: total cholesterol, high density cholesterol (HDL cholesterol), low density cholesterol (LDL cholesterol), triacylglycerols and inflammatory marker) of blood serum were

performed using standard methods in an accredited laboratory using a BioMajesty JCA-BM6010/C automatic biochemical analyzer. The level of lipid and inflammatory parameters was measured using commercial DiaSys kits. Low-density lipoprotein cholesterol was calculated using the Friedewald equation.

The chokeberry juice was made by cold pressing from organic black chokeberry without additives, stabilized only by pasteurization.

The content of nutrients and some important bioactive substances, as well as the juice antioxidant activity were quantified in the Institute of Food Sciences of SUA. The total phenolic content (TPC) was determined by the spectrophotometric method [9] according to the Folin-Ciocalteu method using spectrophotometer Shimadzu UV/VIS-1800 and expressed in mg gallic acid equivalents (GAE)/g. Phenolic compounds were determined by HPLC Agilent 1260 Infinity II (Agilent Technologies GmbH, Waldbronn, Germany) with quaternary solvent manager coupled with degasser, sample manager, column thermostat and DAD detector by slightly modified method according by Gabriele et al. [3]. The antioxidant activity of chokeberry juice was measured using the stable radical 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay and the results were expressed as percentage of inhibition of the radical [1]. Vitamin C content was determined by HPLC system Waters Separations Module 2695 with UV detector 2996.

Statistical analysis

We used the Shapiro-Wilk test to determine whether the variables were normally distributed and the parametric variables were compared by the paired t-test using software Statistica Cz version 14. All data are expressed as the mean ± standard deviation (SD) and p-values of 0.05 or less were considered significant.

RESULTS AND DISCUSSION

Many studies have shown the positive effect of small berries in the prevention of important metabolic disorders associated with obesity, oxidative stress and chronic inflammation [14, 23]. The beneficial effects of berries are related to the antioxidant and anti-inflammatory properties of polyphenols [12]. The effects of chokeberry extracts have been demonstrated both *in vitro* and *in vivo*, especially in cardiovascular diseases [18].

In this interventional clinical trial, women consumed 50 ml of 100% chokeberry juice every day for 8 weeks. The concentration of bioactive compounds and antioxidant activity of chokeberry juice were as follows: total phenolic content 4.90±0.51 mg gallic acid equivalents, ferulic acid 100.37±0.45 $mg \cdot l^{-1}$, rutin 238.69±0.25 $mg \cdot l^{-1}$, benzoic acid 204.71±1.09 $mg \cdot l^{-1}$, neochlorogenic acid 372.36±1.89 $mg \cdot l^{-1}$, chlorogenic acid 497.86±0.52 $mg \cdot l^{-1}$, vitamin C 12.60±0.08 $mg \cdot 100\ g^{-1}$ and antioxidant activity 83.18±0.51%.

Basic characteristics of the probands before intervention are presented in Table 1. The monitored group of probands is characterized by: menopausal and postmenopausal women (from 44 to 63 years old), these are overweight or obese women (BMI from 25.97 to 36.36 $kg \cdot m^{-2}$) with hypercholesterolemia.

Consumption was well tolerated by volunteers and showed no changes in liver and kidney function in blood serum during the intake of chokeberry juice (Table 2).

Table 1. Basic characteristic of probands before intervention

Parameter	Mean ± SD	Min. - Max.
Age (year)	51.21±4.63	44.00-63.00
Body weight (kg)	80.54±9.34	59.50-97.80
Body mass index ($kg \cdot m^{-2}$)	29.65±2.42	25.97-36.36
T-CH ($mmol \cdot l^{-1}$)	6.32±1.05	4.79-8.24
HDL-CH ($mmol \cdot l^{-1}$)	1.61±0.34	0.94-2.27
LDL-CH ($mmol \cdot l^{-1}$)	4.10±0.95	2.68-5.80
TG ($mmol \cdot l^{-1}$)	1.34±0.60	0.63-2.96
ALT ($\mu kat \cdot l^{-1}$)	0.33±0.12	0.16-0.63
AST ($\mu kat \cdot l^{-1}$)	0.36±0.12	0.24-0.62
GGT ($\mu kat \cdot l^{-1}$)	0.36±0.13	0.22-0.75
Urea ($mmol \cdot l^{-1}$)	5.12±1.84	2.5-8.3
Creatinine ($\mu mol \cdot l^{-1}$)	67.55±9.95	51.8-85.2
Uric acid ($\mu mol \cdot l^{-1}$)	315.42±65.03	222-488

values are means ± standard deviation (SD); Min. – minimum; Max. – maximum

Table 2. Metabolic, liver and kidney markers of the subjects before and after chokeberry juice consumption

Parameter	Baseline Mean ± SD	Week 8 Mean ± SD	p-value
ALT (μkat·l^{-1})	0.33±0.12	0.32±0.11	ns
AST (μkat·l^{-1})	0.36±0.12	0.37±0.11	ns
GGT (μkat·l^{-1})	0.36±0.13	0.40±0.36	ns
Bilirubin (μmol·l^{-1})	10.18±3.19	9.25±2.84	ns
Urea (mmol·l^{-1})	4.93±1.69	5.28±1.82	ns
Creatinine (μmol·l^{-1})	66.57±9.24	67.71±8.90	ns
Uric acid (μmol·l^{-1})	305.83±51.26	306.94±52.78	ns
Albumin (g·l^{-1})	48.94±1.81	48.77±1.45	ns

ALT – alanine aminotransferase; AST – aspartate aminotransferase; GGT – gamma glutamyl transferase; values are means ± standard deviation (SD); ns – non significant

Visceral adipose tissue is a hormonally active component of total body fat that affects several processes in the human body. Abnormally high deposition of visceral adipose tissue is known as visceral or abdominal obesity. Visceral fat surrounds internal organs and its increased amount causes health problems, such as glucose metabolism disorders, cardiovascular diseases (ischemic heart disease, hypertension), prostate, breast and colorectal cancer [21].

Jurendić and Ščetar [6] proved that in obese mice treated with aronia extract melanocarpa with a high fat content there was a significant reduction in body weight and improved insulin sensitivity in 46 compared to controls. The study reports the inhibitory activity of polyphenols, in particular proanthocyanidins, against pancreatic lipase, as regards the suppression of fat absorption from foods and strategies against overweight and obesity.

According to the WHO assessment and classification, we recorded 43.3% of overweight probands (25-29.9 kg·m^{-2}), 16.7% of probands with a BMI of 30-34.9 kg·m^{-2} with the obesity of the first degree and 3.3% had obesity II. degree. Regular consumption of chokeberry juice had a statistically significant effect on the reduction of body weight after 8 weeks ($p<0.01$). Weight reduction, however, did not affect skeletal muscle mass, we did not record statistical evidence. Statistically significant differences ($p<0.05$) were observed in the comparison of the average value of the amount of body fat of the probands before the start of consumption and after the end of consumption of chokeberry juice. We noticed a statistically significant reduction especially in the assessment of visceral fat of women during consumption as well as after consumption, the average value decreased from the initial from 126.87 cm^2 to 122.52 cm^2 ($p<0.001$). The effects of chokeberry juice consumption on body composition are shown in Table 3. In this clinical study, we aimed to evaluate the impact of regular consumption of 100% chokeberry

Table 3. Effect of regular consumption of chokeberry juice on body composition of probands

Parameter	Baseline Mean ± SD	4 Weeks Mean ± SD	8 Weeks Mean ± SD	p-value
BW (kg)	80.54±9.34	79.86±9.62	79.75±9.52	<0.01[b]
BFM (kg)	31.73±5.74	31.26±6.11	30.99±6.17	<0.05[b]
BFM (%)	39.24±3.78	38,91±4.03	38.63±4.10	ns
BMI (kg·m^{-2})	29.65±2.42	29.39±2.48	29.36±2.54	<0.01[b]
SMM (kg)	26.85±3.14	26.72±3.09	26.82±3.09	ns
FFM (kg)	48.81±5.26	48.58±5.19	48.76±5.05	ns
VFA (cm^2)	126.87±20.96	124.01±21.27	122.52±20.92	<0.05[a] <0.001[b]
TBW (kg)	35.77±3.88	35.59±3.81	35.73±3.71	ns
ICW (kg)	22.11±2.41	22.02±2.37	22.11±2.32	ns
ECW (kg)	13.67±1.48	13.57±1.46	13.63±1.40	ns
WHR	0.98±0.05	0.97±0.06	0.96±0.05	ns

values are means ± standard deviation (SD); [a]significant difference between weeks 4 and weeks 8; [b]significant difference between baseline and week 8; ns – non significant

juice on selected anthropometric and lipid parameters of overweight and obese women with untreated hypercholesterolemia. Based on anthropometric measurements, we recorded a statistically significant decrease in visceral fat in the monitored group of probands. WHR index ratio waist and hips higher than 0.85 means a risk for the development of cardiovascular diseases. When evaluating the WHR index, we found that the average value of the participants was 0.98 at the beginning of the study, which decreased to 0.96 after stopping the consumption of chokeberry juice without statistical evidence. Our results confirm the results of a clinical study by Kim et al. [7] by the inhibitory activity on adipocyte differentiation by chokeberry extract. The results of the study by Jiao et al. [5] confirmed hypotheses in the prevention of high-fat obesity, focusing on the potential of cyanidin-3-O-galactoside from *Aronia melanocarpa*. Cyanidin-3-O-galactoside attenuated high-fat diet-induced obesity in rats by inhibiting adenosine 5'-phosphorylation monophosphate (AMP) activated protein kinase (AMPK) and inhibited induced inflammation by promoting signal transducer and activator of transcription 3 (STAT3) phosphorylation and suppressing nuclear factor kappa-B p65 (NF-κB p65) in the nucleus. The potential mode of action describes Lim et al. [10] which examined the anti-adipogenic effect of cyanidin-3-O-galactoside *Aronia melanocarpa* extract and its underlying mechanisms were investigated in an in vivo system during an 8-week high-fat diet on body weight and body fat mass in mice. Consequently, increases in body weight and fat mass and subsequent inhibition by supplementation have been reported. White adipose tissue mass (epididymal fat, retroperitoneal fat, mesenteric fat, and inguinal fat) was dose-dependently decreased by chokeberry in mice.

The effect of the consumption of chokeberry juice on the lipid profile is presented in Table 4. The probands of the studied group suffer from hypercholesterolemia and are not pharmacologically treated. In hypercholesterolemia, LDL cholesterol is the main transport form of endogenous cholesterol. Its level effects cholesterol level in the body and increases the risk of cardiovascular and cerebrovascular diseases. HDL cholesterol reverse transports cholesterol from tissue to the liver and breaks it down, which can protect the intimate o vessels and the higher level of HDL cholesterol, the lower the risk cerebral thrombosis and atherosclerosis [17]. In this interventional study, there were no fundamental significant changes in the lipid profile of women. With the short-term consumption of 100% chokeberry juice (after 4 weeks), we recorded an average reduction in total cholesterol and LDL-cholesterol without statistical evidence. We hypothesize that longer-term regular consumption of chokeberry juice may affect the lipid profile, especially risk factors of cardiovascular diseases, similar to what many clinical studies have shown.

Skoczyńska et al. [24] observed the effect of regular consumption of chokeberry juice on the lipid parameters of men with mild hypercholesterolemia, while the clinical study showed a statistically significant reduction in total cholesterol ($p<0.001$) and LDL cholesterol ($p<0.01$) and triglycerides ($p<0.001$) and increase in HDL cholesterol ($p<0.001$) in the blood of the probands. Broncel et al. [2] evaluated the effect of consuming 100 mg chokeberry extract three times a day for 8 weeks. After the end of the study, they confirmed a statistically significant reduction in cholesterol, LDL cholesterol and triacylglycerols in probands with metabolic syndrome. Chokeberry extract contains a significant amount of niacin, which can alleviate cardiovascular disease by reducing lipid activity.

Lipid parameters were also evaluated in a study by Tasic et al. [25], which confirmed after 4 weeks of consumption of chokeberry juice, a statistically

Table 4. Effect of regular consumption of chokeberry juice on lipid profile of probands

Parameter	Baseline Mean ± SD	4 Weeks Mean ± SD	8 Weeks Mean ± SD	p-value
Total cholesterol ($mmol \cdot l^{-1}$)	6.32±1.05	6.18±1.06	6.47±0.89	ns
HDLcholesterol ($mmol \cdot l^{-1}$)	1.61±0.34	1.67±0.29	1.68±0.26	<0.05[b]
LDLcholesterol ($mmol \cdot l^{-1}$)	4.10±0.95	3.91±1.05	4.17±0.89	ns
Triacylglycerols ($mmol \cdot l^{-1}$)	1.34±0.60	1.32±0.59	1.36±0.59	ns
LDL/HDL cholesterol ratio	2.62±0.64	2.40±0.71	2.53±0.59	ns

values are means ± standard deviation (SD); [a]significant difference between weeks 4 and weeks 8; [b]significant difference between baseline and week 8; ns – non significant

Table 5. Effect of regular consumption of chokeberry juice on inflammation markers of probands

Parameter	Baseline Mean ± SD	Week 8 Mean ± SD	p-value
CRP ($mg \cdot l^{-1}$)	5.38±1.46	4.3±1.18	<0.01
IL-6 ($ng \cdot l^{-1}$)	7.15±1.07	6.52±1.25	<0.01
ORM ($g \cdot l^{-1}$)	0.91±0.19	0.66±0.22	<0.001

values are means ± standard deviation (SD); CRP – C-reactive protein; IL-6 – interleukin-6; ORM – orosomucoid

demonstrable decrease in total cholesterol, LDL cholesterol and a favorable increase in HDL cholesterol in women with metabolic syndrome. Many studies have shown that regular consumption of chokeberry products has a positive effect on the lipid profile [2, 16, 24]. However, there were no fundamentally significant changes in the lipid profile of women in this intervention study. With short-term consumption of 100% chokeberry juice (after 4 weeks), we noted a reduction in total cholesterol and LDL cholesterol, but without statistical significant.

Anti-inflammatory activity of chokeberry fruits is primarily associated with the prevention of cardiovascular diseases, diabetes and disorders of the immune system [20]. In a study by Zhang et al. [28] chokeberry juice showed greater anti-inflammatory effects such as rutin or a rutin-magnesium complex. In view of the fact that chokeberry flavonoids reduce the severity of inflammation, regardless of statins, they can be used clinically for secondary prevention of ischaemic heart disease [13]. In the monitored clinical study, we also focused on inflammatory markers at the baseline and after finish of chokeberry juice consumption: C-reactive protein (CRP), interleukin-6 (IL-6) and orosomucoid (ORM). After the chokeberry juice consumption, significant reduction of CRP, IL-6 ($p<0.01$) and ORM ($p<0.001$) were found (Table 5).

CONCLUSIONS

Black chokeberry is a highly biologically valuable plant, suitable for maintaining health and preventing civilization diseases. It is available on the market in various forms, recent attention has focused on the use of fruit juices as a concentrated source of antioxidants. Juice consumption is an effective method to promote fruit and vegetable consumption and is very popular in many countries. In the research, we evaluated the effect of 8-week consumption of 100% chokeberry juice on selected anthropometric and biochemical parameters, focusing on changes in visceral fat and total fat in overweight and obese women. In conclusion, we can conclude that regular consumption of chokeberry extract affects the fat tissue of women of reproductive age. However, further studies with a modified methodology regarding the duration of the intervention and amount of daily intake are needed to more thoroughly examine the effect of consumption of 100% chokeberry juice in the prevention and treatment of CVD.

Conflict of interest

The authors declare no conflict of interest.

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ORIGINAL ARTICLE

ASSESSMENT OF KNOWLEDGE AND NUTRITIONAL STATUS OF STUDENTS BEFORE AND AFTER NUTRITIONAL EDUCATION

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ABSTRACT

Introduction. There are many educational and health programs implemented on a smaller or larger scale in Poland. Many of them address the problem of overweight and obesity in different age groups. Each such activity is considered valuable and important, since the problem of excessive body fat is growing and, in addition, poses a health risk due to the development of other diseases, including diabetes and hypertension. The aim of the study was to test the effectiveness of nutrition education implemented over a 7-week period and to assess the nutritional status of 32 students aged 20-21 from Wroclaw.

Material and Methods. The study group of students participated in a Human Nutrition course that included 30 hours of lectures and 30 hours of exercises. In the study group, the level of knowledge was assessed twice (before and after education) using a questionnaire with 13 closed questions, and body composition analysis was performed twice (before and after education) using an InBody270 analyser.

Results. The study group showed a statistically significant increase in the number of correct answers on the nutrition knowledge questionnaire after the nutrition education (average 9.7 points vs. 11.4 points; $p<0.05$). No statistically significant differences were noted in the studied anthropometric parameters performed before and after nutrition education.

Conclusions. The knowledge gained during nutrition education allowed the surveyed students to give more correct answers in the questionnaire after the Human Nutrition course. The nutritional status of the surveyed students did not change statistically significantly, but two students achieved a weight reduction of 1.5-2 kg during the study period.

Keywords: *education, students, nutritional status*

INTRODUCTION

According to the NCD Risk Factor Collaboration forecast, 28% (25.9% of women and; 30.3% of men) of adults in Poland will be obese in 2025 (http://ncdrisc.org). Compared to 2016, for an average estimate of obesity, the percentage of obese adults is projected to increase by 4%. Relating the data to the population projection compiled by the Central Statistical Office, 6.1-11.4 million adults with obesity are expected in 2025 [1, 2].

There are many methods to assess overweight, obesity or obesity type using anthropometric parameters and body composition [3, 4]. Excessive adipose tissue, the specific location of which predisposes to metabolic disorders, should be diagnosed and treated as soon as possible [5, 6, 7]. Obesity is currently the most important public health problem generating a huge financial burden for the health care system. Due to the projected increase in the prevalence of obesity, an increase in the number of patients with obesity-related diseases is expected in 2025 (compared to 2017), in particular an increase in the number of adult patients with diabetes (estimated increase of 941,000 patients), hypertension (439,000) and knee osteoarthritis (146,000). In total, for these health problems, the estimated increase in the cost of

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services provided to adults in 2025 compared to 2017 will be 327-1,038 million zlotys [1].

There are various strategies related to the treatment of obesity (including diet therapy, physical activity). However, as the above medical statistics show, these interventions are not effective and require continuous evaluation and interdisciplinary cooperation. It seems that the best strategy to combat this condition is prevention and prophylaxis carried out from an early age. Stopping or preventing excessive body fat is individually beneficial in terms of health, but also relieves the burden and provides economic support for the country's health care systems. The need for education in healthy lifestyles, including nutrition education, is indicated by numerous studies [8, 9, 10, 11, 12].

Nowadays, with increasing public awareness, there are significant changes in the concept of health education. Among the most visible changes are a holistic approach to health, with emphasis on the role of psychosocial health, as well as the inclusion of representatives of the social sciences (e.g. psychodietitians) in health education, and a shift from the theoretical transmission of knowledge to the formation of habits, skills and competence to act. This means a shift from teaching to learning, which is particularly important for children and adolescents, since most health-promoting behaviors, including nutrition, begin in childhood.

Aim of the study

The aim of the study was to assess the knowledge of recommended nutrition in a group of 32 students before and after a 7-week education that included 30 hours of theoretical lectures and 30 hours of practical exercises (calculation and analysis). In addition, it was checked using the InBody 270 analyzer to see if the nutritional status of the students under study changed with the acquired knowledge.

MATERIALS AND METHODS

Study design and settings

The study involved second-year students of food technology and nutrition, studying at the Wroclaw University of Life Sciences. The respondents participated in an educational course on Human Nutrition (30 hours of lectures and 30 hours of practical exercises). The CAWI (Computer Assisted Web Interview) method [13] was used to assess nutritional knowledge, and the research tool was a proprietary questionnaire with 13 closed-ended questions. The questions in the survey covered the general knowledge taught in the Human Nutrition course. The student received 1 point for each correct answer, and 0 points for an incorrect one. All participants agreed to participate in the study. Access to the survey was strictly limited by the date and time of the round 1 of the study (13.03.2023) and the round 2 of the study (27.04.2023).

In addition, the nutritional status of the students studied was assessed by electrical bioimpedance using an InBody270 instrument (inbodypoland.pl). Four parameters measured twice before and after education were selected for analysis: body weight (kg), BMI, fat mass (kg) and muscle mass (kg). The range of normal BMI was adopted in accordance with the WHO, i.e. 18.5-24.9, while the other anthropometric parameters were taken in accordance with the manufacturer's instructions for the measuring apparatus. Approval was obtained from the Bioethics Committee to conduct the study no. 14/2023.

Participants

The group of 32 was made up of students aged 20-21, among them were 25 women and 7 men. The inclusion criteria were: student status, attendance at lectures and classes, a written declaration to participate in the study. In addition, each student declared to be in good health, lack of metabolic diseases and not taking any permanent drugs, that could interfere with the assessment of nutritional status.

Statistical analysis

The statistical distributions of the data were examined using the Shapiro-Wilk test. The data distributions deviated from the normal distribution. Wilcoxon non-parametric test was used for further analyses. The level of significance was assumed at $p<0.05$. All analyses were performed using Statistica version 13.3 (TIBCO Software Inc.).

RESULTS

Participants

The questions in the survey covered general knowledge of the principles of human nutrition. The second-year students had not previously taken a course on a similar topic in their curriculum and, prior to the course, relied on their knowledge from elementary school, high school, and the family home, as well as other sources of information. They gave answers individually in a home environment.

Questionnaire

Table 1 shows the number of points obtained for the answers about nutritional knowledge in round 1 (before education) and round 2 (after education) for the whole group without breaking down the respondents by gender. The average score obtained in round 1 was 9.7 out of 13 possible scores. The minimum number of correct answers obtained in round 1 was 5 points. The

Table 1. Scores of nutrition knowledge in round 1 and round 2 of the whole group

Nutrition knowledge	N	Average points	SD	Me	Min	Max	Lower quartile	Upper quartile	Wilcoxon test p<0.05
round 1 (before education)	32	9.7	1.7	10.0	5.0	13.0	8.5	11.0	0.00
round 2 (after education)	32	11.4	1.0	11.5	9.0	13.0	11.0	12.0	0.00

N – number of observations; SD – standard deviation; Me/Min/Max – median, minimum, maximum; p – level of statistical significance

lowest number of correct answers (only 11 students) was shown for the question about the definitions of the RI level of the standard (reference intakes ranges for macronutrients expressed in % energy). In round 2, better results were obtained for all questions. The average score obtained was 11.4 out of 13 possible scores, which was 88% of the possible score (Table 1). There was a statistically significant difference ($p<0.05$) between the average number of points obtained in the study group before and after the education survey (Table 1).

Anthropometric parameters

Nutritional status was analyzed separately for men, and women, due to gender-dependent physiological differences in body composition. Among women, the average body weight was 60.7 kg before education and 59.5 kg after education (Table 2). Among men, 77.9 kg and 77. 8 kg, respectively (Table 3). All subjects had normal BMIs ranging from 18.5-24.9. The average for this parameter BMI in women was 21.6, and in men it was 23.4 in round 1 of study. Body fat mass in the women's group (average 17.1 kg in round 1 and 17.4 kg in round 2) increased slightly, but the correlation was not statistically significant. Muscle tissue content in the women's group decreased slightly (23.9 kg vs. 23.6 kg, respectively), but the difference also did not show statistical significance (Table 2). During the study (7 weeks) in the male group, fat mass and muscle mass did not change in a statistically significant manner, and were 10.5 kg vs. 10.4 kg fat mass and 38.5 kg vs. 38.4 kg muscle mass, respectively (Table 3).

DISCUSSION

A study by Kowalska et al. [14] in a group of 520 high school students showed no effect of a lecture on the principles of healthy eating conducted at school

Table 2. Selected anthropometric parameters in women (n=25) before and after education (round 1 and round 2 of the study)

Anthropometric parameters	Round		Wilcoxon test p<0.05
	1 (before education)	2 (after education)	
	average ± SD (min-max)	average ± SD (min-max)	
Body mass [kg]	60.7±6.7 (50.1-77.3)	59.5±9.0 (50.5-77.5)	0.4352
BMI [kg/m^2]	21.6±2.3 (17.9-25.2)	21.7±2.3 (17.8-25.3)	0.1747
Body fat mass [kg]	17.1±4.7 (10.9-28.2)	17.3±4.7 (10.7-26.9)	0.4929
Body muscle mass [kg]	23.9±2.8 (20.3-34.1)	23.6±3.0 (19.5-34.7)	0.0615

SD – standard deviation; p – level of statistical significance

Table 3. Selected anthropometric parameters in men (n=7) before and after education (round 1 and round 2 of the study)

Anthropometric parameters	Round		Wilcoxon test p<0.05
	1 (before education)	2 (after education)	
	average ± SD (min-max)	average ± SD (min-max)	
Body mass [kg]	77.9±18.7 (53.6-81.4)	77.8±18.9 (54.3-82.9)	0.9165
BMI [kg/m^2]	23.4±5.9 (18.5-23.7)	23.6±5.7 (18.8-23.8)	0.2249
Body fat mass [kg]	10.5±5.4 (8.6-20.1)	10.4±5.2 (8.7-20.4)	0.3454
Body muscle mass [kg]	38.5±9.2 (25.8-43.5)	38.4±9.7 (25.5-44.6)	0.8927

SD – standard deviation; p – level of statistical significance

on increasing the subjects' nutritional knowledge. Zaborowicz et al. [15] conducted an assessment of nutrition behavior and knowledge among 456 students. It was shown that the nutritional knowledge of 1/3 of the subjects was inadequate and could be the cause of the dietary errors found. It was assessed that those with insufficient nutritional knowledge consumed fruit less often and salty snacks more often. Significant conclusions about students' dietary behavior were demonstrated in her study by Orkusz [16]. Students from the University of Economics (n=181), despite knowing the rules of proper nutrition, do not put them into practice. This suggests the need to shape a healthy lifestyle among them.

Similar findings were demonstrated by Likus et al. [17] in a study of Medical University students (n=239). The study found that adolescents aged 19-20 studying medicine, while aware of the importance of proper nutrition and physical activity for health, did not apply this in the practice of daily life. Poplawska et al. [18] studied the nutritional knowledge of students from Warsaw in the first (n=27) and third years (n=29) of a personal trainer course. Most of the students surveyed had a sufficient level of knowledge regardless of their year of study. The authors emphasized the need for further educational efforts to improve the nutritional knowledge and behavior of university students. Similarly, Badrasawi et al. [19] found in assessing the nutritional knowledge of 249 students from Palestine that the evaluation of nutrition knowledge among sports students at An-Najah National University revealed that the overall nutrition knowledge was insufficient. The result of this study recommends including a sports nutrition education course in the program to improve their health, nutritional awareness, and knowledge levels. Priya and Sinha [20] conducted among 60 students (30 boys and 30 girls) from all colleges of RPCAU in India who were residing in University hostel and studying in seventh semester. For testing the nutritional knowledge, 138 questions related to nutrition impacting health were asked through a developed interview schedule. To assess the nutritional status anthropometric and clinical examinations were evaluated by personal meeting. Significant difference in the knowledge level between boys and girls was observed. Knowledge level of girls was better in comparison to boys. Majority of both boys (86.66%) and girls (63.33%) were within the normal BMI range. Among girls there was the case of underweight (23.33%) and overweight (13.34%). Boys were having less nutrition knowledge that clearly showed a negative impact on their health. Hence, it recommended that the knowledge of nutrition is utmost important for all. In the study of Grygiel-Górniak et al. [21] 151 students participated from the medical university (90 female and 61 male subjects). The average BMI in the group of women was obtained at 21.2, body mass 59.5 kg, in men appropriately 24.2 and 80.1 kg and were comparable to our study. The authors stressed that despite improper balanced diets observed in this study, any forms of physical activity should be implemented in the schedule of students. They should be encouraged to participate in a high level of physical activity so as to promote good health.

Limitations

The limitations of the study were the small number of subjects. In future studies, especially the number of men should be increased. Only 7 men took part in the study, so the whole group was subjected to a nutritional assessment without gender breakdown. It should be examined whether gender can be a differentiating factor in nutrition knowledge. It would also be necessary to see if the level of knowledge influences the practical eating behavior of the students surveyed. Also, expanding the group to include students from other fields of study (e.g., humanities) will enable an in-depth analysis of data on students' nutrition knowledge. All students surveyed had good nutritional status (BMI in the range of 18.5-24.9). Two achieved weight reductions of 1.5-2 kg. It should be checked in the long term whether a high level of nutritional knowledge influences the achievement of better anthropometric parameters in the studied students. Due to limited study time and organizational considerations, the pool of questions in the survey was not expanded to include possible changes in eating habits during the study. These topics will be addressed in future studies.

CONCLUSIONS

Nutrition education should be considered a long-term "investment" in the health of society and should be multi-level, starting with the family through kindergarten and, school with the participation of children and teachers, and involve health care personnel, i.e., doctor, nurse, nutritionist, psychologist, as well as mass media. In the present study, an improvement in students' nutritional knowledge was obtained after 7 weeks of education as expressed by an increase in the number of right answers. The study group showed a statistically significant increase in the number of correct answers on the nutrition knowledge questionnaire after the nutrition education (appropriately average 9.7 points vs. 11.4 points; $p<0.05$).

No statistically significant differences were noted in the studied anthropometric parameters performed before and after nutrition education. It should be emphasized that, during the study, all the

anthropometric parameters of the students surveyed were within normal and healthy ranges.

Conflict of interest

The authors declare no conflict of interest.

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ORIGINAL ARTICLE

PESTICIDE EXPOSURE AND BLOOD CHOLINESTERASE LEVELS AMONG ADOLESCENTS FROM FARMING FAMILIES IN NORTHERN THAILAND

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ABSTRACT

Background. Adolescents living in agricultural communities may be at risk for the adverse effects of pesticide exposure because they are involved in agriculture either as a career or to support their families.

Objectives. The purpose of this study was to investigate the association of farm activities related to pesticide exposure on blood cholinesterase (ChE) levels among adolescents from farming families in the north of Thailand.

Material and Methods. This cross-sectional study included 336 adolescents aged 12-19 years from farming families in Chiang Dao District, Chiang Mai Province. Data on pesticide exposure was collected using a questionnaire, and blood ChE activity was assessed using a ChE reactive paper test kit via fingerstick blood sampling.

Results. Overall, 51.2% of participants had abnormal blood ChE levels. Univariable logistic regression analysis revealed that pesticide-related activities on farms associated with abnormal ChE levels were mixing/spraying (OR=10.54; 95%CI=4.63-23.99), assisting or working in areas with pesticide application (OR=5.54; 95%CI=3.45-8.89), and harvesting (OR=3.70; 95%CI=2.35-5.82). In a multivariable model (Nagelkerke R2=0.374), mixing/spraying (OR=4.90; 95%CI=2.03-11.83) and assisting or working in areas with pesticide application (OR=2.61; 95%CI=1.49-4.57) were significantly associated with abnormal ChE levels, but harvesting (OR=1.48; 95%CI=0.84-2.61) was not significant after adjusting for sex, age in years, and entering or walking through a farm.

Conclusions. The findings indicated that Thai adolescents living in farming families are at risk of pesticide exposure, particularly those involved in agricultural activities such as pesticide applicators. An intervention and measure to raise awareness and reduce the risk of pesticide exposure in adolescents is required.

Keywords: *pesticide, organophosphate, carbamate, agriculture, cholinesterase, teenager*

INTRODUCTION

According to the Food and Agriculture Organization of the United Nations (FAO) report, the total amount of pesticide use worldwide over the past 20 years (2011-2020) tends to continually increase [1]. In Thailand, pesticides are widely used to control insects and other agricultural pests, particularly organophosphates (OPs) such as chlorpyrifos and profenofos, and carbamates (CMs) such as methomyl and carbofuran. Farmers can be directly exposed to pesticides, while their farm families can be indirectly exposed through pesticide drift and take-home exposure [2-4]. This raises concerns about potential health risks, especially among children and adolescents living in agricultural communities who are highly susceptible to pesticide exposure and negative effects as a result of developmental, dietary, and physiological factors [5-7]. A previous study found that exposure to OP pesticides among school-aged children living in Thai farming communities is impacted by farming activity, household environments, and child behaviors [8].

Blood cholinesterase (ChE) activity levels in the human body are used as a biomarker to determine internal exposure to OP and CM pesticides, which act as cholinesterase inhibitors. According to prior research, lower levels of ChE activity were observed among children and adolescents residing in rural and agricultural communities [2, 9-10]. In addition, adolescents can become occupationally exposed to pesticides by working on farms to support their families. Previous studies found that adolescent pesticide applicators had decreased ChE levels, which were associated with their health, including

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depression symptoms, neurological symptoms, and poorer neurobehavioral performance [11-18]. As a result, adolescents living in farm communities are at risk of pesticide exposure through a variety of routes, with serious health consequences. However, little is known about pesticide exposure patterns and health monitoring in Thai adolescents living in agricultural settings.

The main purposes of this study were to assess blood ChE levels and investigate the association between farm activities related to pesticide exposure and blood ChE levels among Thai adolescents from farming families in Northern Thailand. An additional goal of the study was to examine demographic factors and environmental pesticide exposure that are associated with ChE levels. The findings of this study can be implemented to the surveillance and prevention of pesticide-related health risks. It is also expected to make recommendations on health policy and planning to minimize pesticide exposure risks among Thai adolescents living in agricultural areas.

MATERIAL AND METHODS

Design, setting, and subjects

This cross-sectional study was conducted on adolescents from farming families in Chiang Dao District, Chiang Mai Province, Northern Thailand. The area of Chiang Dao District was purposively selected because the favorable climate and geographical features provided conditions for cultivation throughout the year, with a large portion of the population engaged in various agricultural activities. These activities include crop cultivation by farmers who use pesticides, particularly OPs and CMs, to increase crop yields and protect against pests. Adolescents are commonly involved in agricultural activities to help their families, such as handling pesticides and assisting with requested tasks on farmland.

Participants for the study were chosen using convenience sampling. Local administrative organizations conducted public relations to invite adolescents who met the inclusion criteria through two schools that serve as gathering places for adolescents all over Chiang Dao District. Adolescents aged 12 to 19 who had spent at least one year living with a family member who worked as a farmer and used agricultural pesticides were eligible for inclusion. The sample size for estimating a population proportion [19] was determined by defining the estimated proportion at 50%, the confidence level at 95%, the level of precision at 0.05%, and the population size at 2,000. Adding 5% of the participants to prevent loss of data yielded a sample size of 340. The final number of adolescents for data analysis was 336, due to errors during blood sampling and testing.

This study received ethical approval from the Committee of Research Ethics, Faculty of Public Health, Chiang Mai University (No. ET014/2023). Before enrollment in the study, subjects aged 12 to 17 provided written informed assent, while those aged 18 to 19 provided written informed consent. For subjects under the age of 18, written informed consent was also obtained from their parents or legal guardians.

Data collection

Data was collected between July and August 2023. In-person interviews were conducted by assistance researchers who had received training prior to data collection. An interviewer-administered questionnaire contained the following sections: (a) demographic characteristics (sex, age, ethnicity, education level, underlying disease, smoking status, and alcohol drinking); (b) environmental pesticide exposure (entering or walking to agriculture area, distance between home and nearest farm, using household insecticides, and house cleaning with a wet cloth); (c) farm activities related to pesticide exposure (mixing and spraying pesticides, assisting or working in areas where pesticides were being applied, and harvesting), along with activity frequency, use of personal protective equipment (PPE), and handwashing and bathing after activities.

A registered nurse collected fingerstick blood samples to assess ChE activity in serum using ChE reactive paper (GPO, Thailand), a standard technique recommended by the Bureau of Occupational and Environmental Diseases, Ministry of Public Health. For laboratory testing, this method had sensitivity, specificity, and positive predictive value of 89.89%, 95.65%, and 94.59%, respectively, while for field screening, it had 77.04%, 90.01%, and 90.38% [20]. Blood ChE levels were classified into four levels based on color indication (Bigg's method): normal (yellow; ≥100 U/ml), safe (greenish yellow; 87.5-99.9 U/ml), risky (green; 75.0-87.4 U/ml), and unsafe (dark green; ≤75 U/ml) [20]. Unsafe and risky levels were defined as "abnormal", while safe and normal levels were defined as "normal".

Data analysis

Descriptive statistics, such as frequency and percentage, were used to analyze the sample variables. *Chi*-square tests were used to investigate the factors (demographic characteristics and environmental pesticide exposure) that are associated with blood ChE levels. Binary logistic regression was then used to determine the strength of the association between pesticide-related farm activities and blood ChE levels. This study examined both a single model, in which each farm activity was tested independently, and a multiple model, in which different farm activities

were tested together. A p-value of less than 0.05 indicated statistical significance, and an odds ratio (OR) with 95% confidence interval (95%CI) was reported. All statistical analyses were performed using SPSS Version 28 (IBM Corp., Armonk, NY, USA).

RESULTS

The demographics and environmental pesticide exposure of the 336 adolescents under study are presented in Table 1. Blood ChE levels were found to

Table 1. Demographic characteristics and environmental pesticide exposure among adolescents, classified by blood ChE levels (n=336)

Variables	n (%)	Blood ChE levels		p-value*
		Normal (n=164) n (%)	Abnormal (n=172) n (%)	
Sex				
Male	104 (31.0)	40 (38.5)	64 (61.5)	0.011
Female	232 (69.0)	124 (53.4)	108 (46.6)	
Age				
12-14 years	69 (20.5)	50 (72.5)	19 (27.5)	<0.001
15-19 years	267 (79.5)	114 (42.7)	153 (57.3)	
Mean ± standard deviation (SD)	15.72±1.56	15.38±1.70	16.05±1.35	
Ethnicity				
Non-ethnic	189 (56.3)	99 (52.4)	90 (47.6)	0.138
Ethnic	147 (43.8)	65 (44.2)	82 (55.8)	
Education level				
Junior high school	103 (30.7)	60 (58.3)	43 (41.7)	0.021
Senior high school	233 (69.3)	104 (44.6)	129 (55.4)	
Underlying disease				
No	320 (95.2)	156 (48.8)	164 (51.2)	0.922
Yes	16 (4.8)	8 (50.0)	8 (50.0)	
Smoking				
No	325 (96.7)	157 (48.3)	168 (51.7)	0.317
Yes	11 (3.3)	7 (63.6)	4 (36.4)	
Alcohol drinking				
No	277 (82.4)	134 (48.4)	143 (51.6)	0.730
Yes	59 (17.6)	30 (50.8)	29 (49.2)	
Entering or walking through a farm				
No	53 (15.8)	41 (77.4)	12 (22.6)	<0.001
≤1 time per month	177 (52.7)	100 (56.5)	77 (43.5)	
>1 time per month	106 (31.5)	23 (21.7)	83 (78.3)	
Home proximity to nearest farm†				
<300 m	76 (23.2)	33 (43.4)	43 (56.6)	0.474
300 m – 1 km	75 (22.9)	36 (48.0)	39 (52.0)	
>1 km	176 (53.8)	91 (51.7)	85 (48.3)	
Use of household insecticides†				
No	74 (22.6)	39 (52.7)	35 (47.3)	0.460
Yes	253 (77.4)	121 (47.8)	132 (52.2)	
House cleaning with a wet cloth†				
<1 time per month	25 (7.6)	7 (28.0)	18 (72.0)	0.209
1-3 times per month	119 (36.4)	60 (50.4)	59 (49.6)	
At least 1 time per week	110 (33.6)	55 (50.0)	55 (50.0)	
Every day or almost every day	73 (22.3)	38 (52.1)	35 (47.9)	

† Unknown answer (n=9); * *Chi*-square test

Table 2. Farm activities related to pesticide exposure among adolescents, with frequency, PPE use, and hygiene behaviors classified by sex

Exposure characteristics	n (%)	Sex	
		Male n (%)	Female n (%)
1. Mixing and spraying			
No	274 (81.5)	73 (70.2)	201 (86.6)
Mixing	39 (11.6)	15 (14.4)	24 (10.3)
Spraying	7 (2.1)	4 (3.8)	3 (1.3)
Mixing and spraying	16 (4.8)	12 (11.5)	4 (1.7)
– Frequency of mixing and applying (n=62)			
Less than once per year	41 (66.1)	18 (58.1)	23 (74.2)
Yearly	15 (24.2)	7 (22.6)	8 (25.8)
Monthly or weekly	6 (9.7)	6 (19.4)	0 (0.0)
– Use of PPE (n=62)			
Goggles	9 (14.5)	3 (9.7)	6 (19.4)
Face mask	37 (59.7)	21 (67.7)	16 (51.6)
Gloves	23 (37.1)	11 (35.5)	12 (38.7)
Long sleeve shirt and long pants	40 (64.5)	20 (64.5)	20 (64.5)
Boots	14 (22.6)	7 (22.6)	7 (22.6)
– Handwashing and bathing after pesticide use (n=62)			
No	7 (11.3)	4 (12.9)	3 (9.7)
Yes	55 (88.7)	27 (87.1)	28 (90.3)
2. Assisting or working in areas with pesticide application			
No	145 (43.2)	38 (36.5)	107 (46.1)
Yes	191 (56.8)	66 (63.5)	125 (53.9)
– Frequency of any work (n=191)			
Less than once per year	121 (63.4)	38 (57.6)	83 (66.4)
Yearly	48 (25.1)	17 (25.8)	31 (24.8)
Monthly or weekly	22 (11.5)	11 (16.7)	11 (8.8)
– Use of PPE (n=191)			
Goggles	6 (3.1)	2 (3.0)	4 (3.2)
Face mask	56 (29.3)	26 (39.4)	30 (24.0)
Gloves	20 (10.5)	5 (7.6)	15 (12.0)
Long sleeve shirt and long pants	98 (51.3)	39 (59.1)	59 (47.2)
Boots	22 (11.5)	13 (19.7)	9 (7.2)
– Handwashing and bathing after work (n=191)			
No	37 (19.4)	10 (15.2)	27 (21.6)
Yes	154 (80.6)	56 (84.8)	98 (78.4)
3. Harvesting			
No	153 (45.5)	36 (34.6)	117 (50.4)
Yes	183 (54.5)	68 (65.4)	115 (49.6)
– Frequency of harvesting (n=183)			
Yearly	157 (85.8)	51 (75.0)	106 (92.2)
Monthly	20 (10.9)	14 (20.6)	6 (5.2)
Weekly	6 (3.3)	3 (4.4)	3 (2.6)
– Use of PPE (n=183)			
Face mask	20 (10.9)	6 (8.8)	14 (12.2)
Gloves	38 (20.8)	12 (17.6)	26 (22.6)
Long sleeve shirt and long pants	82 (44.8)	37 (54.4)	45 (39.1)
– Handwashing and bathing after work (n=183)			
No	32 (17.5)	12 (17.6)	20 (17.4)
Yes	151 (82.5)	56 (82.4)	95 (82.6)

be 0.9% for normal, 47.9% for safe, 42.3% for risky, and 8.9% for unsafe. In total, the prevalence of abnormal ChE levels was 51.2% (95% CI: 45.8-56.6). *Chi*-square tests revealed a significant association between blood ChE levels and demographic variables including gender (p=0.011), age (p<0.001), and education level (p=0.021). We also observed a significant association between entering or walking through agricultural areas and blood ChE levels (p<0.001).

Regarding pesticide-related activities on farms, approximately 18.5% of adolescents had a history of mixing or spraying pesticides, and more than half reported assisting or working in areas where pesticides were being applied (56.8%) and harvesting produce contaminated with pesticide residues (54.5%) (Table 2). No one reported wearing a chemical mask or respirator when handling pesticides or performing activities in areas where pesticides were being applied.

Association between pesticide-related activities on farms and blood ChE levels in adolescents is shown in Table 3. The modeled odds of abnormal ChE activity increased significantly for mixing or spraying pesticides (OR=10.54, 95% CI: 4.63-23.99), assisting or working in areas with pesticide application (OR=5.54, 95% CI: 3.45-8.89), and harvesting (OR=3.70, 95%CI: 2.35-5.82). After controlling for sex, age in years, and entering or walking through a farm, the modeled odds of abnormal ChE activity decreased but remained significant for each of the farm activities.

In the multiple exposure model, abnormal ChE activity was significantly associated all farm activities, including mixing or spraying pesticides (OR=5.75, 95% CI: 2.45-13.49), assisting or working in areas with pesticide application (OR=3.16, 95% CI: 1.86-5.35), and harvesting (OR=1.93, 95%CI: 1.15-3.25), as shown in Table 4. However, harvesting became insignificant after adjusting for sex, age in years, and entering or walking through a farm.

Table 3. Association between pesticide-related activities on farms and blood ChE levels in adolescents: single exposure model

Exposure characteristics	Blood ChE levels		Crude OR (95% CI)	Adj OR[a] (95% CI)	Adj OR[b] (95% CI)
	Normal n (%)	Abnormal n (%)			
1. Mixing/spraying					
No	157 (57.3)	117 (42.7)	1	1	1
Yes	7 (11.3)	55 (88.7)	10.54 (4.63-23.99)*	8.63 (3.75-19.88)*	7.18 (3.03-16.99)*
2. Assisting or working in areas with pesticide application					
No	104 (71.7)	41 (28.3)	1	1	1
Yes	60 (31.4)	131 (68.6)	5.54 (3.45-8.89)*	5.04 (3.11-8.16)*	3.72 (2.21-6.25)*
3. Harvesting					
No	101 (66.0)	52 (34.0)	1	1	1
Yes	63 (34.4)	120 (65.6)	3.70 (2.35-5.82)*	3.34 (2.10-5.31)*	2.20 (1.31-3.71)*

[a] Adjusted for sex and age in years; bAdjusted for sex, age in years, and entering or walking through a farm; *p<0.05

Table 4. Association between pesticide-related activities on farms and blood ChE levels in adolescents: multiple exposure model

Exposure characteristics	Model 1[a]		Model 2[b]		Model 3[c]	
	OR (95% CI)	*p*-value	OR (95% CI)	*p*-value	OR (95% CI)	p-value
Mixing/spraying	5.75 (2.45-13.49)	<0.001	4.97 (2.09-11.80)	<0.001	4.90 (2.03-11.83)	<0.001
Assisting	3.16 (1.86-5.35)	<0.001	3.05 (1.79-5.20)	<0.001	2.61 (1.49-4.57)	0.001
Harvesting	1.93 (1.15-3.25)	0.013	1.88 (1.11-3.18)	0.018	1.48 (0.84-2.61)	0.180
Nagelkerke R^2	0.300		0.316		0.374	

[a] Unadjusted; [b] Adjusted for sex and age in years; [c] Adjusted for sex, age in years, and entering or walking through a farm

DISCUSSION

Our findings revealed that the majority of adolescents from farming families in the north of Thailand had abnormal blood ChE levels, reflecting the degree of OP and CM exposure that entered the body via multiple routes, including inhalation, consumption, and dermal contact. Adolescents' high level of exposure may be due in part to living near pesticide-treated farmland, and families who work in agriculture unintentionally bring pesticide residues home on their clothing, shoes, or equipment [3-4]. It is consistent with previous research, which found lower levels of ChE activity among adolescents residing agricultural communities in Ecuador [2]. Similarly, 42.9% of Indonesian school-age children had low ChE levels [21]. Existing research suggests that environmental exposure to OP pesticides may cause changes in reproductive and thyroid hormone levels in adolescents [22-23]. In terms of occupational pesticide exposure, particularly OPs, decreased blood ChE activity has been linked to neurological symptoms and neurobehavioral effects in adolescent workers [11, 14-15, 24]. Furthermore, other pesticide classes are used in agricultural areas of Northern Thailand, in addition to OP and CM pesticides [25]. These findings indicate that adolescents from farming families are at high risk of health consequences from pesticide exposure, emphasizing the importance of health surveillance for them.

Regarding farm activities related to pesticide exposure, it was found that each one under investigation – mixing and spraying, assisting or working in areas where pesticides were being applied, and harvesting – was significantly associated with blood ChE levels in adolescents. Our findings indicated occupational pesticide exposure among Thai adolescents, which could contribute to their higher health risk. It is consistent with numerous studies that found that adolescent pesticide applicators in Egypt had significantly decreased plasma and erythrocyte ChE levels than non-applicators [11-12, 16]. About 18.5% of the adolescents reported having direct contact with pesticides via mixing or spraying, with most of applications occurring on a low frequency. This is comparable to the findings of adolescent Latino farmworkers, who reported applying or mixing agricultural chemicals at 21.6% [26].

More than half (56.8%) of the adolescents reported assisting or working in pesticide application areas, which was linked to lower blood ChE levels. It is true that common farm activities such as helping parents mix pesticide, pulling pesticide spraying hoses, and cleaning equipment can increase the risk of pesticide exposure through inhalation and dermal contact. This is consistent with a previous study, which found that being present during the application and having any direct contact with the application process was associated with changes in urinary biomarker concentrations in farm families such as children and spouses [27]. Poor PPE use among adolescents, particularly the failure to wear chemical masks or respirators while applying pesticides or engaging in other agricultural activities during the application, has been reported, potentially leading to increased exposure levels and adverse health effects [28-29]. The findings of this study suggest the need for pesticide training or an intervention to improve pesticide application practices, including the adoption of PPE, among adolescents engaged in farm activities, particularly those who are mixers or sprayers [26, 30].

Harvesting activities lost significance in the multiple exposure model after potential factors like entering or walking through a farm were accounted for. This could be due to indirect contact and low dose exposure caused by bodily contact with pesticide-treated crops during harvesting tasks such as picking, cutting, and sorting, which transfers pesticide residues contaminated on crop surfaces to their skin [31-32]. This is consistent with a study conducted among Indonesian schoolchildren, which found that cutting onion leaves (Crude OR=2.41, 95%CI: 1.35-4.29) and carrying onion harvest to other places (Crude OR=1.91, 95%CI: 1.21-3.03) are potential risk factors for OP pesticide exposure measured by metabolites [33]. Likewise, OP pesticides on hands were associated with lower plasma ChE levels among Thai vegetable vendors, implying primarily dermal exposure via hand contact [34]. According to a study by Chetty-Mhlanga et al. [35], picking fruit is the most frequently reported farm activities, and children aged 9-16 who engage in pesticide-related activities, particularly picking crops from the field, may be at a higher risk for health effects. However, 85.8% of the adolescents reported harvesting on a yearly basis, which may result in diminished pesticide exposure.

Demographic characteristics including sex, age, and education were found to be associated with ChE levels in Thai adolescents. This may also be because a higher proportion of male and older adolescents, as well as those with senior high school degree, reported participating in farm activities, leading to greater pesticide exposure and lower ChE activity. According to literature reviews, boys are more likely to participate in activities than girls [36], and men are typically responsible for direct contact with pesticides as farming is physically demanding [37]. Previous research revealed sex-specific effects, with males being more susceptible to OP exposure [38]. In addition, environmental pesticide exposure, like entering or walking through a farm, was associated with lower ChE levels. It is possible that adolescents

come into contact with pesticide-contaminated environments via various pathways while on the farm, such as playing in fields and plantations that have recently been treated with pesticides. A previous study in Thailand observed that toddlers' frequency of following caregivers to the farm was positively associated with CM residue on their feet [39]. This could imply that adolescents who enter or walk through a farm are exposed to pesticide residues via skin contact.

Regarding the study's limitations, this is a cross-sectional study in which causal relationships cannot be inferred. The study omitted many important details about pesticide exposure (for example, the last date of activities, hours and years of field work, length of time working with pesticides, pesticide active ingredient, and spraying method). Generalizability may be limited as a result of sampling by school unit in the investigated region. Therefore, larger-scale studies covering broader geographical areas should be conducted as well as an investigation into the health effects of pesticide exposure among Thai adolescents. Our study focused on blood ChE levels indicating OP and CM exposure; future research should consider using additional biomarkers to assess other pesticide classes, such as metabolites. However, this is a preliminary survey of teenagers from farming families, highlighting pesticide exposure both directly and indirectly as well as concern about a potential health risk, as a major public health issue.

CONCLUSIONS

This study provides evidence of environmental and occupational pesticide exposure among adolescents from farming families in the north of Thailand. Our findings revealed that pesticide-related activities in the fields, such as mixing and spraying, assisting or working in areas where pesticides were being applied, and harvesting, were associated with lower ChE levels. This raises significant concerns about potential health risks, particularly for pesticide applicators. Addressing pesticide exposure in adolescents necessitates raising awareness and public health education among individuals, parents or guardians, and communities.

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Conflict of interest
All authors declare they have no potential competing interest.

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ORIGINAL ARTICLE

EPIDEMIOLOGICAL ASPECTS OF THE ASSOCIATION OF THE HYPERTRIGLYCERIDEMIC WAIST PHENOTYPE WITH METABOLIC SYNDROME AND CARDIOVASCULAR RISK FACTORS IN MOROCCO. CASE THE AMAZIGH POPULATION FROM A GEOGRAPHIC REGION CALLED SOUSS

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ABSTRACT

Background. The global prevalence of metabolic syndrome (MetS) increases susceptibility to non-communicable diseases such as obesity, type 2 diabetes, and cardiovascular disease, posing significant health risks. Effective prevention and management require objective tools. The hypertriglyceridemic waist (TG+WC+) phenotype is proposed as a less expensive approach to identify individuals with metabolic syndrome and other cardiovascular risk factors.

Objective. The current aim of this investigation is to study the epidemiological characteristics of the hypertriglyceridemic waist phenotype and their correlations with cardiovascular risk factors and MetS in the Moroccan Amazigh ethnic group from the Souss region of Morocco.

Material and Methods. A total of 827 Amazigh adults from the Sousse region of Morocco were divided into four distinct phenotype groups: TG-WC-, TG+WC-, TG-WC+, and TG+WC+ (normal TG- or high TG+ triglycerides/normal WC- or high WC+ waist circumference). The association of the different phenotypes with MetS and other cardiovascular risk factors was established by logistic regression analysis.

Results. The prevalence of the TG+WC+ phenotype was 27.7% and varied according to age group and sex. Among subjects with the TG+WC+ phenotype, most were 41-60 years old (53.3%) and in women (74.2%). Participants with the TG+WC+ phenotype had the highest prevalence of dyslipidemia (87.3%), hypoHDLaemia (69.9%), and general obesity (37.12%). The three phenotypes TG-WC-, TG+WC- and TG-WC+ were less associated with MetS and other cardiovascular risk factors. Moreover, people with the TG+WC+ phenotype had a very high odds ratio for MetS.

Conclusion. These findings suggest that the TG+WC+ phenotype exhibits a robust correlation with MetS and additional variables connected to cardiovascular risk. The TG+WC+ phenotype serves as a valuable clinical instrument for detecting individuals vulnerable to MetS and cardiovascular diseases.

Keywords: *hypertriglyceridemic waist phenotype, cardiovascular risk factors, metabolic syndrome, epidemiology, Morocco*

INTRODUCTION

Metabolic syndrome (MetS), is characterized by a range of interconnected clinical and biochemical irregularities and dysfunctions. The combined impact of these elements represents a notable factor at risk for the onset of cardiovascular disease (CVD), type 2 diabetes (T2D), and additional health complications [1]. Metabolic syndrome prevalence is on the rise across the globe, varying from 12.5% to 31.4%, contingent upon the adopted definition and the geographical region studied. The Eastern Mediterranean and the Americas show the highest prevalence rates, which correlate with the standard of living and income levels [2].

The growing prevalence of MetS globally is attributed to the shift in epidemiological and nutritional patterns known as the epidemiological and nutritional transition that accompanies rapid economic growth. This phenomenon arises from unhealthy lifestyle choices, including sedentary behavior and inadequate

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dietary habits, which have become pervasive across all nations due to continuous cultural globalization [3].

Morocco, like all other nations, has undergone an epidemiological transition, leading to adverse outcomes for both public health and the economy [3]. It was reported that Moroccan population presents a high risk of cardiovascular disease (37%), significantly elevated in older adults [4]. It is evident that cardiovascular disease continues to be the major reason behind mortality [5, 6]. Also, the prevalence of MetS varies by age, gender, ethnicity, and area [7]. However, there is no national average, for example, among adult women in southern Morocco, it was 16.3% in 2002 [8], but among post-menopausal women in northern Morocco, it was 74.18% in 2020 [9].

Metabolic syndrome (MetS) is generally indicated by the coexistance of at least three of the following characteristics, although other health organizations may have different diagnostic standards: obesity, either general or central located, high triglyceride (TG), low HDL-cholesterol, hypertension, and hyperglycemia [10]. The main causes of MetS are considered to be central obesity and insulin resistance [11]. According to Engin [12], central obesity is the most observed aspect of MetS. Waist circumference (WC) measurement is considered the classic marker screening for MetS and a more effective parameter for predicting CVD risk indicators [13, 14]. But which index best predicts MetS remains controversial. While WC is one of the components of MetS [15], its limitations lie in its inability to distinguish between subcutaneous and visceral abdominal fat [16]. Indeed, it has been demonstrated that visceral fat is strongly linked with various cardiovascular risk triggers and CVD [17, 18, 19]. The presence of elevated TG and WC is known as the hypertriglyceridemic waist phenotype (TG+WC+). Lemieux et al. in 2000 [20] suggested that males displaying atherogenic metabolic traits and facing a heightened risk of coronary heart disease might be detectable through an inexpensive screening approach involving the assessment and analysis of waist circumference and triglyceride levels.

Recent studies have employed the TG+WC+ phenotype, an inexpensive index to identify hypertension [21], type 2 diabetes [22, 23] and MetS as part of initial population screening efforts. For identifying metabolic problems, hypertriglyceridemic waist phenotypes may be more predictive value than individual anthropometric markers [24, 25]. One of the most potent indicators of breast cancer risk is the TG+WC+ phenotype [26]. For screening the risk of cardiovascular disease, this marker may be a useful tool [27].

The objective of this study is to elucidate the epidemiological characteristics of the TG+WC+ phenotype and investigate its possible associations with MetS and cardiovascular factors at risk in a cross-sectional sample drawn from the Moroccan Amazigh ethnic group in the Souss region of Morocco.

MATERIAL AND METHODS

The target population

This is a prospective epidemiological study carried out between January 2023 and December 2023 at the Hassan 1st Provincial Hospital in Tiznit city. The study involved a population of Amazigh origin, 827 healthy individuals (254 men and 573 women aged between 20 and 80). The study was carried out in conjunction with a lipid panel. Participants are volunteers and each of them had written consent prior to their inclusion in this study. We excluded pregnant women, disabled people, senile individuals over 80, hemodialysis patients, cancer patients, and cardiac patients.

Anthropometric measurements

Weight measurements were taken utilizing an electronic scale with a sensitivity of 0.1 kg, with participants being weighed wearing only light underwear. The height of each subject was assessed with precision, to the nearest millimeter using a wall measuring device. The subjects were placed with their backs to the wall, with their feet, buttocks, back, shoulders and head in contact with the surface. WC was determined using a tape measure halfway between the last rib of the thorax and the tip of the hip bone at the end of a natural exhalation. The test subject is required to stand with their feet 25 cm [28].

Blood pressure measurement

The automated sphygmomanometer (MICOLIF, Germany) was utilized to test the blood pressure. While the subjects were at rest, measurements were taken. Three measurements of the blood pressure were made, and the analysis was done using the mean value.

Blood tests

After a 12-hour overnight fast, blood samples were obtained by venipuncture from trained medical personnel. The lipid profile analysis was conducted at the medical biology unit of the Provincial Hospital in Tiznit city using an automated analyzer (BioSystems BA400).

Definitions

MetS is defined according to the harmonized criteria outlined in the Joint Interim Statement (JIS) definition [29], considering specific WC cut-off points for Mediterranean region. When three or more of the following risk-factors indicators were met, the patient was considered with MetS: WC in women ≥80 cm and in men ≥94 cm; fasting plasma glucose (FPG) ≥1 g/L or taking anti-diabetic drugs; systolic blood pressure

(SBP) ≥130 mmHg and/or diastolic blood pressure (DBP) ≥85 mmHg or confirmed treatment history of hypertension, elevated fasting triglycerides (FGT) ≥1.5 g/L or under treatment for hypertriglyceridemia and low HDL-cholesterol <0.40 g/L in male and <0.5 g/L in female or under therapy for hypoHDLemia. Elsewhere, hypertension is manifested by systolic pressure value ≥140 mmHg, diastolic pressure value ≥ 90 mmHg, or taking anti-hypertensive drugs [30], type 2 diabetes is defined as a fasting blood glucose ≥1.26 g/L, HbA1c ≥6.5%, or taking anti-diabetic drugs [31].

Anthropometric indices

The body mass index (BMI), also referred to as the Quetelet index, is calculated by dividing an individual's weight in kilograms by the square of their height in meters (kg/m²) [32]. Thus, general obesity has been defined by a BMI≥30.0 kg/m².

Definition of the triglyceridemic-waist circumference phenotype

To identify triglyceridemic-waist phenotypes TGWC, subjects were divided into four phenotypic categories according to strict JIS criteria based on fasting serum TG levels and WC [29].

- Phenotype TG-WC- was considered normal phenotype: WC<94 cm in male and <80 cm in female; TG<1.50 g/L.
- Phenotype TG+WC-: WC<94 cm in male and <80 cm in female; TG≥1.5 g/L.
- Phenotype TG-WC+: WC≥94 cm for male and ≥80 cm for female; TG<1.5 g/L.
- Phenotype TG+WC+ known as hypertriglyceridemic waist phenotype: WC≥94 cm for male and ≥80 cm for female; TG≥1.50 g/L.

Statistical analysis

The data is presented in the form of mean values accompanied by standard deviation or as a numerical percentage. Analysis of variance (ANOVA) was performed to compare quantitative characteristics and the *chi*-square test for qualitative characteristics. The link between MetS and cardiovascular factors at risk with the TG+WC+ phenotype were tested using logistic regression models. Model 1 can fit. In Model 2, adjustments were made for both age and sex. Model 3 further expanded upon Model 2 by incorporating additional adjustments for all examined variables: MetS, diabetes mellitus, dyslipidemia, general obesity, hypertension, hypercholesterolemia, hyperLDLemia, and hypoHDLemia.

The results are expressed as odds ratios (OR) along with their corresponding 95% confidence intervals (CI) in both univariate and multivariate analyses. IBM SPSS Statistics 25.0 software was utilized for all statistical computations and data analysis.

RESULTS

Our study has enrolled 827 adults (mean age 52.9±14.7); 30.7% were men (mean age 56.5±14.9 years) and 69.3% were women (mean age 51.2±14.4 years). Subjects were grouped into four groups based on their phenotype (TGWC). The prevalence of the TG- WC-, TG+ WC-, TG- WC+ and TG+ WC+ phenotypes were 14.4%, 1.9%, 56%, and 27.7%, respectively (Tables 1 and 2). Among subjects with the TG+WC+ phenotype, 8.7% were people aged 20 to 40 years, 53.3% were adults aged 41 to 60 years and 38% were senior adults aged 61 to 80 years (Figure 1.a). According to the sex, the distribution was unequal. Indeed, in men the prevalence was 23.2%, while in women it was 29.7% (Figure 1.b). However, women had the highest TG-WC+, 81.2%, closely followed by TG+WC+, 74.2%. Conversely, men had the highest TG+WC- (87.5%) and TG-WC- (79%) (Table 2).

Based on the four TGWC phenotypes, Table 1 presents the anthropometric and biochemical measurements of the subjects. It was observed a significant increase in all measured quantitative indicators within the four groups. The TG+WC+ phenotypic participants showed the highest values, while, the TG-WC- phenotype participants had the lowest values. Table 2 shows that the TG+WC+ phenotypic participants had the highest prevalence of cardiovascular risk-factors including 69.9% hypoHDLemia, 37.12% obesity, and 87.3%

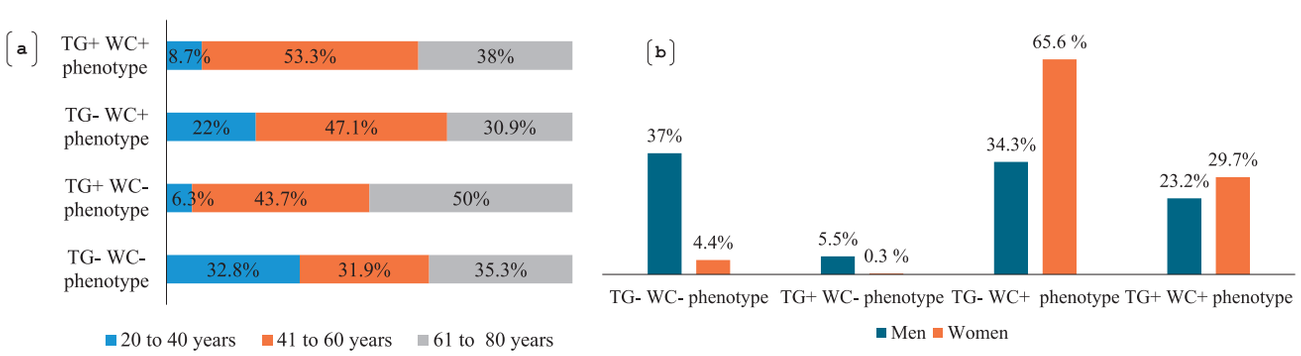

Figure 1. Distribution of triglyceridemia-waist circumference phenotypes as a function of age (a) and sex (b)

Table 1. Baseline characteristics of study participants in the triglyceridemia-waist circumference phenotypes

	ALL=827 (100 %)				TG-WC- phenotype N=119 (14.4%)				TG+WC- phenotype N=16 (1.9%)				TG-WC+ phenotype N=463 (56%)				TG+WC+ phenotype N=229 (27.7%)				p-value
	Min	Max	Mean ±SD	95% CI	Min	Max	Mean ±SD	95% CI	Min	Max	Mean ±SD	95% CI	Min	Max	Mean ±SD	95% CI	Min	Max	Mean ±SD	95% CI	
Age (years)	20	80	52.86 ±14.71	51.85 to 53.86	20	79	49.52 ±18.25	46.21 to 52.83	39	80	59.75 ±12.28	53.21 to 66.29	20	80	51.78 ±14.73	50.44 to 53.13	21	80	56.28 ±11.76	54.74 to 57.81	0.000
Clinical data																					
Weight (kg)	42	125	71.87 ±13.42	70.96 to 72.79	42	92	61.87 ±9.69	60.11 to 63.62	48.5	90	66.13 ±10.66	60.44 to 71.81	44	125	72.72 ±13.39	71.49 to 73.94	47	116	75.76 ±12.74	74.10 to 77.42	0.000
Height (m)	1.45	1.9	1.63 ±0.08	1.62 to 1.63	1.46	1.83	1.66 ±0.08	1.65 to 1.67	1.47	1.84	1.65 ±0.1	1.59 to 1.70	1.46	1.9	1.62 ±0.08	1.61 to 1.63	1.45	1.84	1.62 ±0.08	1.612 to 1.6	0.000
WC (cm)	64	135	97.26 ±11.59	96.47 to 98.05	64	93	81.81 ±6.59	80.62 to 83.01	74	92	85.19 ±6.26	81.85 to 88.52	80	133	99.12 ±9.97	98.20 to 100.027	80	135	102.37 ±9.63	101.11 to 103.62	0.000
BMI (kg/m^2)	16.162	45.312	27.19 ±4.74	26.86 to 27.51	16.298	32.466	22.4 ±2.85	21.88 to 22.91	20.761	32.051	24.28 ±3.03	22.67 to 25.89	16.162	43.58	27.73 ±4.52	27.315 to 28.14	18.671	45.312	28.79 ±4.41	28.212 to 29.36	0.000
SBP (mm Hg)	59	135	83.99 ±11.06	83.25 to 84.74	65	121	82.76 ±12.2	80.54 to 84.97	74	110	89.19 ±10.65	83.51 to 94.86	59	135	83.07 ±10.33	82.13 to 84.02	62	131	86.12 ±11.53	84.62 to 87.62	0.001
DBP (mm Hg)	87	207	139.9 ±20.125	138.53 to 141.27	102	196	134.95 ±21.04	131.13 to 138.77	110	170	152.31 ±16.24	143.66 to 160.96	87	207	138.68 ±19.71	136.88 to 140.48	100	206	144.08 ±19.74	141.51 to 146.65	0.000
Laboratory data																					
TC (g/L)	0.33	4.59	1.94 ±0.45	1.91 to 1.98	0.33	2.63	1.65 ±0.47	1.57 to 1.74	1.46	3.43	2.19 ±0.57	1.89 to 2.50	0.8	4.59	1.9 ±0.41	1.861 to 1.9	0.9	3.39	2.17 ±0.41	2.12 to 2.22	0.000
FPG (g/L)	0.7	5.33	1.39 ±0.59	1.35 to 1.43	0.7	3.41	1.28 ±0.57	1.18 to 1.39	0.7	5.33	1.84 ±1.23	1.19 to 2.50	0.75	3.98	1.32 ±0.52	1.27 to 1.36	0.87	3.91	1.57 ±0.6	1.49 to 1.65	0.000
HDL-C (g/L)	0.12	1.41	0.47 ±0.12	0.460 to 0.48	0.12	1.41	0.5 ±0.16	0.471 to 0.530	0.25	0.66	0.45 ±0.1	0.394 to 0.505	0.2	0.88	0.48 ±0.11	0.471 to 0.492	0.17	0.86	0.42 ±0.1	0.411 to 0.437	0.000
LDL-C (g/L)	0.43	3.97	1.239 ±0.36	1.214 to 1.264	0.43	1.9	1.07 ±0.32	1.009 to 1.126	0.6	2.35	1.35 ±0.49	1.085 to 1.608	0.44	3.97	1.23 ±0.34	1.198 to 1.261	0.57	2.52	1.34 ±0.38	1.291 to 1.390	0.000
TG (g/L)	0.08	5.72	1.29 ±0.67	1.25 to 1.34	0.08	1.5	0.85 ±0.3	0.78 to 0.90	1.52	2.78	2 ±0.35	1.81 to 2.19	0.34	1.5	0.98 ±0.28	0.96 to 1.01	1.51	5.72	2.11 ±0.65	2.020 to 2.19	0.000

BMI – body mass index; WC – waist circumference; SBP – systolic blood pressure; DBP – diastolic blood; FPG – fasting plasma glucose; TC – total cholesterol; TG – triglyceride; HDL-C – high-density lipoprotein cholesterol; LDL-C – low-density lipoprotein cholesterol; p-value <0.05 significant across four phenotypes

Table 2. Prevalence of cardiovascular risk factors in the triglyceridemia-waist circumference phenotypes

	Total N=827 (100%)	Phenotype TG-WC- N=119 (14.4%)	Phenotype TG+WC- N=16 (1.9%)	Phenotype TG-WC+ N=463 (56%)	Phenotype TG+WC+ N=229 (27.7%)	p-value
Men	254 (30.71%)	94 (79%)	14 (87.5%)	87 (18.8%)	59 (25.8%)	0.000
Women	573 (69.29%)	25 (21%)	2 (12.5%)	376 (81.2%)	170 (74.2%)	0.000
Clinical data						
Metabolic syndrome	548 (66.26%)	11 (9.2%)	13 (81.3%)	300 (64.8%)	224 (97.8%)	0.000
Diabetes mellitus	356 (43.05%)	40 (33.6%)	10 (62.5%)	169 (36.5%)	137 (59.8%)	0.000
Dyslipidemia	517 (62.5%)	45 (37.8%)	11 (68.8%)	261 (56.4%)	200 (87.3%)	0.000
General obesity	225 (27.21%)	1 (0.84%)	1 (6.25%)	138 (29.81%)	85 (37.12 %)	0.000
Central obesity	692 (83.68%)	0 (0%)	0 (0%)	463 (100%)	229 (100 %)	0.000
Hypertension	447 (54.05%)	47 (39.5%)	12 (75%)	244 (52.7%)	144 (62.9%)	0.000
Hypercholesterolemia	105 (12.7%)	7 (5.9%)	3 (18.8%)	37 (8%)	58 (25.3%)	0.000
HyperLDLemia	128 (15.5%)	9 (7.6%)	3 (18.8%)	54 (11.7%)	62 (27.1%)	0.000
HypoHDLemia	427 (51.6%)	33 (27.7%)	5 (31.3%)	229 (49.5%)	160 (69.9%)	0.000

dyslipidemia. Except for hypertension and diabetes mellitus which showed the highest values in TG+WC- phenotypic group (75.0% and 62.5%, respectively), their prevalence in TG+WC+ group were 62.9% and 59.8%, respectively. Concerning the TG-WC- phenotype group had the lowest values. In Figure 2, it was observed that the TG+WC+ phenotype group was associated with the highest prevalence of MetS (97.8%), whereas the TG-WC- phenotype was associated with the lowest prevalence (9.2%).

The use of univariate and multivariate binary logistic regression to identify any relationship between cardiovascular risk factors or MetS and the four TGWC phenotypes has showed that the three phenotypes TG-WC-, TG+WC- and TG-WC+ were less associated to MetS and the cardiovascular disease risk factor with an odds ratio (OR) ranging from 0.01 to 0.86 (Table 3). After caring out adjustment in Models 2 and 3, there was a significant increased risk of 13.89 times (OR) of dyslipidemia in the TG+WC- phenotype. Furthermore, the TG+WC+ phenotype showed a stronger and statistically significant positive association with MetS ($p<10^{-3}$) and the other cardiovascular risk factors ($p<10^{-3}$) in both Model 1 and adjusted Model 2. Indeed, individuals with the TG+WC+ phenotype had very high odds ratio for MetS and dyslipidemia, as well as high odds ratio for the other risk factors of cardiovascular diseases. The probability of participants with the TG+WC+ phenotype to develop the MetS was higher in Model 1; OR=37.89 for a 95% CI: [15.39 to 93.25]; ($p<10^{-3}$) and Model 2; OR=35.47; for a 95% CI: [14.28 to 88.12]; ($p<10^{-3}$). Moreover, for diabetes mellitus, the OR was 2.59; 95% for a CI: [1.67 to 3.52]; ($p<10^{-3}$); adjusted by age and gender OR=2.31; for a 95% CI: [1.67 to 3.22]; ($p<10^{-3}$). For dyslipidemia, the OR was 6.11; for a 95% CI: [4.01 to 9.32]; ($p<10^{-3}$), adjusted by age and gender OR=5.88; for a 95% CI: [3.84 to 8.99]; ($p<10^{-3}$). The probability that participants exhibiting the TG+WC+ phenotype developing was for hypertension OR=1.65; for a 95% CI: [1.21 to 2.25]; ($p<10^{-3}$), adjusted by age and gender OR=1.43; for a 95% CI: [1.03 to 1.99]; ($p<0.05$); for hypercholesterolemia OR=3.98; for a 95% CI: [2.61 to 6.06]; ($p<10^{-3}$), adjusted by age and gender OR=3.69; 95% CI: [2.41 to 5.66]; ($p<10^{-3}$); for hyperLDLemia OR=2.99; for a 95% CI: [2.03 to 4.41]; ($p<10^{-3}$), adjusted by age and gender OR =2.71; for a 95% CI: [1.83 to 4.02]; ($p<10^{-3}$); and for hypoHDLemia OR=2.87; for a 95% CI: [2.08 to 3.98]; ($p<10^{-3}$), adjusted by age and gender OR=2.86; for a 95% CI: [2.05 to 3.98]; ($p<10^{-3}$). It should be considered that, according to the data of this study, the TG+WC+ phenotype is highly correlated with MetS, and the underlying risk factors with cardiovascular disease. After adjustment by all predictors in analysis Model 3, the association of MetS ($p<10^{-3}$), dyslipidemia ($p<10^{-3}$), for hyperLDLemia ($p<10^{-3}$), hypoHDLemia ($p<10^{-3}$), and TG+WC+ persisted. The risk of MetS was doubled, dyslipidemia and hypertension halved, while hypercholesterolemia remained stable.

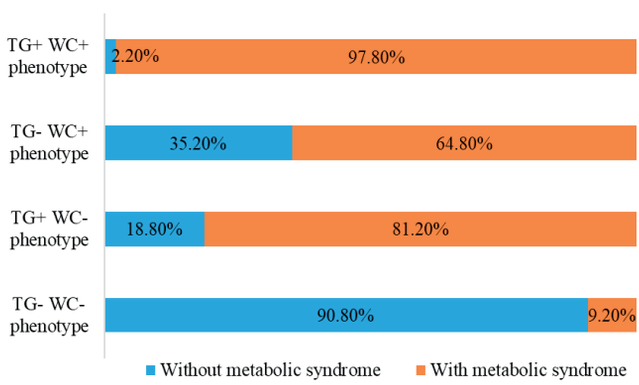

Figure 2. Prevalence of metabolic syndrome in the triglyceridemia-waist circumference phenotypes

Table 3. Binary logistic regression analysis evaluating associations between triglyceridemia-waist circumference phenotypes and cardiovascular risk factors and metabolic syndrome

		Phenotype TG-WC- N=119		Phenotype TG+WC- N=16		Phenotype TG-WC+ N=463		Phenotype TG+WC+ N=229	
Characteristics		Odds ratio (95%CI)	p-value	Odds ratio (95%CI)	p-value	Odds ratio (95%CI)	p-value	Odds ratio (95%CI)	p-value
Metabolic syndrome	Model 1	0.03 (0.02 to 0.06)	0.000	0.03 (0.02 to 0.06)	0.000	0.86 (0.64 to 1.15)	0.314	37.89 (15.39 to 93.25)	0.000
	Model 2	0.02 (0.01 to 0.04)	0.000	0.02 (0.01 to 0.05)	0.000	0.72 (0.52 to 1.01)	0.055	35.47 (14.28 to 88.12)	0.000
	Model 3	0.01 (0 to 0.02)	0.000	5.10 (0.91 to 28.55)	0.063	1.09 (0.69 to 1.74)	0.703	68.21 (15.66 to 297.05)	0.000
Diabetes mellitus	Model 1	0.63 (0.42 to 0.94)	0.026	0.63 (0.42 to 0.94)	0.026	0.54 (0.41 to 0.72)	0.000	2.58 (1.89 to 3.52)	0.000
	Model 2	0.57 (0.35 to 0.93)	0.025	0.57 (0.35 to 0.93)	0.025	0.57 (0.42 to 0.77)	0.000	2.31 (1.67 to 3.22)	0.000
	Model 3	2.64 (0.96 to 7.26)	0.061	0.46 (0.12 to 1.84)	0.273	0.58 (0.4 to 0.83)	0.003	1.39 (0.92 to 2.1)	0.113
Dyslipidemia	Model 1	0.30 (0.20 to 0.45)	0.000	0.30 (0.2 to 0.45)	0.000	0.55 (0.41 to 0.73)	0.000	6.11 (4.01 to 9.32)	0.000
	Model 2	0.37 (0.23 to 0.58)	0.000	0.37 (0.23 to 0.58)	0.000	0.43 (0.31 to 0.59)	0.000	5.88 (3.84 to 8.99)	0.000
	Model 3	1.41 (0.21 to 9.3)	0.720	13.89 (1.28 to 150.37)	0.030	0.37 (0.18 to 0.76)	0.006	3.97 (1.76 to 8.95)	0.001
Obesity general	Model 1	0.02 (0.00 to 0.13)	0.000	0.02 (0 to 0.13)	0.000	1.35 (0.99 to 1.85)	0.059	1.93 (1.39 to 2.68)	0.000
	Model 2	0.03 (0.00 to 0.18)	0.000	0.03 (0 to 0.18)	0.000	1.07 (0.77 to 1.49)	0.686	1.79 (1.28 to 2.52)	0.000
	Model 3	0.03 (0.00 to 0.33)	0.004	0.36 (0.04 to 3.51)	0.382	1.05 (0.72 to 1.53)	0.813	1.48 (0.98 to 2.25)	0.065
Hypertension	Model 1	0.50 (0.34 to 0.75)	0.001	0.5 (0.34 to 0.75)	0.001	0.88 (0.67 to 1.16)	0.379	1.65 (1.21 to 2.25)	0.000
	Model 2	0.34 (0.21 to 0.55)	0.000	0.34 (0.21 to 0.55)	0.000	1.09 (0.8 to 1.48)	0.591	1.43 (1.03 to 1.99)	0.030
	Model 3	1.14 (0.47 to 2.76)	0.771	1.15 (0.26 to 5.14)	0.850	1.30 (0.9 to 1.88)	0.161	0.58 (0.38 to 0.88)	0.012
Hypercholesterolemia	Model 1	0.39 (0.176 to 0.86)	0.019	0.39 (0.18 to 0.86)	0.020	0.38 (0.25 to 0.58)	0.000	3.98 (2.61 to 6.06)	0.000
	Model 2	0.44 (0.19 to 1.07)	0.060	0.44 (0.19 to 1.04)	0.060	0.33 (0.21 to 0.52)	0.000	3.69 (2.41 to 5.66)	0.000
	Model 3	1.46 (0.22 to 9.78)	0.697	0.84 (0.08 to 8.42)	0.884	0.47 (0.24 to 0.89)	0.021	2.58 (1.3 to 5.11)	0.006
HyperLDLemia	Model 1	0.41 (0.20 to 0.82)	0.012	0.4 (0.2 to 0.82)	0.012	0.52 (0.35 to 0.76)	0.001	2.99 (2.03 to 4.41)	0.000
	Model 2	0.48 (0.22 to 1.04)	0.063	0.48 (0.22 to 1.04)	0.063	0.46 (0.31 to 0.69)	0.000	2.71 (1.83 to 4.02)	0.000
	Model 3	0.83 (0.14 to 4.99)	0.843	0.22 (0.01 to 3.42)	0.283	1.02 (0.57 to 1.82)	0.952	0.92 (0.5 to 1.69)	0.785
HypoHDLemia	Model 1	0.31 (0.20 to 0.47)	0.000	0.20 (0.47 to 0.00)	0.306	0.82 (0.62 to 1.08)	0.159	2.87 (2.08 to 3.98)	0.000
	Model 2	0.38 (0.24 to 0.62)	0.000	1.62 (4.18 to 0.00)	2.604	0.66 (0.49 to 0.89)	0.006	2.86 (2.05 to 3.98)	0.000
	Model 3	1.75 (0.28 to 10.74)	0.547	0.05 (0.01 to 0.45)	0.008	1.20 (0.63 to 2.31)	0.574	0.75 (0.38 to 1.49)	0.409

The abbreviations are as presented in Table 1. Model 1 is applicable. Model 2 was adjusted for age and sex. Model 3 was adjusted for Model 2 in addition to all variables studied, namely metabolic syndrome, diabetes mellitus, dyslipidemia, general obesity, hypertension, hypercholesterolemia, hyperLDLemia, and hyoHDLemia

DISCUSSION

To our knowledge, there is no comparative data linking triglyceridemic-waist circumference (TGWC) phenotypes with MetS or cardiovascular risk factors among different ethnic groups in Morocco. This epidemiological investigation is the first study conducted among the Amazigh population in the geographic region called Souss, aiming to examine the relationship between TGWC phenotypes and MetS or some other cardiovascular diseases risk factors.

According to the finding of this survey, 27.7% of Amazigh participants presented a WC+TG+ phenotype, with a higher prevalence among women (29.7%) compared to men (23.2%).

According to the previous published papers, the prevalence of WC+TG+ phenotype in our studied sample was close to that observed in Norfolk, UK, which is 27.81% in women and 31.20% in men [33], higher to the ones found in a studies carried out in Nigeria 23.4% [34], in the municipality of Shanghai 21.2% [35] and almost double that the ones recorded in China 15.22% and 15.93% [36, 37]. However, it is lower than that observed in other countries or regions like in Spain (38.2%) [38], South Asian Indians (35.4%) [39], and in Quebec City metropolitan area (80%) [20].

Regarding the MetS prevalence, the lowest (9.2%) was observed in individuals with the TG-WC- phenotype, and the highest value (97.8%) was recorded within individuals with the TG+WC+ phenotype group which indicates that individuals with the TG+WC+ phenotype are predominantly affected by multiple abnormal metabolic parameters. This same finding was also reported in South African by Prakaschandra and Naidoo in 2022 [39] in which the prevalence of MetS was equal to 88.7% in the TG+WC+ phenotype group. Therefore, it may be suggested that the TG+WC+ phenotype constitutes a more accurate predictor of MetS than previously thought and can be used as a way for early screening and identification of individuals with an elevated risk of developing MetS. It is well known that MetS is a condition that includes a cluster of risk factors specific for cardiovascular disease, thus this study reports higher, even alarming, prevalence of cardiovascular risk factors in individuals exhibiting the TG+WC+ phenotype compared to those who are considered normal (TG-WC-). Indeed, the prevalence of seven risk factors investigated in this study were 87.3%, 62.9%, 69.9%, 59.8%, 37.12%, 25.3% and 27.1% for dyslipidemia, hypertension, low HDL levels, type 2 diabetes, obesity, hypercholesterolemia and hyperLDLemia, respectively.

Also, subjects with the TG+WC+ phenotype seems to be at a higher risk than those with other phenotypes to develop MetS (37 to 68 times more), dyslipidemia (4 to 6 times more), type 2 diabetes (3 to 2 times more), hypertension (1 to 2 times more), hypercholesterolemia (4 times more), general obesity (2 to times more), hyperLDLemia and low HDL (3 times more).

This finding confirms what was previously published from several studies. Gazi and all reported that people with the TG+WC+ phenotype presented also a dyslipidemia, high LDL-cholesterol, and low HDL cholesterol [40, 41]. In a meta-analysis study carried out by Ma and all on a sample of 242 879 subjects from the general population of 19 countries, the pooled odds ratio for type 2 diabetes related to the TG+WC+ phenotype was equal to 2.89 in non-diabetic subjects [42]. In a prospective cohort study of 95 015 participants in China reported that the TG+WC+ phenotype is associated with a nearly 1.24 to 2 times increased risk of developing CVD [43]. Moreover, in a cohort study conducted in Norfolk, UK, it was observed that men and women who had the TG+WC+ phenotype faced 2.4 and 3.84 times higher risk of developing coronary heart disease in comparison to healthy population (TG-WC-) [33] confirming the ability of TG+WC+ phenotype to identify individuals at elevated risk factors for CVD [17, 33, 37].

CONCLUSIONS

This study presents novel findings regarding the prevalence of the TG+WC+ phenotype and confirms its relation to metabolic syndrome and cardiovascular diseases risk factors among the Amazigh population living in Morocco. The findings suggest that the TG+WC+ phenotype is associated with a heightened risk of MetS and cardiovascular diseases risk factors, persisting even after adjusting for confounding factors such as sex, age, and other relevant risk factors.

The simplicity, reproducibility, and low cost of the TG+WC+ phenotype make it a valuable and practical tool to identify individuals at an elevated risk of cardiovascular disease. However, further studies involving other ethnic groups from Morocco are recommended to confirm the present findings and evaluate the efficacy of using TG+WC+ phenotype as a clinical or epidemiological tool.

This study presents the first data on the prevalence of the TG+WC+ phenotype and its association with MetS and cardiovascular diseases risk factors in a representative sample of the Amazigh ethnic group from the geographical region of Souss in Morocco.

Ethics statement

The protocol for this survey has been reviewed and validated by the Ethics Committee (N° IRB00012973) and authorized by the Ministry of Health and Social Protection. Participants were informed of the aims and objectives of the study, and informed consent was obtained in writing.

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Conflict of interest

The authors disclose no conflicts of interest.

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ORIGINAL ARTICLE

HEPATITIS B: A PILOT STUDY ON AWARENESS AND ATTITUDE AMONG STAFF NURSES AND STUDENTS OF A TEACHING HOSPITAL IN INDIA

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ABSTRACT

Background. Hepatitis B is a serious public health concern and health care professionals especially nurses are at higher risk of acquiring this infection. Basic knowledge, awareness and a positive attitude are required to prevent this disease.

Objective. The present pilot study was conducted to assess awareness and attitude regarding hepatitis B among staff nurses and nursing students of a teaching hospital in north India.

Material and Methods. A total of 205 subjects who gave consent to participate were included in this descriptive study. A self-structured pre-tested close ended questionnaire comprising of two parts was used to collect information from the study subjects. First part collected information on demographic details of the study subject and second part assessed subjects' awareness and attitude on various aspects of hepatitis B infection including vaccination. *Chi*-square test and multiple linear regression analysis was used for statistical analysis.

Results. The mean age of the study subjects was 25.8±5.6 years. Awareness regarding HBV infection was present among 92.6% (190) of subjects. Long-term effects of hepatitis B were known to 58.4% (119) of subjects and 79.1% (162) of subjects reported taking universal precautions. Half of the subjects (105), were getting regularly tested for hepatitis B antigen.The odds of getting regularly tested for hepatitis B antigen were 3.26 times greater in female subjects and 3.45 times greater in subjects who were GNMs.

Conclusion. Low awareness levels were reported regarding some important aspects of hepatitis B among study subjects though more than 90% were aware of it. There is an urgent need for education and training programs for nurses designed to increase their knowledge about HBV infection.

Keywords: *hepatitis B, infection, awareness, vaccination, nurses*

INTRODUCTION

Hepatitis B infection is a serious global healthcare problem which is caused by hepatitis B virus (HBV). World Health Organization (WHO) has estimated that more than 254 million people are living with chronic hepatitis B infection, each year adding 1.2 million new infections [1]. In 2022, 1.1 million deaths were reported globally, mostly from cirrhosis and hepatocellular carcinoma surpassing malaria and tuberculosis. According to recent estimates, sixty-one million people are infected with hepatitis B in the WHO South-East Asia Region only [2].

Hepatitis B infection shows variable clinical manifestations ranging from asymptomatic HBV carriers to complete liver failure. It begins as acute infection, but in some people, it becomes chronic and often progresses to chronic hepatitis, cirrhosis, and ultimately hepatocellular carcinoma [3]. It has been estimated that 40% of health care workers (HCWs) are infected with hepatitis B in developing countries [4]. Transmission of HBV in HCWs occurs mainly

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through the skin prick with an infected, contaminated needles and syringes or through accidental inoculation of minute quantities of blood during any surgical procedure [5]. Physicians, dentists, nurses, laboratory staff, and dialysis center personnel are at high risk of acquiring infection.

About two-thirds of patients with acute HBV infection show mild, asymptomatic and subclinical illness that usually goes undetected [6]. Medical history of the patient is unreliable in identifying exposure to HBV infection, therefore all the patients should be considered as potential HBV carriers regardless of their medical history [7]. Proper diagnosis for HBV should be based on testing for a series of serological markers of HBV and by additional testing to exclude alternative etiological agents such as hepatitis A and C viruses [8].

Infection rates remain high, especially in countries with lower socio-demographic profiles such as India which has over 50 million hepatitis B cases. This is despite the fact that an effective vaccine against this deadly disease has been available for over 30 years. Healthcare workers are four times more likely to be infected with HBV compared to the general population. Effective and extensive use of HBV vaccine can dramatically control and reduce the number of incident infections in countries worldwide [9]. Knowledge and attitudes of HCWs plays a key role in prevention of spread of hepatitis B infection. Nurses with direct patient contact fall into particular risk group, therefore, a pilot study was conducted to assess the awareness and attitude and among staff nurses and nursing students of a teaching hospital regarding hepatitis B.

MATERIAL AND METHODS

Ethical clearance and study setting

The present descriptive cross sectional study was conducted among staff nurses and students of a teaching hospital after obtaining ethical clearance from the institutional ethics committee. The purpose of the study was thoroughly explained to the study subjects and informed consent was obtained from every subject prior to the start of the study. Subjects were also assured of confidentiality of their data and it would only be used for research purposes. The study was conducted for a period of two months.

Study population and study sample

Study population consisted of all the staff nurses and nursing students of a teaching institute. The subjects were called in the Out Patient Department (OPD) of the hospital according to availability from different departments of the hospital to participate in the study. This was done by using the following formula to calculate the required sample size:

$$n = \frac{Z^2pq}{d^2}$$

where Z is the standard normal deviation (1.96), p=0.915, percentage of knowledge from previous literature (91.5%) [7], q=1-p and d=0.05 degree of error (5%).

Based on the calculations, a sample size of 121 was arrived at initially. To ensure maximum participation of subjects (working in the hospital) in the study and also take into account non-responders, we doubled this figure on the recommendations of the statistician which was finally 242. The response rate was 85%, therefore the final sample comprised of 205 subjects which seemed adequate. A two-stage random sampling technique was employed:

Stage 1: Seven clinical departments and three laboratories were selected using simple random sampling (ballot method).

Stage 2: From each of the selected departments and laboratories, subjects were stratified according to their working profile and proportionately enrolled in the study using simple random sampling.

Preliminary survey and pre testing of the questionnaire

Preliminary survey was conducted on 10% of the study population to determine the feasibility of the study. The questionnaire was based on previously validated instrument and modifications done accordingly with the suggestions of our experts [10]. Cronbach's coefficient was found to be 0.81 which signifies an acceptable internal reliability of the questionnaire. The content validity ratio was also calculated by using itemrated content validity indices to test the validity. This was attained by noting the responses on the dichotomous scales where the academician indicated whether an item is favorable (score of +1) or unfavorable (score of 0). A panel of four academicians were invited from the department of Infectious Disease and Microbiology to calculate the content validity ratio (0.84). In addition, there were no changes required in the questionnaire as a result of pretest.

Data collection and analysis

The questionnaire, written in English language was distributed by a single investigator among the study subjects. The subjects were told to approach the investigator in case of any doubt regarding the content of the questionnaire which was divided in two parts:

Part 1: 'General Section' which was made to collect socio-demographic details of the subjects (age, gender, educational profile, experience).

Part 2: Comprised of 12 questions on awareness and attitude of the subjects regarding hepatitis B infection.

The principal investigator made sure that the subjects answered all the questions and none of the questionnaires were incomplete. The response of subjects towards the questionnaire was assessed on a two-point Likert scale (Yes or No).

Statistical analysis

Data were entered into Microsoft Excel Spreadsheet version 2010 and was assessed using a statistical package (SPSS, version 21.0, Chicago, IL, USA). Categorical measurements were calculated using number and percentages. *Chi*-square test as used to evaluate the relationship between some demographic factors on some aspects of hepatitis B infection. The significance level was set at <0.05. Multivariate logistic regression analysis was also performed to assess the effect of various independent variables on getting regularly tested for hepatitis B antigen. Odds ratio with 95% CI were also generated.

Table 1. Socio-demographic details of the study subjects

Characteristics	Number	Percentage
Age (years)		
18-35	152	74.1
36 and above	53	25.9
Gender		
Male	47	23
Female	158	77
Qualification		
GNM	82	40
BSc	39	19
MSc	24	11.7
Student	60	29.3
Experience (years)		
<1	62	30.2
1-2	70	34.1
3-5	33	16.1
More than 6	40	19.5

RESULTS

Socio-demographic details of the study population is depicted in Table 1. The present study was conducted among 205 subjects of a teaching institution. The mean age of the study subjects was 25.8±5.6 years. A vast majority of the subjects were females (N=158, 77%) as compared to males. GNMs (General Nursing and Midwifery) constituted 40% of the study population (N=82) and only 11.7% (N=24) had qualification in Masters in Nursing Science (MSc). More than one-third of subjects (N=70, 34.1%) had an experience between one and two years.

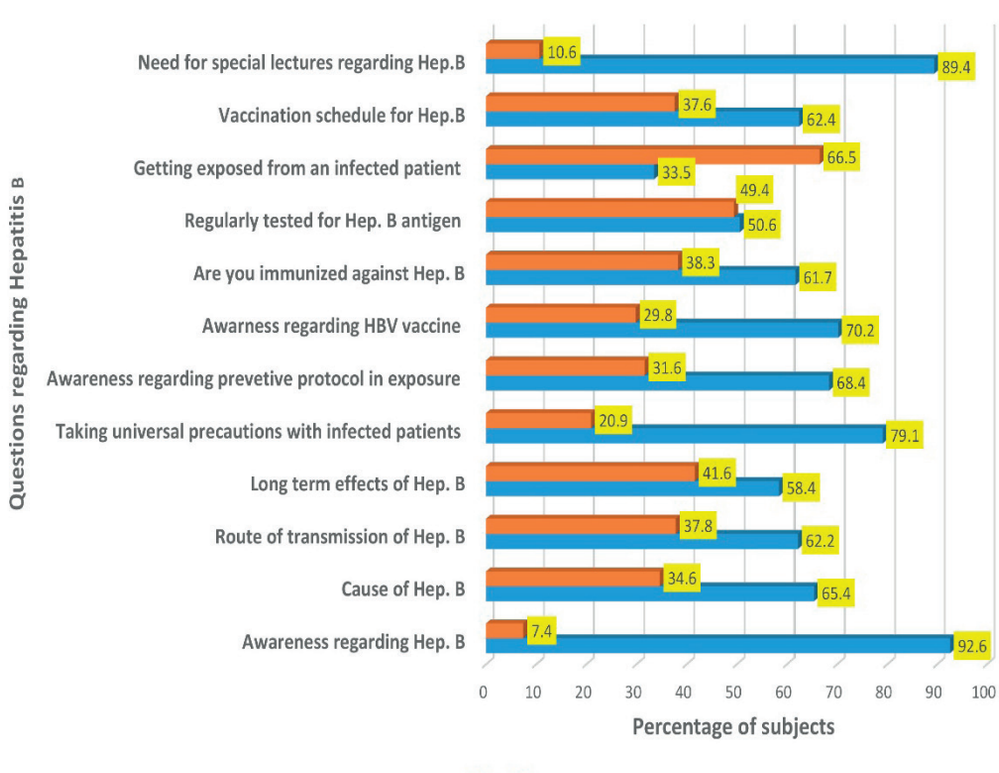

Figure 1. Subjects' responses towards the questionnaire on hepatitis B

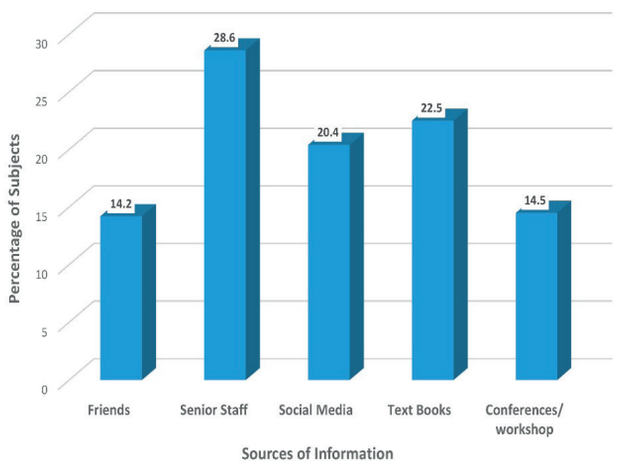

Figure 2. Various sources of information cited by study subjects on hepatitis B

Subjects' response to the questionnaire

Various responses of the subjects towards the questionnaire are shown in Figure 1. More than 90% of subjects (N=190) were aware regarding HBV infection, however only 65.4% (N=134) of subjects were aware regarding its cause. Long-term effects of hepatitis B were known to only 58.4% (N=119) of subjects and 79.1% (N=162) of subjects reported taking universal precautions while dealing with infected patients. More than 70% (N=143) of subjects were aware of vaccine to prevent hepatitis B but 37.6% (N=77) were unaware regarding the vaccination schedule. More than one-third of subjects (N=68, 33.5%) reported of getting exposed from an infected patient and only 61.7% (N=126) of subjects were fully vaccinated for HBV. Half of the subjects (N=105), were getting

Table 2. Association of qualification with awareness regarding cause, long term effects and vaccination schedule for hepatitis B

Qualification	Cause of hepatitis B		Long term effects of hepatitis B		Vaccination schedule for hepatitis B	
	Yes	No	Yes	No	Yes	No
GNM	74 (90.2%)	8 (9.8%)	66 (80.4%)	16 (19.6%)	72 (87.8%)	8 (12.2%)
BSc	24 (61.5%)	15 (38.5%)	23 (60%)	16 (40%)	27 (69.2%)	11 (30.8%)
MSc	20 (83.3%)	4 (26.7%)	16 (66.7%)	8 (33.3%)	19 (79.1%)	5 (21.9%)
Student	16 (26.6%)	44 (73.4%)	14 (23.4%)	46 (74.6%)	10 (16.6%)	50 (83.4%)
p value[1]	0.032*		0.014*		0.074	

[1] *Chi*-square test; *statistically significant (p<0.05)

Table 3. Multiple logistic regression analysis on getting regularly tested for hepatitis B antigen

Variable	Odds Ratio (OR)	95% CI	p value
Age			
18-35	1.78	0.82-3.89	0.071
36 and above	1	Ref	
Gender			
Male	1	Ref	0.022*
Female	3.26	0.72-4.31	
Qualification			
GNM	3.45	0.18-4.07	0.001*
BSc	2.67	0.34-7.53	
MSc	3.14	0.19-6.75	
Student	1	Ref	
Experience			
<1	1	Ref	0.027*
1-2	1.34	4.67-8.34	
3-5	2.56	4.98-7.45	
More than 6	4.23	2.56-5.34	

*statistically significant (p<0.05)

regularly tested for hepatitis B antigen. Senior staff and text books were cited as the two main sources of information regarding HBV by 28.6% (N=58) and 22.5% (N=46) of study subjects as compared to other sources. (Figure 2).

A vast percentage of subjects who were GNMs had awareness regarding cause of hepatitis B (90.2%), long term effects (80.2%) and vaccination schedule (87.8%) as compared to subjects who were qualified as BSc, MSc and students of nursing. Also, there was statistically significant association of qualification of subjects with the awareness regarding cause (p=0.032) and long term effects of hepatitis B infection (p=0.014) (Table 2).

Multiple logistic regression analysis

Multiple regression analysis was performed to assess the effect of various independent variables on getting regularly tested for hepatitis B antigen. Odds ratios were also generated (Table 3). The odds of getting regularly tested were 3.26 times greater in females as compared to males and 3.45 times greater in subjects who were GNMs as compared to others. Moreover, subjects having experience of more than 6 years were showed more willingness towards getting regularly tested as compared to less experienced subjects. (OR: 4.23).

DISCUSSION

The hepatitis B virus is world's most common and highly contagious disease which has caused significantly mortality. In health care setting, hepatitis B infection may occur due to the lapse in the sterilization technique of instruments or due to improper disposal of hospital waste [11]. Hepatitis B is not only the most transmissible infection, but also the only one that can be prevented by vaccination [12]. The present pilot study was conducted among staff nurses and nursing students to assess their awareness and attitude regarding hepatitis B infection as they fall into high risk group. A close ended questionnaire was used to record the responses of the subjects. Such questions are easy to analyse and may achieve a quicker response from subjects.

More than 90% of the subjects were aware regarding hepatitis B infection in the present study. Similar findings were observed in a recent study conducted among primary health care nurses in Saudi Arabai [13]. However, another study conducted at an apex health-care institute in Central India among medical and nursing students found contrasting results [14]. This could be because of lack of educational and training programmes on hepatitis conducted in the work place. Findings of another study conducted in Ghana revealed that more than 90% of staff nurses knew the cause of hepatitis B [15]. This is contrary to the findings of our study.

'Universal precautions' are designed to prevent infection from inoculation; contact with mucous membranes such as mouth or eye, or through skin damages such as cuts [16]. Approximately 80% of subjects in the present study followed universal precautions while dealing with hepatitis B patients. Results of another study conducted among nurses and midwives in Sudan are in congruence with our study [17].

Awareness regarding hepatitis B vaccine was reported from more than 70% of subjects in the present study. This is in contrast to the findings of some other study conducted among nurses in Nigeria [18]. Moreover, only 61.7% of the subjects in the study were reported to be immunized with hepatitis B vaccine in spite of the fact that more than one-third of subjects reported getting exposed from an infected patient. This finding is consistent with similar studies conducted in different low and middle income countries (LMICs) that reported hepatitis B vaccine coverage ranging from 56.9% to 69.1% [19-21]. However, a study conducted among Polish HCWs reported vaccine coverage of 90% [22].

The findings of the study posited that the qualification of subjects was significantly associated with awareness regarding cause and long term effects of hepatitis B. Similar findings were also observed in a hospital-based study conducted in Nigeria among HCWs including nurses [23]. Senior staff and text books were cited as the two main sources of information regarding HBV in our study. However, reports of another study conducted among midwives and nurses in Turkey revealed continuing education programs and brochure reading as their main sources of information apart from formal education [24]. A vast majority of subjects in our study showed need for special lectures to update their knowledge regarding hepatitis B, which is an encouraging finding.

Multiple logistic regression analysis revealed that odds of getting regularly tested for hepatitis B antigen were 3.26 times higher in female subjects as compared to their male counterparts which is analogous to a previous study conducted in Iran [25]. Furthermore, subjects who were GNMs and those who had experience of more than 6 years demonstrated a higher likelihood of getting themselves regularly tested for hepatitis B antigen as compared to others. Prolonged education, training and experience provide individuals with the necessary skills and knowledge to effectively implement preventative measures and strategies, like the good practice of getting regularly tested for hepatitis B antigen [26].

The present study had some limitations. Though the study was conducted in an accredited teaching

institution in north India, the results of the present study may not be generalizable to the entire nursing staff employed in other hospitals because of limited sample size and difference in study settings. As the study relied upon self-reported data and researchers mainly relied upon information provided by the subjects, some subjects could have given socially acceptable responses. Moreover, due to the cross-sectional design of the study, we cannot determine a cause-effect relationship.

CONCLUSION

Though a vast majority of subjects had awareness regarding hepatitis B infection, low awareness levels were witnessed regarding some important aspects like cause, long term effects of hepatitis B infection, vaccination schedule etc. Suboptimal attitude was also found in significant number of subjects regarding immunization and getting regularly tested for hepatitis B antigen.

Recommendations

There is an urgent need of educational and training programs (continuing education programs and workshops) for nursing staff and students focusing more on prevention of hepatitis B in order to protect themselves and others. There should also be mandatory regular screening of all health care staff for HBV and provision of free or subsidized vaccination services. These should be included in the institutional or hospital policy. It is recommended that future research involving a larger sample (including more hospitals and other medical professionals) should be conducted to gather more valuable information on hepatitis B infection.

Conflict of interest

The authors disclose no conflicts of interest.

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