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The Editorial Board kindly informs that since 2014 *Nowiny Lekarskie* has been renamed to *Journal of Medical Science*.

The renaming was caused by using English as the language of publications and by a wide range of other organisational changes. They were necessary to follow dynamic transformations on the publishing market. The Editors also wanted to improve the factual and publishing standard of the journal. We wish to assure our readers that we will continue the good tradition of *Nowiny Lekarskie*.

You are welcome to publish your basic, medical and pharmaceutical science articles in *Journal of Medical Science*.

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The Journal of Medical Science applies the ethical principles and procedures recommended by COPE (Committee on Conduct Ethics), contained in the Code of Conduct and Best Practice Guidelines for Journal Editors, Peer Reviewers and Authors available on the COPE website: https://publicationethics.org/resources/guidelines

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The occurrence of abnormal body weight values and selected eating habits and physical activity of nurses

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ABSTRACT

Aim. The aim of the research was to analyze the incidence of abnormal body weight values and to assess eating habits and physical activity of nurses.
Material and Methods. The studies involved all first-year nursing students of the second degree daily studies, working as a nurse. The women's eating habits were analyzed based the diet. The questionnaire allowed the researchers to determine daily consumption of each particular dietary component (proteins, carbohydrates, fats, fatty acids, vitamins) as well as the women's calorie consumption.
Results. The analysis showed that the group of underweight participants was comprised of 5 students (group I), the group of participants with normal body weight — of 43 students (group II), and the group of participants with excessive body weight — of 10 students (group III). Group II students spent the longest time on (moderate and intense) physical activity (on average 378 minutes/week), whereas group III students — the shortest (on average 203 minutes/week). While analyzing the average protein intake, it was found that all groups exceeded its daily requirement. The intake of sodium and cholesterol was exceeded more than twice of the recommended amount. A very low intake of vitamin D — covering from 40 to 48% of the daily requirement — was observed in all groups.
Conclusions. Despite the fact that the nurses' diet includes all nutrients necessary for the body, it is not properly balanced which obliges to raising awareness of types of consumed food.

Keywords: BMI; lifestyle; nutrition; public health; vitamin.

Introduction

Contemporary lifestyle is the subject of numerous studies and discussions. Rush, excessive responsibilities, stress as well as lack of sleep or physical activity are factors affecting health [1, 2]. Other negative factors also include poor eating habits, such as: low consumption of vegetables and fruit, eating highly processed products, high consumption of fast food products, excessive coffee drinking, complementing nutrient deficiencies by means of dietary supplements. Such a lifestyle leads to occurrence of abnormal, especially excessive, body weight values, cardiovascular diseases including hypertension, lipid disorders, diabetes, and even cancer [3]. An important element of prevention of the irregularities is health education which should start as early as in childhood and result in positive behaviors later in life [4]. However, numerous studies indicate that the above statements are far from real practice. The level of students' knowledge about healthy

lifestyle is very low. This applies both to students of technical departments, the humanities, and often also to students of medical departments, including nursing [3, 5, 6]. Nurses are expected to take positive actions contributing to health, such as risk assessment of unhealthy lifestyle, health education and disease prevention [7, 8]. However, the number of responsibilities including work and professional training as well as irregular sleep prevent nurses from leading a healthy lifestyle themselves and make them set a bad example to others and risk their own health and life.

Aim

The aim of the research was to analyze the incidence of abnormal body weight values and to assess eating habits and physical activity of nurses who work and enhance their professional skills at second degree studies.

Material and Methods

The studies involved all first-year nursing students of the second degree daily studies, working as a nurse, in the academic year 2016/2017. The students of second degree studies are nurses with *Bachelor's degree in nursing* who improve their qualifications pursuing Master's degree studies. Altogether, 58 women aged 22–41 were involved in the study.

The study exclusion criteria included not being employed as a nurse and taking dietary supplements with vitamins and minerals in the period of 6 months preceding the study.

The women's eating habits and dietary composition were analyzed based the diet. Portion sizes were verified using "The Album of Photographs of Food Products and Dishes" [9]. The women filled in the questionnaire on the basis of the album, specifying the quantity and quality of the food consumed. The questionnaire allowed the researchers to determine daily consumption of each particular dietary component (proteins, carbohydrates, fats, fatty acids, vitamins) as well as the women's calorie consumption. The questionnaire included a list of products grouped according to the following food groups: milk and dairy products, eggs, meat, sausages, offal, fish, seafood, animal and vegetable fats, vegetables, fruit and fruit-products, potatoes and potato-based products, seeds, legumes, cereals and cereal-products, pre-cooked ready-meals, salty snacks, nuts and grains, sugar and sweets, soft drinks, alcohol, soup concentrates, sauces and spices. The study participants had to record how frequently they consumed each product, with the options being: daily, several times a week, once a week, 2–3 times a month or never.

The DIETA FAO program, which includes data on 1067 typical Polish dishes or food products, was used to estimate the quantity of the aforementioned components. Dietary consumption was validated via the 'Food Intake Frequency Questionnaire', a 7-day nutritional survey. The method employed consisted in writing down all the products and dishes which were consumed each day, for a period of 7 days. The DIETA FAO program allows to specify the vitamin and mineral content in particular components of a diet. Energy and nutrient requirement was determined for women weighing 65 kilograms. The obtained average intake values were compared with United States Department of Agriculture dietary guidelines for people with moderate physical activity and with *Normy żywienia dla populacji polskiej* (Nutrition standards for Polish population) [10, 11]. The research consisted in conducting measurement of body weight and height of each student and calculating the BMI value which was categorized in accordance with WHO recommendations: underweight < 18.5, norm 18.5–24.9, overweight 25.0–29.9, obesity ≥ 30.0.

The measurement of physical activity was conducted on the basis of the Polish version of the *International Physical Activity Questionnaire* — IPAQ, approved by the IPAQ Committee, which was filled in by the respondents. The duration of physical activity was presented as the weekly number of minutes (MET — minutes/week) [12].

Excel v. 2010 spreadsheet was used for statistical analysis, the Pearson correlation coefficient was calculated. The statistical significance was determined at $p < 0.05$.

Ethical Consideration

According to the Polish legal system this research did not require the approval of the Bioethics Review Board. All participants gave the informed consent for taking part in the research, the participation in this study was voluntary and the anonymity of the participants was preserved.

Results

The average age of the tested subjects was 25 years and 8 months. The conducted analysis showed that the group of underweight participants was comprised of 5 students (group I), the group of participants with normal body weight — of 43 students (group II), and the group of participants with excessive body weight — of 10 students (group III).

In group I — participants with underweight — the average BMI value was 17.6. The diet of students of this group contained on average 2005.7 kilocalories which accounted for 90% of the energy requirement standard. In group II — comprised of students with normal body weight — the caloric value of a daily food intake was 2430.7, which accounted for 110% of the norm, while the average BMI value was 21.62. In the case of group III — students with excessive body weight — the average BMI value was 27.91 and the caloric value of daily food intake was 3116.94 kilocalories, which accounted for 141% of the energy requirement standard.

Group II students spent the longest time on (moderate and intense) physical activity (on average 378 minutes/week), whereas group III students — the shortest (on average 203 minutes/week). Group I students spent the last time travelling by car. All students consumed an amount of water which only covered 64–76% of the daily requirement. While analyzing the average protein intake, it was found that all groups exceeded its daily requirement. At the same time, the

Table 1. Average values of selected nutrients intake and coverage of the daily demand in the analyzed groups of students

Tested variable/ tested group	Group I		Group II		Group III	
MET-min./week	average	coverage of norms	average	coverage of norms	average	coverage of norms
BMI	17.6	-	21.62	-	27.91	-
Energy [kcal]	2005.7	90%	2430.7	110%	3116.94	141%
Moderate activity [MET-min/week]	210	-	245	-	189	-
Intense activity [MET-min/week]	126	-	133	-	14	-
Driving time [MET-min/week]	340	-	575	-	360	-
Water [l]	1.73	64%	1.83	68%	2.05	76%
Total protein [g]	94.49	205%	97.56	212%	107.99	234%
Animal protein [g]	67.48	ND	66.98	ND	74.79	ND
Vegetable protein [g]	27.01	ND	30.48	ND	32.97	ND
Fat [g]	82.85	71%/	101.3	87%/	127.41	111%/
Carbohydrates [g]	240.29	184%	300.93	231%	413.06	317%
Sodium [g]	3.27	218%	4.04	269%	4.6	307%
Potassium [g]	3.17	68%	3.64	77%	3.83	82%
Calcium [mg]	697.01	87%	691.41	86%	929.41	116%
Phosphorus [mg]	1478.75	254%	1565.68	269%	1793.72	309%
Magnesium [mg]	303.3	114%	335.43	126%	390.64	147%
Ferrum [mg]	11.9	110%	13.6	136%	14.93	149%
Zinc [mg]	11.41	165%	13.18	193%	15.87	233%
Copper [mg]	1.04	148%	1.27	181%	1.49	212%
Vitamin A [µg]	928.09	185%	1297.9	259%	1738.24	347%
Vitamin B6 [mg]	2.07	159%	2.43	186%	3.11	239%
Vitamin B12 [mg]	3.57	178%	4.18	209%	5.77	288%
Vitamin C [mg]	46.87	78%	57.18	95%	70.56	117%
Vitamin D [µg]	4.77	48%	4.08	40%	4.36	44%
Vitamin E [mg]	9.61	80%	11.01	93%	14.86	123%
Iodine [µg]	126.43	132%	132.62	138%	143.31	150%
Folates [µg]	271.89	85%	310.98	97%	309.87	97%
Saturated fatty acids [g]	35.09	ND	40.32	ND	53.46	ND
Monounsaturated fatty acids [g]	28.91	ND	39.33	ND	46.91	ND
Polyunsaturated fatty acids [g]	12.27	ND	14.63	ND	18.91	ND
Cholesterol [mg]	575.39	246%	508.21	220%	468.48	202%
Dietary fibre [g]	21.07	84%	23.33	93%	36.73	146%

amount of animal protein intake exceeded the amount of vegetable protein intake twice. The intake of sodium and cholesterol was exceeded more than twice of the recommended amount. It was observed that in group III the recommended intake was exceeded by more than 300% in the case of sodium (307%), phosphorus (309%) and vitamin A (347%). The average intake value of fat, potassium and calcium in groups I and II was below standard. A very low intake of vitamin D — covering from 40 to 48% of the daily requirement — was observed in all groups (**Table 1**).

Statistical analysis revealed existence of both positive and negative correlations in the analyzed variables. In group I there are negative correlations between moderate and intense physical activity, and the BMI value as well as positive correlations between total protein intake and animal protein, and the BMI value. Negative correlation means that the BMI value decreases along with

Table 2. Value of the Pearson correlation coefficient showing the correlation between physical effort and the amount of protein consumed and the BMI value in the tested group (I) of students with p below 0.05

Value of the correlation coefficient in the group of students	Moderate physical activity and the BMI value	Intense physical activity and the BMI value	The overall amount of protein consumed and the BMI value	The amount of animal protein consumed and the BMI value
Group I	-0.822	-0.655	0.536	0.618

Table 3. The Pearson correlation coefficient value showing the relation between the amount of monounsaturated and saturated fatty acids intake and the BMI values in the I groups of students. p below 0.05

Value of the correlation coefficient in the group of students	The amount of saturated fatty acids consumed and the BMI value	The amount of monounsaturated fatty acids intake and the BMI value
Group I	0.763	0.905
Group II	0.594	0.830
Group III	0.682	0.904

Table 4. The Pearson correlation coefficient value showing the average consumption of selected nutrients in given groups of students. p below 0.05

Correlation coefficient value	Group I/Group II	Group II/Group III	Group I/Group III
Moderate activity [MET-min/week]	-	-	-0.59
Intense activity [MET-min/week]	0.73	-	-
Driving time [MET-min/week]	-	-	-0.65
BMI	-	0.58	-
Energy [kcal]	-	-	0.55
Animal protein [g]	0.75	-	-0.54
Vegetable protein [g]	-	-	0.76
Fat [g]	0.53	-	0.63
Carbohydrates [g]	-0.69	-0.69	-
Potassium [g]	-0.66	-	0.54
Calcium [mg]	-	-	-0.72
Magnesium [mg]	-	-	0.93
Copper [mg]	-	-	0.61
Vitamin B6 [mg]	-	-	0.68
Vitamin B12 [mg]	0.67	-	-0.65
Vitamin C [mg]	-0.55	-	-0.67
Vitamin D [µg]	0.75	-	-
Vitamin E [mg]	-0.67	-	0.51
Iodine [µg]	-0.68	-	-
Saturated fatty acids [g]		-	0.79
Polyunsaturated fatty acids [g]	-	-	0.97
Cholesterol [mg]	-	-0.72	-
Dietary fibre [g]	-0.78	-	-
Financial situation	-	-	0.97

the increase of physical activity, whereas positive correlation means that the BMI value increases along with the increase of the amount of protein intake (**Table 2**).

In all three analyzed groups there is positive correlation between the intake of saturated and monounsaturated fatty acids and the BMI value, which means that along with the increase of the above mentioned fatty acids intake the BMI value also increases (**Table 3**).

The conducted statistical analysis also examined the existence of a correlation between particular groups of students. While comparing group I with group II 11 correlations were found — among others: positive correlations of intense physical activity, average animal protein and fat intake, and negative correlations referring to average carbohydrate intake. When comparing group II and III only 3 correlations were observed. The highest number of correlations (17) were found when comparing group I and group III. They include positive correlations pertaining to average vegetable protein and fat intake, and negative correlations of moderate physical activity, the time spent on moving by car, average animal protein intake (**Table 4**).

Discussion

Polish research conducted in 2009 showed that 38.4% of women were overweight and 29.1% were obese [13]. The WOBASZ II (the abbreviation of Polish: Multicenter National Research on Health Status of the Population) research conducted in the years 2013–2014 demonstrated BMI above 30 (obesity) in 23.4% of women, and BMI above 25 (overweight) in 29.5% of women. Compared to the WOBASZ 2003–2004 research the distribution of body weight shifted to higher values in the case of obesity by 1.1% and overweight by 1.8% [14]. On the basis of the analysis of data on prevalence of excessive body weight from the period 1995–2010 it was stated that the percentage of obese women in Poland amounted to 23.3%. Thus, unless any changes are implemented, the percentage will increase to 27.3% of women in 2020, and to 31.4% in 2030 [15]. No occurrence of obesity was observed in the authors' own research.

The American National Health and Nutrition Examination Survey (NHANES) conducted in the years 1999–2008 revealed an average BMI value of 27.7 in the United States in women aged 16–49, which indicates the occurrence of overweight [16]. Research on a group of Warsaw students conducted at the turn of 2008 and 2009 revealed an average BMI value of 24.19 [17], i.e. a lower value than the one revealed by American studies. The average BMI value in the authors' own research was 22.38, which is even lower than the value in the group of Warsaw students. Research carried out in Calcutta in India in the years 2011–2012 on a group of students aged 20–22 found that 69.23% of the participants were overweight [2]. In the authors' own research overweight was observed in 17.2% of respondents, however, underweight was relatively common and concerned 8.6% of the tested women. It should also be noted that the average age of respondents was higher and amounted to 25 years and 8 months. Moreover, the tested group was selected according to particular criteria — it was comprised of nurses developing their professional skills pursuing second degree studies.

The European Health Interview Survey (EHIS) was carried out in Poland in 2014 in accordance with recommendations of the European Union. The survey revealed that 30.1% of women were overweight, 15.6% were obese and 4.2% were underweight. These results indicate that body weight of Polish women continues to increase [18]. No occurrence of obesity was observed in the authors' own research. However, the increase of the number of people with excessive body weight is considerable and pertains to most of the European countries [15].

The increase in the number of people with excessive body weight is connected with positive energy balance. It should be noted, however, that while determining the size of consumed portions — and thus the number of consumed nutrients — it is crucial to check whether the whole portion was eaten. The analysis of publications covering the period of 1995–2010 conducted by Krzysztoszka et al. demonstrated that factors which may affect the positive energy balance — and thus the excessive body weight — include stress and lack of sleep both resulting in an increased appetite for high-energy products, especially for carbohydrates [15]. The authors' own research demonstrated that the level of coverage of daily energy requirement was exceeded in the group of women with excessive body weight and the group of

women with the correct BMI value. Whereas daily carbohydrate requirement was exceeded in all analyzed groups. It confirms that an excessive carbohydrate intake results in positive energy balance.

Research carried out in Portugal on a group of students aged 18–25 demonstrated that most of the students had correct body weight values. The students also had relatively good eating habits and were physically very active [19]. Physical activity is a significant element of human life. People devoting their time to physical activity have greater work motivation and life satisfaction. Activity at an appropriate level prevents occurrence of the so called metabolic syndrome. Public health campaigns aiming at promoting and increasing physical activity are held. However, studies show that attempts at making the adult part of the society more active are not very effective. It is thus important to raise awareness of the benefits of physical activity as well as of increasing physical activity to a recommended level [1].

In 2010, while conducting research on a group of 100 students, Ślusarska et al. concluded that 23% of the tested students were overweight, 3% obese and 7% underweight. Despite all of the participants being students, the group was not homogenous because the tested subjects studied both at medical and non-medical departments. It might be assumed that medical students, as future health educators, should demonstrate a greater concern for their own health status and, at the same time, correct body weight. Although the research authors point out that the majority of respondents (84–94%) were aware of the factors causing obesity, including lack of physical activity, regardless of the department they studied at, the students were totally unaware of the role of regulation of the caloric value of meals in obesity prevention [6].

The authors' own research conducted among nurses pursuing second degree studies revealed that participants with correct BMI value devoted the largest proportion of time to physical activity and, at the same time, spent the largest proportion of time on moving by car. Even though the tested group included 100% of students of a given year, the group was relatively small. Subjective determination of time devoted to physical activity also seems to be problematic.

A study conducted in Bydgoszcz in the group of 230 participants demonstrated that the lowest level of activity was characteristic for students of pedagogy and computer science. The more often physical activity was taken up, the more attention was paid to proper nutrition. At the same time, the authors of the research concluded that the level of physical activity decreased along with age [20]. Research carried out among students of Gdańsk University of Physical Education demonstrated that students had lower BMI values in 2000 than in 2010. Motivation to take up physical activity was different — in 2000 it was the concern for health and in 2010 — the need for self-realization. The reason for lack of physical activity remained the same — lack of money and time, despite the fact that, for example, running does not require any significant expenditure of funds [21]. Research on physical activity of nursing students of the second degree studies conducted in Biała Podlaska revealed that 73.2% of students were very active physically and 26.8% — moderately active physically. However, 76.4% of the respondents pointed to an insufficient amount of time and only 12.2% of them declared to have enough free time to engage in physical activity [22].

In the authors' own research the analyzed group included studying and working people, i.e. people who do not have a large amount of free time. In spite of this, the tested subjects took up physical activity, which may be connected with the awareness of the importance of the activity for health, not only with the desire to lose weight. In fact, a crucial reason for avoiding physical activity is the inability to quickly lose weight which results in the loss of motivation [13].

According to the studies conducted by Biernat and Tomaszewski no instances of obesity occurred among Warsaw students, overweight was observed in 10.3% and underweight, much more frequently, in 20.3% of the tested subjects. Average time devoted to moderate physical activity in the group of students with underweight was 69.2 minutes/week, with the correct BMI value — 68.1 minutes/week and in the group with overweight — 61.7 minutes/week, which in each case is a very small amount of time [23]. In the authors' own research 210 MET (minutes/week) was observed in the case of moderate activity in people with underweight, 245 MET in people with correct body weight and 189 MET in people with

overweight. It can thus be concluded that the Warsaw students spend significantly less time on physical activity.

Correct eating habits, i.e. proper nutrition and hydration of the organism constitute an important element of a healthy lifestyle. The studies show that replacing sweetened drinks and juices with water results in the reduction of daily caloric value of meals by 10–13% [24]. According to the authors' own research too little amount of water is drunk per day. The basis of the diet is protein — building material of muscles and immunoglobulins. Deficiencies in protein, concerning primarily people with underweight, may cause decrease in respiratory muscles strength leading to ventilation disorders and reduction of the immune response of the organism [25]. According to the authors' own research, protein intake in all tested groups exceeded daily requirement by more than 100%. In the case of the average daily fat intake, daily requirement was exceeded only in the group of people with overweight. Standards of polyunsaturated fatty acids intake are not defined in literature but a deficiency of the acids in the diet may result in fat tissue atrophy and humoral response disorders [25]. On the basis of the authors' own research it was found that the average daily polyunsaturated fatty acids intake was between 12.27 grams in the group of women with underweight and 18.91 grams in the group of women with overweight.

Important elements of nutrition are also dietary supplements which are to complement deficiencies in vitamins and minerals. Poznań studies conducted in 2011, which were aimed at assessment of vitamin and minerals intake, including dietary supplements, demonstrated improvement only in vitamin consumption [26]. In the authors' own research the analyzed group did not apply dietary supplements, therefore the supplements portions were not defined. Due to universal access to vitamin and mineral supplements the risk of overdosing or even poisoning increases [27, 28].

But in people who want to complement deficiencies supplements may cause even greater deficits as the analysis of selected preparations showed that none of them included the declared quantity of active substances [29].

The amount of consumed vegetables and fruit influences the vitamins and minerals intake. Adults consume vegetables and fruit relatively seldom. Only 2/3 of women eat them every day [18]. According to the authors' own research, all women ate fruit or vegetables at least once a day.

The study conducted in Wrocław, aiming at demonstrating differences in vitamins and minerals intake among students depending on a place of residence (family house, rented apartment, campus) did not show significant differences. All students demonstrated low intake of vitamins B1, B2, B6 and niacin (50–75% of daily requirements) and low intake of calcium and ferrum (51% and 63% of daily requirements respectively). Too high intake of sodium (250%) and phosphorus (118%) was found [22].

Exceeding of daily intake standards was observed in the case of vitamins B6 and B12, whereas vitamin B1 intake was not determined. In the case of calcium intake, its deficiency was noted in the diet of students with low or proper body weight. Intake of sodium and phosphorus was very high, it exceeded daily requirements by more than 100%, and in the group of students with overweight — even by 200%.

The results of the Poznań research showed exceeding of the daily standard of vitamin A intake by 76% and of vitamin E by 26%. Similarly, in the case of vitamins of the B group: intake of thiamine was exceeded by 8%, of niacin by 12% and of riboflavin by as much as 46%. The intake of vitamin B12 and folic acid was very low — the former covered 32% of the daily requirement and the latter — only 17%. Vitamin D was consumed in the amount covering 29% of the daily requirement. Vitamin C intake covered 153% of the daily requirement [30].

Vitamin D intake was also very low in the authors' own research — it covered only 40–48% of the daily requirement, whereas the amount of consumed vitamin C covered the daily requirement only in the group of students with overweight. It is worth remembering that both vitamin D and vitamin C are crucial dietary elements. Vitamin D demonstrates pleiotropic effect, reduces the risk of lung cancer and reduces the risk of developing colorectal cancer. High doses of vitamin D and low doses of retinol reduce the risk of colorectal cancer. Vitamin C is also important for the body as it, among others, affects wound healing process, causes tumor apoptosis and is a cofactor of numerous reactions occurring in the body [28].

An important element of female nutrition, especially in the reproductive period, is the intake of folates. It was demonstrated that a low level of folates is associated with an increased risk of neural tube defects in the offspring [30]. In the authors' own research no tested group reached the recommended value in the diet. Both deficiency and excess of vitamins and minerals in the diet have adverse effects on health. Vitamin A - which is essential in the human body owing to its influence on the proper condition of skin and hair, as well as diminishing the risk of lung and digestive tract cancer — if consumed in excess can cause poisoning whose symptoms can be nausea, vomiting or liver disorders [28]. In the research on Poznań students a recommended daily intake of vitamin A was not shown [30]. According to the authors' own research the vitamin A intake in the group of students with overweight exceeded the norm by 200%. In the other groups the average daily intake was also at a high level (185% and 259% of the daily requirement). Results of the authors' own research in the scope of cholesterol intake are also alarming — they exceed from 102% to 146% (depending on the tested group) of the daily intake norm, which significantly increases the risk of developing atherosclerosis. While analyzing the intake of selected nutrients, Przysławski et al. considered losses resulting from thermal treatment — they assumed 10% loss of vitamin D, 15% of vitamins of the B group, 20% of vitamins A and E, 55% of vitamin C and 65% of folic acid [30]. Such losses were not included in the authors' own research. However, taking the losses into consideration in the case of vitamins with exceeded norm of daily intake would nonetheless not result in the intake below 100% of the daily requirements. In the case of vitamins with daily intake below the norm, the intake level would have been significantly decreased. The Poznań research carried out at the turn of 2009 and 2010 demonstrated that 41–47% of students were aware of their lack of knowledge on proper nutrition, which indicates the necessity to introduce educational activities directed to students of both non-medical and medical departments [13, 31, 32].

Conclusions

1. The body weight of studying nurses is in most cases appropriate, whereas in the group with overweight both reduction of caloric intake and increase of physical activity are recommended.
2. Despite the fact that the nurses' diet includes all nutrients necessary for the body, it is not properly balanced which obliges to raising awareness of types of consumed food.
3. Medical studies education curricula pertaining proper qualitative and quantitative nutrition should definitely be broadened.

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The authors declare no conflict of interest.

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Minerals in the diet of adolescents aged 15

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ABSTRACT

Introduction. Studies which focus on the supply of minerals in the diet of adolescents show that the diet is deficient in such macroelements as calcium and magnesium and such microelements as copper, zinc, iodine and iron. Inadequate supply of minerals may increase the risk of development of diet related diseases at a mature age.

Material and Methods. The questionnaire on the consumption of food products consumed every day for the period of 7 days was filled in by the person conducting the survey. The needs for minerals were defined individually for every child with reference to recommended dietary allowance.

Results. The norm of daily sodium intake among 15-year-old adolescents was exceeded in all tested groups, however, in the group of boys sodium intake was at a higher level than in the group of girls. Potassium intake in the group of both girls and boys did not reach the recommended daily value, whereas the recommended daily consumption of manganese was exceeded twice. In all tested subjects, the ratio of calcium to phosphorus was very low. In the group of girls who are underweight, daily consumption of iron did not cover 50% of the demand.

Conclusions. Inadequate supply of minerals recorded in all tested 15-year old subjects, regardless of their BMI, may lead to developmental disorders and diet related diseases at a mature age.

Keywords: children, food, nutrition, malnutrition, minerals.

Introduction

Minerals, in addition to basic nutrients, play an important role in the diet of children, adolescents and adults. Adequate supply of nutrients conditions proper development of the human organism [1]. Research on nutrition of adolescents shows that their diet is characterised by many errors, especially related to the nutritional and energetic value of meals and to the content of minerals in the diet [2]. Proper nutrition in the period of adolescence is particularly important as it conditions the predisposition to learn at school at the same time having impact on the occurrence of diseases at a later age. The main errors in nutrition of adolescents include unbalanced nutrient consumption, too high supply of fats and sugars and too low consumption of complex carbohydrates, fibre, calcium, magnesium, iron and certain vitamins [3]. Another important problem concerning the diet of adolescents aged 13–15 is too high consumption of sodium which leads to cardiovascular diseases in adolescence and at a later age may be the cause of strokes [4]. Excessive consumption of manganese also has adverse effects on human organism as it may lead to the development of dementia, schizophrenia or exacerbate

symptoms of Parkinson's disease, its deficiency may destabilize DNA and decrease the synthesis of proteins [5].

Studies which focus on the supply of minerals in the diet of adolescents show that the diet is deficient in such macroelements as calcium and magnesium and such microelements as copper, zinc, iodine and iron [3]. Iodine deficiency in diet may lead to the occurrence of mental retardation and inhibit growth of the organism. Deficiency in some minerals may be associated with the occurrence of excessive body weight [5]. Inadequate supply of minerals may increase the risk of development of diet related diseases at a mature age [6].

Aim

The aim of the study was to assess daily intake of selected minerals depending on BMI value in the diet of selected adolescents aged 15.

Research subjects and Methods

The study was conducted in the Spring of 2017 in a representative middle school in the city of Bielsko-Biała. All third grade students were included in the study.

Participation in the study was voluntary. In total 133 students aged 15 (57 girls and 76 boys) were included in the study. Measurement of body mass and height of all of the students was taken and the BM value, classified in accordance with WHO and US Department of Health and Human Services and U.S. Department of Agriculture was calculated. BMI below 5 percentile — underweight, BMI between 5 and 85 percentile — appropriate body mass, overweight — BMI between 85–95 percentile and obesity — BMI value above 95 percentile [7, 8].

The questionnaire on the consumption of food products consumed every day for the period of 7 days was filled in by the person conducting the survey. In order to determine the amount of minerals consumed, the size of the portion was verified with the use of „The Album of Photographs of Food Products and Dishes" [9] and the DIETA FAO programme containing information about 1067 typical food products. A detailed description of the study was included in earlier publications. The needs for minerals were defined individually for every child with reference to recommended dietary allowance. Obtained mean intake values were compared with nutrition standards for people with moderate physical activity defined by the United States Department of Agriculture as well as with *Normy żywienia dla populacji polskiej (Nutrition Standards for Polish Population)* [8, 10].

The analysis was performed with the following software: PQStat 1.6.4; PSPP 0.10.4; MS Office 2013 (RS for Excel), "R". The analyzed variables were measured on nominal and quantity scales. Materiality level p in all analyzes was < 0.05. Normality of the variable distribution was verified using Shapiro-Wilk's test. If variables were other than normal, the nonparametric Kruskal-Wallis test was used. For qualitative variables the $\chi 2$ test was applied and – in the case of failure to meet its objectives – the exact Fisher's test.

Ethical Consideration

The study was approved by the Ethics Committee of the University of Bielsko-Biala (No: RNN/10/2017) which is in accordance with the Declaration of Helsinki. All participants gave their informed consent to taking part in the research, participation in this study was voluntary and anonymity of participants was preserved.

Adolescents participating in the study were divided into groups according to gender and BMI values. Among boys the groups were as follows: BMI below 5th percentile, BMI between 5th and 85th percentile and BMI between 85th and 95th percentile. Among girls the groups were as follows: BMI below 5th percentile, BMI between 5th and 85th percentile and BMI above 95th percentile (**Table 1**).

Results

In the tested group of 15-year-olds 68.4% (n = 39) of girls and 65.8% (n = 50) of boys had correct BMI value. In 8.8% (n = 5) of girls cases of obesity were recorded and in 15.8% (n = 12) of boys - cases of overweight. Cases of underweight were more frequently observed in the analyzed group of young people. Underweight occurred in a similar number of girls (n = 13) and boys (n = 14) (**Table 1**).

The analysis of the intake of selected minerals demonstrated that in all groups of girls and boys, regardless the BMI value, there were cases of both shortage and excessive intake of minerals (**Tables 2** and **3**).

Table 1. Presentation of the studied groups of boys and girls

Percentile BMI	Below 5		Percentile 5–85		Percentile 85–95		Above 95		Total	
	n	%	n	%	n	%	n	%	n	%
Boys	14	18.4	50	65.8	12	15.8	–	–	76	100
Girls	13	22.8	39	68.4	–	–	5	8.8	57	100
Total	27	20.3	89	66.9	12	9	5	3.8	133	100

In all groups of boys excessive intake of zinc (12.28–16.4 mg) and manganese (4.68–5.55 mg) as well as insufficient intake of magnesium (297–370 mg) and potassium (2054–3436 mg) was recorded. In the case of sodium the recommended daily intake was exceeded more than three times and amounted to 4475–5303 mg.

In all groups of boys daily intake of phosphorus exceeded daily demand and amounted to 1334–1654 mg, while daily intake of calcium amounted to 535–828 mg and covered only 41–64% of the daily demand (**Table 2**). An important element in nutrition is maintaining a proper ratio of calcium to phosphorus. The ratio of daily intake of calcium to phosphorus in the group of boys with underweight was 0.5:1, in the group of boys with normal body weight it was 0.4:1 and in the group of boys with overweight the ratio was 0.37:1. Statistically significant differences were observed in the intake of minerals between the group of boys with various BMI values. In the group of boys with underweight the intake of potassium, calcium, magnesium and manganese did not cover the recommended daily intake, yet, in spite of this, it was the highest compared to the group of boys with normal weight and overweight – the differences were statistically significant. In all groups of boys the recommended daily intake of manganese was more than double and the difference in the intake between the group of boys with underweight and the group of boys with overweight was statistically significant. In the analyzed groups of boys the intake of iron covered daily demand, in the case of iodine intake daily demand was covered in the diet of boys with normal body weight (**Table 2**).

In all groups of girls an excessive intake of zinc (9.55–11.14 mg) and manganese (3.52–3.85) and insufficient intake of potassium (2513–2621 mg) and magnesium (239.23–258.06 mg) were reported. The intake of sodium (2309–3655 mg) in the group of girls exceeded daily demand, however, it was not as high as in the group of boys (**Tables 2** and **3**).

Table 2. Illustration of the median and IQR of daily consumption of selected minerals in the tested groups of boys divided according to the BMI value

Studied variable and unit	Group A BMI below 5 percentile Median (IQR)	Group B BMI 5–85 percentile Median (IQR)	Group C BMI 85–95 percentile Median (IQR)	Statistical significance	Recommended dietary allowance RDA
Sodium (mg)	4810.60 (3230.23–6390.98)	4475.19 (4119.64–5852.99)	5303.81 (4208.61–5303.81)	No	1500 mg
Potassium (mg)	3436.92 (3138.54–3735.31)	3344.34 (2616.90–4013.62)	2054.57 (2054.57–2632.53)	Yes A and C; B and C	4700 mg
Calcium (mg)	828.89 (696.40–961.38)	571.85 (496.66–1000.80)	535.91 (295.33–535.91)	Yes A and C; B and C	1300 mg
Phosphorus (mg)	1654.42 (1493.79 - 1815.05)	1334.76 (1244.87–1628.39)	1437.65 (1098.53–1437.65)	Yes A and B; A and C	1250 mg
Magnesium (mg)	370.96 (318.64–423.27)	326.57 (271.06–359.97)	297.67 (272.268–297.67)	Yes A and C; B and C	410 mg
Iron (mg)	12.51 (11.34–13.68)	13.75 (13.67–16.44)	13.59 (11.60–13.59)	No	12 mg
Zinc (mg)	14.907 (13.22–16.59)	12.28 (11.35–14.06)	16.40 (12.61–16.4)	Yes A and B; B and C	11 mg
Copper (mg)	1.28 (1.12–1.44)	1.23 (1.20–1.83)	1.04 (1.04–1.05)	Yes A and C	0.9 mg
Manganese (mg)	5.55 (4.13–6.98)	5.09 (3.51–6.45)	4.68 (3.80–4.68)	Yes A and C	2.2 mg
Iodine (µg)	138.94 (111.19–166.68)	154.29 (126.75–212.74)	147.56 (122.83–147.56)	No	150 µg

mg – milligram, µg – microgram

Table 3. Illustration of the median and IQR of daily consumption of selected minerals in the tested groups of girls divided according to the BMI value

Studied variable and unit	Group D BMI below 5 percentile Median (IQR)	Group E BMI 5–85 percentile Median (IQR)	Group F BMI above 95 percentile Median (IQR)	Statistical significance	Recommended dietary allowance RDA
Sodium (mg)	2309.58 (2309.58–3135.59)	3655.49 (3135.59–4801.52)	3655.49 (3065.79–4209.75)	Yes D and E; D and F	1500 mg
Potassium (mg)	2621.74 (2513.62–2621.74)	2550.24 (2513.62–2586.86)	2513.62 (2365.10–2857.76)	No	4700 mg
Calcium (mg)	656.68 (623.34–656.68)	499.19 (499.19–623.34)	574.34 (496.38–627.02)	Yes D and E	1300 mg
Phosphorus (mg)	993.06 (993.06–1106.25)	1121.33 (1106.25–1376.99)	1106.25 (1043.12–1294.75)	Yes D and E; D and F	1250 mg
Magnesium (mg)	258.06 (239.23–258.06)	254.72 (239.23–311.45)	239.23 (214.93–279.89)	No	360 mg
Iron (mg)	7.16 (5.89–7.16)	22.00 (5.89–26.71)	14.05 (6.730–22.00)	Yes D and E; D and F	15 mg
Zinc (mg)	9.55 (5.11–9.55)	10.690 (5.11–10.69)	11.14 (5.85–11.36)	Yes D and F	9 mg
Copper (mg)	1.15 (0.80–1.15)	0.89 (0.80–1.01)	0.88 (0.82–0.96)	Yes D and E; D and F	0.9 mg
Manganese (mg)	3.85 (1.96–3.85)	3.52 (1.96–3.52)	3.66 (2.32–4.22)	Yes D and E	1.6 mg
Iodine (µg)	89.27 (89.27–218.47)	104.33 (104.339–218.47)	168.82 (50.67–216.35)	No	150 µg

mg – milligram, µg – microgram

In the group of girls with underweight and girls with normal body weight insufficient intake of iodine (89.27–104.33 µg) was observed. Daily intake of phosphorus (993–1121 mg) as well as of calcium (499–656 mg) did not cover the daily demand. However, in the case of calcium intake the demand was not covered even in 50% (**Table 3**). Among girls the most beneficial ratio of calcium to phosphorus (0.66:1) in the diet was observed in the group of girls with underweight. In the group of girls with normal body weight the ratio of calcium to phosphorus was 0.44:1, whereas in the group of girls with obesity 0.52:1.

In a group of girls with underweight very low iron intake (7.16 mg) was observed, it did not cover even 50% of the recommended daily intake. Statistically significant differences in the intake of minerals between the particular groups of girls were demonstrated. They included unfavourable differences concerning phosphorus and iron intake in the group of girls with underweight. In other cases of statistically significant differences minerals intake in the group of girls with underweight was the highest compared to the other groups of girls (**Table 3**). Manganese intake in the group of girls exceeded the recommended daily intake, but it was not at such a high level as in the group of boys (**Tables 2** and **3**).

Discussion

A diet which is properly balanced in terms of nutrition and energy, with particular emphasis on minerals, is an element ensuring proper physical and mental development of children and adolescents [2]. Both excess and shortage of minerals can adversely affect health.

Studies by Wielgos et al. assessing the coverage of minerals demand in Polish adolescents in the region of Lesser Poland demonstrated that sodium intake norm was exceeded 6 times both in the group of boys and girls. After being converted to salt, the obtained results amounted to 6.8 g for girls and 7.9 g for boys. The sodium came mostly from sodium chloride contained in bread, salty snacks and meat preparations [6]. American research on a group of adolescents aged 2–18 demonstrated that daily intake of sodium increased with age, and in the group of 12- to 18-year-old adolescents it amounted to 3545 ± 79 mg per day [4]. The norm of intake was exceeded more than three times. Such an amount of sodium corresponds to 9 grammes of salt.

In the authors' own research similar average daily sodium intake in all analyzed groups of boys (regardless of the BMI value) was observed. In the group of girls sodium intake was not at such

a high level as in the group of boys, however, it also exceeded the recommended daily intake. The lowest daily intake of sodium was reported in the group of girls with underweight (2309 mg), and this difference, compared to a group of girls with normal weight and with obesity, was statistically significant. Higher intake of sodium connected with higher weight values was reported by Haidong et al. leading research on a group of adolescents aged 14–18 in the State of Georgia [11]. According to Grimes et al., higher sodium intake causes intense thirst which contributes to increased consumption of sugar-sweetened beverages (SSBs) connected with the occurrence of overweight [4]. Consumption of sweetened beverages every day increases the risk of overweight by more than 60% compared to consumption of sweetened beverages once a week [12].

Epidemiological studies proved that high intake of sodium is associated with an increased risk of cardiovascular diseases and in particular with the occurrence of arterial hypertension. High intake of sodium combined with low intake of potassium may contribute to the occurrence of elevated values of blood pressure. Low intake of potassium initiates mechanisms leading to its retention in the organism, with simultaneous retention of sodium. Due to water retention sodium leads to an increase in the amount of circulating blood and to increased blood pressure. A diet rich in potassium is related to the occurrence of lower blood pressure [3, 13].

The analysis of the authors' own materials demonstrated that average daily consumption of potassium in the group of both girls and boys did not reach the recommended daily value of 4700 mg. Similar results were achieved by Italian researchers – estimated average daily intake of potassium among children aged 2–18 was lower than the recommended in more than 96% of boys and 98% of girls [14]. Maintenance of proper values of blood pressure is also dependent on the proper amount of consumed calcium. Calcium plays a major role in muscle contractility and vascular tension. Calcium is an important element of the skeleton structure. Adolescence is a period during which peak bone mass is achieved. Achieving high peak bone mass in this period to a large extent prevents osteopenia and osteoporosis in old age. To achieve a proper bone mass phosphorus is also necessary. An inadequate ratio of calcium to phosphorus in the diet, which in the case of children should be at least 1.2:1, results in impaired bone transformation [3, 15].

Examining the consumption of calcium in Polish girls aged 13–15, Czeczuk et al determined that despite an increased content of this element in the diet, its supply is still low [16]. Wang at al demonstrated that the percentage of insufficient consumption of calcium is still very high (> 96%) [17].

Authors own research demonstrated that average daily consumption of calcium in the group of girls (656 mg) and boys (828 mg) with underweight was statistically higher than in the other groups of adolescents, nonetheless, it did not satisfy the daily demand of 1300 mg. An improper ratio of calcium to phosphorus was also observed. The most appropriate ratio was noted in the analysed groups with underweight - which was 0.5:1 for boys, and 0.66:1 for girls. An improper ratio of calcium to phosphorus is connected with the risk of resorptive mechanisms in bones [3, 15].

The recommended daily consumption was not achieved in the studied group in the case of iron. Iron consumption by girls did not even satisfy 50% of the demand. Studies by Wang at al conducted on the group of Chinese adolescents aged 4–17 demonstrated that consumption of minerals increases with age. Consumption of iron was changing over time and in the group of boys aged 14–17 the percentage related to inadequate intake of iron significantly increased, however, an increase up to 30.8% in the percentage was observed in girls. The symptoms occurring in girls, caused by decreased consumption of iron, are additionally strengthened during menstruation. Low consumption of iron, even in the absence of anaemia, results in slower growth, lower immunity to infections, reduced cognitive abilities and in hormonal imbalance. The associated overall fatigue impairs efficiency of learning [18].

Polish studies which were carried out on high school students from the region of Mazovia divided into two age groups (16–18 years old and 19 years old) showed that sodium intake exceeded the Estimated Average Requirement (EAR), at the same time, insufficient consumption of calcium, potassium and iron was observed. Insufficient iron intake was reported in the case of the group of 19-year-old adolescents, in the younger group (16–18 years old) daily intake of iron covered the EAR, which was 8 mg [2]. In the authors' own research the recom-

mended daily intake of iron was defined according to RDA which is 12 mg for boys and 15 mg for girls. If compared to the EAR level, iron intake in the tested group would cover the recommended consumption in the authors' own research. In the case of sodium, calcium and potassium comparison of EAR and RDA values did not show such large differences as in the case of iron.

Magnesium deficiency significantly affects development and, at the same time, leads to aggravation of ADHD (Attention Deficit-Hyperactivity Disorder) symptoms in children. It is believed that insufficient intake of magnesium plays an important role in etiology of cardiovascular diseases, diabetes and thyroid diseases [19].

According to the authors' own research, consumption of magnesium in the group of boys was at a higher level than in the group of girls, however, it did not cover the daily demand in any of the tested groups. In the group of girls with underweight and normal body weight, insufficient intake of iodine was also recorded. Reduced intake of iodine might be caused by reduction of salt intake, however, this was not observed in the tested groups as the sodium intake norm in both tested groups of girls was exceeded.

Iodine is essential for proper functioning of thyroid and synthesis of hormones influencing proper growth and development of the body. Insufficient consumption of iodine results in development of thyromegaly, which is connected with the occurrence of hypothyroidism. Iodine deficiency in children may lead to inhibition of growth and mental development as well as to cretinism [20].

Research conducted in Australia revealed that iodine deficiency concerned 14.8% of adolescents aged 14–18. Despite introduced mandatory iodine fortification of salt used in bread there are differences in the consumption of iodine in the diet, which is caused, inter alia, by insufficient consumption of bread [21]. In Poland, owing to the Minister of Health Decree of 1997, an obligation to iodize salt for direct consumption was introduced. This obligation does not apply to salt used in food processing.

American studies conducted among adolescents aged 13–19, which were to determine the relation between the concentration of minerals in blood and the occurrence of obesity, demonstrated that an increase in manganese concentration in blood was related to obesity in the tested adolescents [5]. According to the authors' own research, the recommended daily intake of manganese in the group of boys, regardless of the BMI value, was exceeded more than twice. In the group of girls the recommended daily intake of manganese was also exceeded more than twice in all tested groups. It should be noted, however, that recommended daily intake of manganese in the group of boys is 2.2 mg and is higher than in girls (1.6. mg). Consumption of manganese was statistically much higher in the group of adolescents with BMI which was an indication of overweight, than it was in the other groups. The current state of knowledge does not allow to demonstrate a link between the consumption of manganese, an increase in its concentration in blood and the occurrence of overweight. Nonetheless, manganese is known to play an important role in the metabolism of carbohydrates, proteins and lipids [5].

There exists a correlation between the consequences associated with deficiency and excess of minerals and the occurrence of metabolic disorders. The risk related to deficiency in minerals increases with consumption of highly processed food. In addition, the risk is even higher due to insufficient knowledge about the right choice of nutrients and balancing diet.

Summary

The norm of daily sodium intake among 15-year-old adolescents was exceeded in all tested groups, however, in the group of boys sodium intake was at a higher level than in the group of girls. Potassium intake in the group of both girls and boys did not reach the recommended daily value, whereas the recommended daily consumption of manganese was exceeded twice. In all tested subjects, regardless the BMI value, the ratio of calcium to phosphorus was very low. In the group of girls who are underweight, daily consumption of iron did not cover 50% of the demand, whereas in the group of girls with underweight and appropriate body weight a relatively low consumption of iodine was recorded.

Conclusions

1. Inadequate supply of minerals recorded in all tested 15-year old subjects, regardless of their

BMI, may lead to developmental disorders and diet related diseases.
2. Existing nutrition errors in the diet of adolescents point to the need to introduce educational programmes about rational nutrition addressed both at adolescents as well as their caregivers.

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ORIGINAL PAPER

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Comparison of antibiotic resistance and virulence in vancomycin-susceptible and vancomycin-resistant *Enterococcus faecium* strains

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ABSTRACT

Aim. Today, infections caused by vancomycin-resistant *Enterococcus faecium* (VRE) are a major problem in the healthcare system. The aim of this study was to compare the antibiotic resistance and virulence traits between vancomycin-susceptible *E. faecium* (VSE) and VRE clinical isolates.

Material and Methods. Studies were performed on 66 *E. faecium* (32 VRE and 34 VSE) strains. Susceptibility testing and identification were performed, and strains were examined for ß-lactamase, hemolysin and biofilm production. Isolates were tested for the presence of 5 *van* genes, 8 virulence genes and 6 aminoglycoside-modifying enzyme (AME) genes. Obtained amplicons were subjected to electrophoretical separation and DNA sequencing.

Results. Among 32 VRE isolates, 28 were found to have the VanA phenotype, and 4 the VanB. The most frequent resistance and virulence profile among VRE strains was resistance to ampicillin, imipenem, gentamicin, streptomycin, teicoplanin, and vancomycin with enterococcal surface protein (*esp*), endocarditis antigen (*efaA*), collagen adhesin (*acm*), and hialuronidase (*hyl*) genes; among VSE: resistance to ampicillin, imipenem, gentamicin, streptomycin with *esp*, *efaA*, *acm*, and *hyl* genes.

Conclusions. Our findings prove that both VRE and VSE strains were well equipped with virulence and resistance genes, although VRE strains were characterized by a greater variety and a higher number of these genes. However, statistical analysis revealed no significant differences between VSE and VRE strains ($p > 0.05$). Nevertheless, our results suggest that VRE strains may slowly acquire and incorporate resistance and virulence genes, due to their ability to survive in a hospital environment for a long time.

Keywords: *Enterococcus faecium*, VRE, VSE, resistance, virulence.

Introduction

Enterococcus are nowadays the fourth most common etiological factor in nosocomial infections in Europe [1]. Although these bacteria are natural inhabitants of the normal flora of the gastrointestinal and genitourinary tracts, they can lead to serious infections such as bacteriemia, endocarditis, infections of the urinary tract, and wounds [1, 2]. For a long time, the majority of

infections were caused by *Enterococcus faecalis*. In the last few years, *Enterococcus faecium* have evolved as a common nosocomial pathogen and partially replaced *E. faecalis* as a cause of hospital-associated infections [3, 4]. This change is related to the fact that *E. faecium* has a number of mechanisms of intrinsic resistance and is also able to acquire resistance by mutations or incorporation of genes located on plasmids, transpozons, or integrons [3]. The largest threats are strains resistant to glycopeptides (VRE - vancomycin-resistant *Enterococcus*) [1, 5].

During the past 15 years the knowledge about genetic background and molecular mechanisms responsible for glycopeptide resistance has been increasing [5, 6]. *Enterococcus* resistant to glycopeptides produce cell-wall precursors with decreased affinity for the drug, which prevents the antibiotic from blocking cell-wall synthesis [7]. Nowadays, there are ten known types of enterococcal resistance to glycopeptides: VanA, VanB, VanC, VanD, VanE, VanF, VanG, VanL, VanM and VanN [8–10]. VanA and VanB types occur most frequently; VanA is responsible for a high level of resistance to both vancomycin and teicoplanin, whereas VanB confers only a low level of resistance to vancomycin while susceptibility to teicoplanin is conserved. Other types are rarely found in *Enterococcus* species [10]. *van* genes are most often located on transpozons (e.g., *vanA* on Tn*1546*, *vanB* on Tn*1549*/Tn*5382*) or on plasmids, and they transfer between enterococcal isolates by plasmid conjugation or transposition [5, 7].

In the treatment of enterococcal infections, the use of a cell-wall active agent (ß-lactam, glycopeptide) with an aminoglycoside results in synergistic antibacterial activity [11]. *E. faecium* has high-level resistance to many β-lactams as a consequence of modification of penicillin-binding proteins (PBP), or very rarely, by the production of a β-lactamase enzyme [15]. High-level aminoglycoside resistance (HLAR), caused by production of aminoglycoside-modifying enzymes (AMEs), makes therapy with aminoglycosides and ß-lactams ineffective [11]. At present, over 70 such enzymes have been discovered; their genes are also located on mobile genetic elements and are widespread among *Enterococcus* [11, 12]. Two of the most prevalent AME genes, *aac(6')-Ie* and *aph(2'')-Ia*, encode a bifunctional 2'-phosphotransferase/6'-acetyltransferase that confers resistance to a broad spectrum of aminoglycosides [13, 14]. Recently, new AMEs genes such as *aph(2'')-Ib*, *aph(2'')-Ic,* and *aph(2'')-Id* have been discovered and they are responsible for high-level gentamicin resistance [11, 14]. Although no single enzyme can inactivate all available aminoglycosides, most VRE strains can produce multiple enzyme types and consequently have the HLAR phenotype [11, 14].

Additionally, *E. faecium* have the abilities to produce several virulence factors and to form strong biofilm structure [4, 15–17]. The most common virulence determinants are: cytolysin (Cyl), endocarditis antigen (EfaA), enterococcal surface protein (Esp), aggregation substance (As), collagen adhesin (Acm), gelatinase (GelE) and hialuronidase (Hyl) [4, 18]. Cyl is a toxin, encoded by an operon localized on a plasmid or chromosome, which shows haemolytic and bactericidal activity [17]. As, encoded by a plasmid *as* gene, causes binding to the host epithelium [19]. Acm (*acm*) and EfaA (*efaA*) have been identified as the main virulence factors connected with infective endocarditis [4, 15, 20]. Hyl (*hyl*) degrades hyaluronic acid and, consequently, is associated with tissue damage [21]. Esp (*esp)*, and GelE (*gelE),* a zinc metalloprotease, are involved in the process of biofilm formation [4, 18, 22].

The increasing role of *E. faecium,* especially VRE and HLAR strains, in nosocomial infections calls for constant monitoring of their susceptibility and virulence. Astonishingly, in the literature there are many conflicting reports about the levels of resistance and virulence among VRE isolates in comparison with susceptible strains (VSE - vancomycin-susceptible *Enterococcus*) [6, 13, 21, 23–26]. Moreover, the data about VRE infections in Poland are still very limited [13]. Additionally, it should be noted that many recent studies are based only on phenotypic observations, which have some known limitations and are not fully conclusive [27–29]. This prompted us to perform a study about the exact comparison of resistance and virulence traits between VRE and VSE clinical isolates with the use of molecular and phenotypic methods.

Material and Methods

Strains

A total of 66 *E. faecium* strains (32 VRE and 34 VSE strains) isolated from November 2012 to May

2014 from hospitalized patients from various departments of the Medical University of Bialystok Clinical Hospital, were investigated.

Most of the VRE strains were collected from the haematology (50%) and intensive care units (31.3%), and were isolated mostly from rectal swabs (56%), whereas VSE strains were gathered from intensive care (53%) and surgery (20.7%) units and were isolated from blood (29%) and wound swabs (18%).

Identification and susceptibility testing

The identification and susceptibility testing of study isolates were performed using the VITEK2 system (bioMerieux, France) according to the manufacturer's guidelines using VITEK2 GP and AST-P516 cards, respectively. *E. faecalis* ATCC 29212 was used as a reference strain. Later, identification was confirmed by polymerase chain reaction (PCR) with primers targeted to *ddl* (d-Ala-d-Ala ligase) chromosomal genes [30].

ß-lactamase production

Strains were tested for ß-lactamase production by a chromogenic cephalosporinase method [31] using nitrocefin discs (OXOID, United Kingdom) as per the manufacturer's instruction.

Hemolysin production

Hemolysin production was evaluated on Columbia blood agar supplemented with 5% sheep blood (OXOID) [32].

Biofilm production

Biofilm formation was determined using two methods: the tube method and the Congo red agar method as described previously [33, 34]. Each experiment was repeated 3 times for each strain. Strains that demonstrated the ability to produce biofilm by both methods were considered as biofilm-positive isolates.

DNA extraction

Genomic DNA was extracted from overnight *E. faecium* cultures using a commercial kit (Genomic Mini Kit, A&A Biotechnology, Poland).

PCR detection of vancomycin resistance genes, virulence genes, and AME genes

A PCR reactions was used for *vanA*, *vanB*, *vanC*, *vanD* and *vanE* detection as described previously [35]. VRE *Enterococcus faecalis* ATCC 51299 was used as a positive control. Genes encoding virulence factors were investigated as described by Camargo [23], Zou [36] and Ozden Tuncer [37], revealing the presence of *gelE*, *acm*, *esp*, *efaA*, *hyl* and *cylA*, *cylLl*, *cylLs*. PCR was also used to detect genes encoding the AMEs: *aac(6')-Ie-aph(2'')-Ia*, *aph(2'')-Ib*, *aph(2'')-Id*, *aph(3')-IIIa*, *ant(4')-Ia,* and *aph(2'')-Ic* [38].

Sequencing

DNA sequencing was carried out on PCR products by GENOMED S.A. Company, Poland. The sequences were aligned and compared with reference sequences achieved using GenBank with the Basic Local Alignment Search Tool (BLAST) algorithm.

Statistical analysis

STATA 13.1 (StataCorp LP, USA) was used for statistical analysis. Differences in the prevalence of antibiotic resistance and virulence factors between VRE and VSE strains were assessed by the Chi-square test and Fisher's exact test. Results with $p < 0.05$ were considered significant.

Results

Among 32 VRE strains, 28 were found to be resistant to both vancomycin and teicoplanin; 4 strains were resistant only to vancomycin. Therefore, multiplex PCR for detecting the vancomycin-resistant genes confirmed that those 28 strains had VanA phenotype and 4 strains the VanB phenotype. Both *vanA* and *vanB* genes were not detected in any of the tested isolates.

Resistance and virulence patterns among all VRE and VSE strains are shown in **Table 1**. VSE strains carried 2 or more of the virulence genes, whilst VRE isolates had at least 4 virulence genes. The most frequent antibiotic-resistance profile among VRE strains was AMP^R IPM^R CN^R S^R TEC^R VA^R (resistance to ampicillin, imipenem, gentamicin, streptomycin, teicoplanin, vancomycin), which was detected in 17 (53.1%) strains. Four (12.5%) of these strains had the following virulence genes: *esp, efaA, acm, hyl* and had the ability to form biofilm and hemolyze. The 2 most frequent resistance and virulence patterns of VSE isolates, which occurred in 7 (20.6%) strains, were

Table 1. Characteristics of resistance and virulence patterns among VRE and VSE strains

Antibiotic resistance										Virulence factors									
Number of inactive antibiotics	Resistance pattern						AME genes		Number of genes	Genes detected by PCR								Phenotypic frequency	Number of strains
VRE (n = 32)																			
6	AMP	IPM	CN	S	TEC	VA	aac(6')/aph(2'')	aph(3')	6	esp	efa			gelE	cLs	cLi	cA	HB	2
	AMP	IPM	CN	S	TEC	VA	aac(6')/aph(2'')	aph(3')	5		efa			gelE	cLs	cLi	cA		1
	AMP	IPM	CN	S	TEC	VA	aac(6')/aph(2'')	aph(3')	4	esp	efa	acm	hyl					HB	4
	AMP	IPM	CN	S	TEC	VA	aac(6')/aph(2'')	aph(3')		esp	efa	acm	hyl					H	2
	AMP	IPM	CN	S	TEC	VA	aac(6')/aph(2'')	aph(3')		esp	efa	acm	hyl					HB	1
	AMP	IPM	CN	S	TEC	VA	aac(6')/aph(2'')	aph(3')		esp	efa	acm	hyl						1
	AMP	IPM	CN	S	TEC	VA	aac(6')/aph(2'')	aph(3')	3	esp	efa	acm						HB	2
	AMP	IPM	CN	S	TEC	VA	aac(6')/aph(2'')	aph(3')		esp		acm	hyl					HB	1
	AMP	IPM	CN	S	TEC	VA	aac(6')/aph(2'')	aph(3')			efa	acm	hyl					H	3
5	AMP	IPM		S	TEC	VA	aac(6')/aph(2'') aph(2')-Ib	aph(3')	5	esp	efa	acm	hyl	gelE				HB	1
	AMP	IPM	CN	S		VA	aac(6')/aph(2'')	aph(3')	4	esp	efa	acm	hyl					HB	2
	AMP	IPM		S	TEC	VA	aac(6')/aph(2'')	aph(3')		esp	efa	acm	hyl					HB	3
	AMP	IPM	CN		TEC	VA	aac(6')/aph(2'')			esp	efa	acm	hyl					HB	2
	AMP	IPM		S	TEC	VA	aac(6')/aph(2'')	aph(3')	3	esp		acm	hyl					HB	1
	AMP	IPM		S	TEC	VA	aac(6')/aph(2'')	aph(3')		esp	efa		hyl						1
	AMP	IPM		S	TEC	VA	aac(6')/aph(2'')	aph(3')	2		efa	acm						HB	1
	AMP	IPM		S	TEC	VA	aac(6')/aph(2'')				efa	acm						HB	1
4	AMP	IPM	CN			VA	aac(6')/aph(2'')	aph(3')	4	esp	efa	acm	hyl					HB	2
	AMP	IPM			TEC	VA	aac(6')/aph(2'')	aph(3')		esp	efa	acm	hyl					H	1
VSE (n = 34)																			
4	AMP	IPM	CN	S			aac(6')/aph(2'')	aph(3')	4	esp	efa	acm	hyl					HB	7
	AMP	IPM	CN	S			aac(6')/aph(2'')	aph(3')		esp	efa	acm	hyl					H	3
	AMP	IPM	CN	S			aac(6')/aph(2'')	aph(3')	3	esp	efa	acm						HB	1
	AMP	IPM	CN	S			aac(6')/aph(2'')	aph(3')		esp		acm	hyl					HB	3
	AMP	IPM	CN	S			aac(6')/aph(2'')	aph(3')			efa	acm	hyl					HB	1
	AMP	IPM	CN	S			aac(6')/aph(2'')	aph(3')		esp	efa	acm						H	1
3	AMP	IPM		S			aac(6')/aph(2'')	aph(3')	4	esp	efa	acm	hyl					HB	7
	AMP	IPM		S			aac(6')/aph(2'')	aph(3')		esp	efa	acm	hyl					H	2
	AMP	IPM		S			aac(6')/aph(2'')	aph(3')		esp	efa	acm	hyl						1
	AMP	IPM		S				aph(3')		esp	efa	acm	hyl					HB	1
	AMP	IPM	CN				aac(6')/aph(2'')			esp	efa	acm	hyl					HB	1
	AMP	IPM		S			aac(6')/aph(2'')	aph(3')	3	esp	efa	acm						HB	1
	AMP	IPM		S			aac(6')/aph(2'')	aph(3')			efa	acm	hyl					HB	1
	AMP	IPM	CN				aac(6')/aph(2'')	aph(3')			efa	acm	hyl					HB	1
2	AMP	IPM							4	esp	efa	acm	hyl					H	1
1		IPM							4	esp	efa	acm	hyl					B	1
0									2		efa	acm						H	1

AMP – ampicillin, IPM – imipenem, CN – gentamicin, S – streptomycin, VA – vancomycin, TEC – teicoplanin, *aac(6')/aph(2'') - aac(6')-Ie-aph(2'')-Ia, aph(3') - aph(3')-IIIa, esp* – enterococcal surface protein, *efa* – endocarditis antigen, *acm* – collagen adhesin, *hyl* – hyaluronidase, *gelE* – gelatinase, *cA, cLi, cLs* – cytolysin, H – hemolysis ability, B – biofilm-forming ability.

AMP^R IPM^R CN^R S^R and AMP^R IPM^R S^R with *esp, efaA, acm, hyl* genes. The highest resistance and virulence (resistance to 6 antibiotics and 6 virulence genes) were found in VRE strains. **Table 1** shows that VRE isolates were also characterized by a greater variety of resistance and virulence patterns than VSE strains.

The exact comparison of antibiotic susceptibility between VRE and VSE isolates revealed that all (100%) strains showed the highest susceptibility to linezolid and tigecycline. VRE isolates were resistant to ß-lactams, whereas, interestingly, two (5.9%) VSE isolates were found to be susceptible to ampicillin and one to imipenem. High-level gentamicin resistance (HLGR) was detected in 2 (5.9%) VSE and 4 (12.5%) VRE strains. High-level streptomycin resistance (HLSR) appeared more frequently in VSE strains (13 (38.2%) and 8 (25%), respectively), while high-level resistance to all aminoglycosides (HLAR) occurred more frequently in VRE strains (16 (47%) and 19 (59.4%)). However, these differences were not statistically significant ($p > 0.05$).

This study demonstrates that *aac(6')-Ie-aph(2'')-Ia* and *aph(3')-IIIa* genes occur more frequently than others (**Table 1**). The coexistence of these 2 genes was observed in 29 (85.3%) VSE and 29 (90.6%) VRE strains. Interestingly, one VRE strain carried 3 AME genes: *aac(6')-Ie-aph(2'')-Ia, aph(3')-IIIa,* and *aph(2'')-Ib.* One VSE isolate and 3 (9.4%) VRE isolates had only *aac(6')-Ie-aph(2'')-Ia* gene. *aph(3')-IIIa* gene alone was found in one VSE strain, and the remaining 3 (8.8%) VSE strains did not carry any aminoglycoside-resistant genes. However, no statistically significant differences were found between these two groups of *E. faecium* ($p > 0.05$). It should also be noted that newer AME genes such as *aph(2'')-Ic, aph(2'')-Id,* and *ant(4')-Ia* were not detected among our study isolates.

Hemolytic activity and biofilm-forming ability were similar between tested groups; α-hemolysis occurred in 29 (90.6%) VRE and 32 (94.1%) VSE strains; biofilm production in 24 (75%) VRE and 25 (73.5%) VSE strains. The ability to produce ß-lactamase was not detected in any of the tested isolates. *cylA, cylLl, cylLs* and *gelE* genes were only detected in the case of VRE strains; 2 (6.3%) strains carried the Cyl genes and 3 (9.4%) strains - the *gelE* gene. All (100%) of the *VSE* and 28 (87.5%) VRE strains had the *acm* gene. Occurrence of *efaA, esp and hyl* genes were on similar levels: *efaA* was detected in 31 (91.2%) VSE and 30 (93.7%) VRE strains, *esp* in 30 (88.2%) and 26 (81.3%), and *hyl* in 30 (88.2%) and 25 (78.1%) strains, respectively. These differences were not statistically significant ($p > 0.05$).

Discussion

The present study focused on comparison of antibiotic resistance and virulence traits between VRE and VSE clinical isolates. Comparison of HLAR between VSE and VRE groups showed that more HLAR and HLGR strains were in the VRE group, and more HLSR strains in the VSE group, although these differences were not statistically significant. This is in accordance with Baldir [39] who also did not find a significant difference in HLGR and HLSR rates among VRE and VSE strains, but did find that the HLAR phenotype occurred significantly more often in VRE isolates. In another study [26], all three aminoglycoside-resistant phenotypes occurred significantly more frequently among VRE strains. Likewise, Tripathi [40] revealed that resistance to gentamicin prevailed in VRE isolates. Differences between these results may indicate that the resistance to vancomycin does not always correlate with resistance to aminoglycosides, and determination of HLAR among *E. faecium* strains must always be performed.

We have demonstrated that the bifunctional enzyme coding gene *aac(6')-Ie-aph(2'')-Ia* and *aph(3')-IIIa* gene occurred the most frequently, and none of the tested isolates carried newer AME genes such as *aph(2'')-Ic, aph(2'')-Id,* and *ant(4')-Ia*. Similar results were reported by other authors [37, 41–43]. In our study we observed strains with the *(6')-Ie-aph(2'')-Ia* or *aph(3')-IIIa* gene but without respected phenotypic resistance towards gentamicin. This may be due to low levels or downregulation of these genes' expression or by inactive gene products.

In our study all of the VRE strains and 94.1% of VSE strains were resistant to ampicillin and imipenem. None of the investigated *E. faecium* strains showed β-lactamase activity. These results are in agreement with other studies [24–25]. It should be noted that in this study the majority of tested strains that were resistant to β-lactams also had HLAR phenotype. The occurrence of co-resis-

tance between ampicillin and aminoglycosides in VRE isolates is worrisome because it eliminates the synergistic effect between β-lactams and aminoglycosides in the treatment of patients. Moreover, Leavis [44] showed that increasing numbers of β-lactam resistant *E. faecium* proceeded the growing rates of VRE both in the USA and in Europe. Precisely, high-risk enterococcal clonal complex (CC17), associated with hospital outbreaks of VRE on 5 continents, was strongly correlated with ampicillin resistance. Moreover, ampicillin resistance was followed by resistance to fluoroquinolones and then acquisition of the *vanA* or *vanB* gene [44]. Nowadays, *Enterococcus* spp. isolates resistant to β-lactams, aminoglycosides and glycopeptides are considered as multidrug resistant (MDR), and their increased prevalence and dissemination worldwide cause the necessity of searching for new treatment strategies, including combination therapy [44–46].

The presence of *cyl* and *gelE* genes among *E. faecium* strains is rare. Vankerckhoven [47] did not find any *cyl* and *gelE* genes with PCR in 271 *E. faecium* isolates. In our study, both VSE and VRE isolates were shown to be hemolysin producers (>90%), but only 2 of the VRE strains carried the genes of the *cyl* operon. This may be due to the expression of other hemolysin genes that are yet not known or not so well studied. Interestingly, these *cyl*-positive strains also had the *gelE* gene. A small percentage of strains with the *gelE* gene has also been reported by other researchers [6, 13, 41] but without the coexistence of *cyl* genes. A recent study performed by Saba Copur [48] showed that more VSE than VRE strains possessed the *gelE* (25% and 2.2%, respectively) and *cyl* (50% and 0%) genes, which is not in concordance with our results. On the other hand, Biswas [49] revealed that 44.4% of VRE and 16.4% of VSE isolates contained the *gelE* gene ($p < 0.001$).

We reported that the prevalence of *esp* and *hyl* genes among tested strains groups was almost equal and, consequently, these differences among isolates were not statistically significant. Similar proportions and lack of significant differences between VSE and VRE isolates were seen by other researchers [6, 21, 48], but Vankerckhoven [47] and Biswas [49] found that the *esp* gene was significantly more prevalent among VRE isolates than among VSE strains (77% VRE versus 53% VSE, and 27.8% VRE versus 8.9% VSE, respectively). These contradictory results indicate that the presence of all tested virulence genes cannot be unambiguously correlated with the occurrence of vancomycin resistance among *E. faecium* strains.

Bacterial *ability to form biofilm* is considered to be an important factor in the pathogenesis of enterococcal infection [6, 17, 21]. In our study, over 70% of strains from both VRE and VSE groups were found to produce biofilm. These results did not coincide with studies by others [6, 15, 21]. Di Rosa [15] found that VRE strains were able to produce biofilm less frequently (28.8%). Praharaj et al [21] also showed that 13 out of 32 VRE and 70 out of 125 VSE isolates formed biofilm. However, similarly to our results, there was no significant difference when comparing VRE and VSE groups. Collectively, these data suggest that VRE strains do not produce biofilm more often than VSE isolates; nevertheless, potential impact of biofilm forming ability among *E. faecium* isolates should be *taken into account* when developing treatment options or infection-control procedures.

VRE was first identified in *Europe* in *1986, spread* rapidly, and is now widespread across Europe [50]. Unfortunately, according to European Centre for Disease Prevention and Control report (2015), resistance to glycopeptides has significantly increased over the last 4 years, especially in Bulgaria, Croatia, Denmark, Hungary, Ireland, Italy, Slovakia and the United Kingdom. The percentage of VRE varied between 0% in Estonia, Finland, Malta, and Iceland to 45.5% in Ireland. A decrease in the prevalence of the VRE strains, compared to previous years, was reported only in France. In Poland, the first VRE strains were reported in 1996 [51]. Nowadays, the percentage of VRE isolates in Poland has also increased and ranges between 8 and 19%. These varieties and changes in VRE epidemiology are a reflection of differences in antibiotic and infection control policies and remain a major challenge throughout Europe.

In conclusion, both VRE and VSE isolates were well equipped with virulence and resistance genes, although VRE strains were characterized by greater variety and a higher number of these genes. This variety, especially when it occur in combination with phenotypic high-resistance level to antimicrobials, might increase the ability to adhere to artificial surfaces, lead

to persistence and spreading in hospital environments, and cause more serious nosocomial infections. Moreover, these results suggest that VRE strains slowly acquire and incorporate resistance and virulence traits, due to their ability to survive in hospital environments for a long time. However, statistical analysis revealed no significant differences in the occurrence of tested features between VSE and VRE strains. This can be related to an insufficient number of isolates that could have compromised the statistical analysis. Studies with a higher number of heterogeneous VRE and VSE strains should be performed to clarify each type of strain's role in pathogenesis of enterococcal infections. Further studies are also needed on regulation and expression of virulence and resistance genes, how to prevent the spread of MDR enterococcal infections, and on treatment alternatives. Novel pharmacotherapy targeted at specific virulence factors such as anti-adhesins may play preventative or even therapeutic role in the elimination of MDR enterococcal infections.

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REVIEW PAPER

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Separate growth charts and cephalometric norms for children with Down syndrome

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ABSTRACT

Different phenotypic features characterizing the body structure of children with Down's syndrome, which include low growth, small head, short limbs, as well as the tendency to obesity and other systemic diseases or congenital malformations, prompted the WHO to develop separate standards including growth charts for children with this syndrome. Selected authors in their studies also compare orthodontic parameters, and more precisely cephalometric parameters, between children with Down's syndrome and healthy individuals. They note a tendency to repeated deviations from the accepted norms, including the skeletal class, antero-posterior dimensions of the jaw, the length of the base of the skull, the cranial base angle, and ANB, SNA, SNB angle. It is related to the occurrence of specific features of the skull skeleton structure, typical for children with Down's syndrome. The described tendency of changes in cephalometric parameters, in correlation with the already developed separate growth charts to assess the growth of children with Down's syndrome, leads to considerations on the need to develop separate standards in the field of orthodontics, adequately defining the skeletal structure of the facial part of the skull of these children.

Keywords: growth charts, Down's syndrome, cephalometric analysis.

Introduction

Properly constructed and appropriate growth charts are necessary to assess the correctness of body growth, and also indicate optimal physical development of the child, health and nutrition [1, 2]. Monitoring and assessment of child development is one of the most important tasks of medical care. These growth charts, presenting graphically developed development norms, are constructed in such a way that successive percentile lines determine the percentage of children in each age group below their level, i.e. if the measurement value of the tested feature is on the 10th percentile, it means that in this calendar age 10% of peers are characterized by a lower value of this feature [3]. Limits of the so-called narrow standard are defined by 25th and 75th centile. The growth chart, also known as percentile or centile chart, gives the opportunity to compare the selected parameter, e.g. weight or height of the child in relation to other children of the same age and sex [4]. The basic method of assessing the physical development of a child is to compare his individual phenotypic image with the developmental norm (reference system), however it should be emphasized that the developmental norm may depend on comorbid diseases, which include genetic syndromes [3].

A specific group is represented by children with various mental disabilities co-occurring with specific genetic syndromes, e.g. children with Down's syndrome. The prevalence of this syndrome is estimated to be 11–16 per 10,000 [5, 6]. There are many characteristics of children with Down's syndrome, which include, among others, low growth, small head, single transverse palmar crease, almond shaped eyes caused by a fold over the eyelid, weakened muscle tone. An increased risk of congenital heart disease, gastroesophageal reflux, recurrent middle ear infections, hyperthyroidism syndrome and thyroid gland diseases are also reported [1, 2, 7, 8]. High risk of occurrence of numerous impairments in the functioning of individual body systems does not remain indifferent to the process of proper growth and development. Separate growth charts for children with Down syndrome

Children with Down's syndrome (Ds) are born with a smaller birth weight, but they develop overweight when they are 3 to 4 years old. The tendency to overweight is quite common — at the age of 19, it occurs in 31% of men with Ds and in 36% of the female with Ds [9]. The tendency towards the specific features of body structure in children with this syndrome, prompted the World Health Organization to construct dedicated growth charts for children with Down's syndrome. Van Gameren-Oosterom et al. describe that the first charts for Dutch children with Down syndrome were published and introduced in 1996 [2].

The growth scheme of children with Down syndrome is distinguished by a significant impairment of their developmental pace, starting from the moment of birth to adolescence, intensified in particular in the age range from 6 months to 3 years and also during puberty. According to the WHO guidelines for the assessment of excessive body mass, the BMI mass index is most commonly used to classify the nutritional status of children, adults and the elderly [9]. It is emphasized that obesity is a common condition among children with Down's syndrome [10]. The tendency to present typical features of body structure is one of the important arguments for the rightness of constructing separate growth chart for children with Down's syndrome.

Zemel et al. describe that the characteristic features of the phenotypic image of children with Down's syndrome in relation to healthy children include, among others, shorter limbs, which undoubtedly affects a different distribution of body mass in relation to weight [10]. As shown by Bertapelli et al., the uninterrupted scheme of BMI growth observed in children with Down's

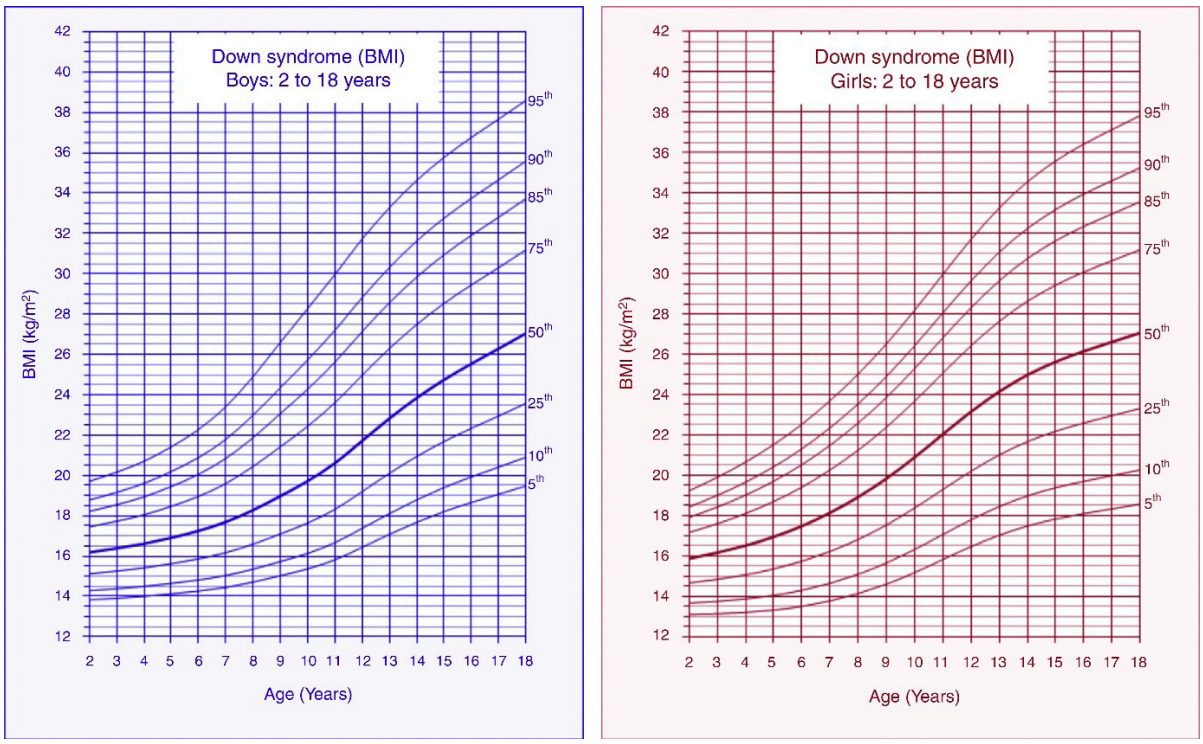

Figure 1. Growth charts expressing BMI values for boys and girls with Down's syndrome aged 2–18 years [11]

Figure 2. CDC Growth Charts – growth charts expressing BMI values for healthy boys and girls, without Down's syndrome, aged 2 to 20 years [12]

syndrome is definitely different compared to BMI standards defined for the general population without this syndrome (**Figures 1, 2**). International growth references indicate a rapid acceleration of the central percentile of BMI in the first year of life, followed by a decline by the age of 5 years, and a subsequent acceleration in later life. These age-specific BMI curves are descriptive growth standards in children and adolescents with Down syndrome. However, these curves do not indicate the optimal standard of weight to which all children with this syndrome should grow. The use of these curves, however, allows us to compare and monitor the status of body mass [11].

Cephalometric parameters in children with Down's syndrome

The literature describes a number of characteristic features of skull structure in children with Down's syndrome, including hypoplasia of the middle part of the face, flattening of the skull base, skeletal class III together with the co-occuring open frontal bite.

Quintanilla et al. [13] assessed the morphology of the facial part of the skull of patients with Down's syndrome based on the results of cephalometric analysis, the study group included children from 7 to 18 years of age. The average size of the overbite and overjet parameters was -1.01 and 1.73 respectively, with respect to the Ricketts standards (overbite = overjet = 2.5 mm) adopted by the authors, which are therefore lower values, and the negative overbite confirms the reverse overjet. The inter-incisal angle, whose mean value in the patients tested was 126.4°, was underestimated with respect to the Ricketts standard of 130°. The average length of the anterior segment of the skull base of children with Down's syndrome was 52.12 mm, which was slightly lower than the accepted standard of 55 mm. The authors did not include the control group corresponding to the age of the respondents, hence all the values referred only to the adopted standards developed by Ricketts. In addition, the analysis does not include the parameters describing the base angle of the skull, the anterolateral relation of the mandible to the jaw, as well as the relationship of the mandible and jaw to the base of the skull. They describe

that, unlike other authors, they did not obtain classification results for the III skeletal class, which was explained by the fact that this group included people in the period of the growth of the skull.

Similar research was undertaken by Suri et al. [14], who compared the results of the analysis of 25 cephalometric X-rays of children with Down's syndrome in the age range from 11 to 18 years. The results obtained were referred to the control group of healthy children of similar age, with I skeletal class. The results showed a reduction in the linear dimension of the anterior length of the skull base and a slight increase in the skull base angle value in children with Down's syndrome, in relation to children without this syndrome. It should be emphasized that the norm of skull base angle according to Segner and Hasund is within 128–136°, while the value of this parameter for children with Down's syndrome was 140.31°, being an inflated value, for children from the control group it was within the normal range. All dimensions concerning the jaw were interpreted by the authors as significantly smaller in the group of children with Down's syndrome, its length was reduced by 17.4% in relation to the control group, amounting to 47.8 mm. The SNA angle for children with Down syndrome was on average 82.47° and showed no significant difference in values relative to the control group. The limit of the standards according to Segner and Hasund is 79–85°. The average value of the SNA angle for both children with Down syndrome and without this syndrome is within this limit. The dimensions of the SNB angle were higher in the test group relative to the control group, but the results of both groups were within the normal range of 77–83° according to Segner and Hasund. Co-occurring anterior mandibular rotation has been recognized by the authors as a factor favoring the occurrence of its prognathism. In the group with Down's syndrome, 48% patients had anterior cross-bite.

In another paper, Melo de Matos et al., [15] analyzed cephalometric X-rays of 15 patients with Down's syndrome in the age range from 21 to 34 years, and the results were referred to a control group of 15 healthy people, appropriately assigned by age, of the Brazilian population. On the basis of own observations they assessed that in Down's syndrome the values of the length of the anterior and posterior base of the skull are reduced, while the value of the base angle of the skull is increased. For people with Down's syndrome the mean value of this angle was 151.5°, in relation to the standards of Segner and Hasund developed for Europeans amounting to 128–136° for the NSBa angle, which is definitely above the upper limit of the norm, also for the control group it was 140.3°, being in the Brazilian population higher than in the norms adopted for Europeans. They also obtained lower values of SNA and SNB angles in people with Down's syndrome compared to the control group, which they estimated as a distal position of the maxilla and mandible relative to the base of the skull. With regard to standards developed by Segner and Hasund, the SNA angle is 79–85°, and the SNB angle is 77–83°, the mean values of these angles for people with Down's syndrome are lower, while for people without this syndrome they fall within the reference values. The authors' analysis of the relation of the mandible to the maxilla based on the ANB angle showed a significant reduction of this angle in relation to the group of healthy people, which was interpreted as a tendency of III skeletal class occurrence. The inter-incisal angle in the group of subjects was lower in relation to the control group, which was caused by protrusion and proclination of the upper central incisors. The norm of values of the inter-incisal angle according to Segner and Hasund is 125–141°, while the average value of this parameter for people with Down's syndrome is below the lower limit of the norm and amounts to 119.3°. For the control group, it is 125.5°, i.e. within the reference values. The authors qualified people with Down's syndrome in their adulthood, hence it is impossible to refer and compare the results to previously reported studies, including children in developmental age. The obtained results were compared only between the test and control groups, omitting a reference to valid cephalometric standards, e.g. in the analysis of Segner and Hasund.

Development of norms of cephalometric parameters for children with Down syndrome

A clear tendency to deviations of certain cephalometric parameters, resulting from a different skeletal structure of the facial part of the skull, which was described in the literature, may suggest the need to develop separate norms of cephalometric parameters for children with Down's syndrome, as well as growth charts. The values that show a tendency to deviate seem to include the length of the anterior cranial base, cranial base angle, antero-pos-

terior dimensions of the jaw, ANB angle, SNA angle and SNB angle, as well as the inter-incisal angle. It is worth noting that the cited studies of different authors demonstrate no unification of the age group of the persons with Down's syndrome, as well as no systematic reference of the obtained results, as some authors refer them to adopted and generally known norms, e.g. developed by Ricketts, and others compare them only between the test and control group, omitting the adopted standards. It would be noteworthy to conduct a study on children with Down's syndrome in the appropriate age range, including the growth period, and allowing proper cooperation with the child to obtain reliable lateral cephalometric X-rays. A similar issue concerns the physical development of the body of children with Down's syndrome, which, showing typical phenotypic traits, should not be compared with the values developed for healthy children, hence separate percentiles were created for them, taking into account the typical body structure and the tendency for a different growth scheme. Based on the above, the question arises whether, due to the tendency for a different skeletal structure of the facial part of the skull described by many authors, it is not worth considering and directing attention to the desirability of developing separate values of cephalometric parameters that would be considered a norm for children with Down's syndrome. This is an open question that requires proper research and, above all, the gathering of a sufficiently large group of subjects, a control group and a comparison of values between them and references to generally accepted norms.

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REVIEW PAPERS

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Dental anxiety — conditions, models and therapy

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ABSTRACT

Dental anxiety is a condition suffered by many dental patients. It causes psychological discomfort and avoidance of dental appointments, which in turn may lead to oral health issues. Dental anxiety has not yet been fully explored and seems to be still posing challenge to both dentists and psychologists. The aim of this article is to review dental anxiety studies, paying particular attention to the conditions, social, demographic and psychological correlations, as well as the ramifications of this type of anxiety. The article presents the most common psychological models of dental anxiety, methods to measure this type of anxiety and therapy techniques used with patients suffering from dental anxiety.

Keywords: dental anxiety, discomfort, therapy.

Introduction

Dental anxiety is considered a global challenge in the area of dental care [1, 2]. It is said to be a serious, common form of medical stress. Dental anxiety is defined as patient's reaction to a specific kind of dental-related stress [1, 3], and according to McNeil and Berryman [4], it is an emotional reaction to dental stimuli or experiences combined with a cognitive evaluation of such stimuli and experiences. Dental anxiety is a complex phenomenon, which has a somatic, psychological and social dimension, which is why psychology may be of particular use to dentistry [5] in the process of studying dental anxiety.

Due to the fact that dental anxiety is so common a condition, many believe it should be a central focus for dentists [6]. It is estimated that strong dental anxiety is suffered by approximately 20% of patients [7, 8] and anxiety before dental appointments experienced by about 40% of patients [9, 10]. Kelly et al. [11] cite survey results which show that 64% of respondents experience uneasiness provoked by dental appointments, and 49% feel afraid before going to the dentist.

Analysing dental anxiety brings both theoretical and practical benefits, partly due to the fact that this type of anxiety is a common problem and partly because it has serious negative consequences. Research show that dental anxiety leads to dental care avoidance [12], negligence of oral health [13] and lower overall quality of life [5, 12, 14, 15]. Dental anxiety is also related to pain which impels people to seek dental help only after they start to feel it [16]. It might be said that dental anxiety bears somatic consequences, i.e. deterioration in the health of the oral cavity and the entire body, psychological consequences, i.e. lower self-esteem related to external appearance, and social consequences consisting in the deterioration in the quality of social relations and withdrawal from interpersonal relations.

This article aims to carry out a psychological analysis of dental anxiety based on the studies conducted to date. In the first part, the paper presents the conditions provoking dental anxiety, together with its social, demographic and psychological correlations. Further on, the article describes the most common models of dental anxiety and the best-known psychological methods of this anxiety measurement. The final part concentrates on the illustration of selected therapeutic techniques used in the treatment of this type of anxiety.

Conditions provoking dental anxiety

Weiner and Sheehan [17] suggest that dental anxiety may be exogenous or endogenous. The exogenous type of dental anxiety is conditioned by a negative dental experience, whereas the endogenous anxiety accounts for the vulnerability to react with fear to potentially dangerous situations. The most frequent cause of dental anxiety is past, usually childhood, traumatic experiences related to dental care [4, 17, 18]. In the studies carried out by De Jongh, Aartman, Brand [19], 87% of patients with a dental phobia reported an extremely aversive past dental appointment. From among the above group, 46% of patients displayed one or more symptoms of the post-traumatic stress disorder (PTSD). However, study results also show that many of those with a dental phobia did not experience an aversive dental treatment in the past. Analogically, among the patients who experienced a negative dental experience, there are people who do not suffer from a dental phobia [20].

It is found that some people may have learnt dental anxiety from a member of their family who was dentally anxious [21]. Thomson, Locker, Poulton [22] state that psychological factors contribute to the aetiology of dental anxiety more substantially than aversive dental experiences. Vulnerability to feel dental anxiety is related to certain predisposing personality characteristics, mainly a general susceptibility to anxiety [18]. Fearful people exaggerate the intensity of aversive incidents and dental anxiety may be very deeply rooted and related to other problems of psychological nature. These could be for example the fear of losing control, crossing borders of intimacy, etc.

Correlators of dental anxiety

Results of the studies carried out by Hagglin et al. [23] indicate that the level of dental anxiety decreases with age; elder people experience less dental anxiety than younger people. Results of other studies, however, suggest a different correlation. Hittner and Hemo [24] proved that dental anxiety correlates significantly and positively with age. In dental anxiety questionnaires, women obtain higher results than men. Furthermore, results show a significant negative correlation between the amount of income and dental anxiety [24].

Abundant research has been conducted concerning psychological correlators of dental anxiety. Research proves that dental anxiety correlates significantly and positively with neuroticism [18, 23]. Economou [26] proved a significant positive correlation between dental anxiety and self--awareness, understood as seeing yourself as an object observed by other people. De Jongh [27] showed a significant correlation between dental anxiety and forcing out thoughts about dental appointments and the frequency of negative intrusive thoughts about dental treatment. In an interesting experiment conducted by Muris et al [28], both the subjects who did not experience deep dental anxiety and the ones who experienced a dental anxiety were asked to suppress negative thoughts associated with dental appointments. As a result, the persons who did not have a deep dental anxiety began to experience acute anxiety before dental care, and patients with a dental anxiety did not note a higher level of fear. Authors of this experiment explain the paradox, stating that in order to suppress feelings in the experiment – as requested – patients with a anxiety applied certain remedial reactions they had previously worked out, whereas the ones who did not suffer from a dental anxiety had not developed and could not use any techniques to deal with the dental-related contents provoking fear. Such an interpretation might raise doubts, though. If the patients suffering from a dental anxiety had worked out certain techniques for dealing with fear, they would not have been suffering from dental anxiety; the fact that they were not feeling increased dental anxiety may have stemmed from the fact that their thoughts, paradoxically updated by the experimental instruction to suppress, were *de facto* always present in their minds (hence the

anxiety) and the instruction itself did not activate such thoughts, which could in turn occur in the case of the patients with weak dental anxiety.

In the studies published by Hittner and Hemo [24], significant positive correlations were proved between dental anxiety and life satisfaction, forcing out negative thoughts about dental treatments and the internal locus of control. In the said studies, the more satisfied the subjects were with life, the more often they forced out negative thoughts about dental care and the more internal locus of control they felt, the deeper fear they experienced. One of the explanations to the above correlations offered by Hittner and Hemo [24] was that w people fear that their life satisfaction may deteriorate because of dental issues. A person may, for instance, fear that poorer oral health will hinder their ability to derive pleasure out of life. Anticipating the events which may deteriorate your quality of life may increase the level of dental anxiety. When it comes to a rather intriguing correlation between placing control internally and dental anxiety, Hittner and Hemo [24] explain that fear may be related to feeling responsible for one's own conduct, including for one's own oral health care. Such responsibility is adopted by people with internal control who assume responsibility for aversive experiences from the past, as well as the ones which will happen in the future. When summarising the results of their studies, Hitner and Hemo [24] emphasized that dental anxiety may be also positively stimulating, as it may motivate to exercise care of oral health.

Crofts-Barnes et al [16] studied the correlation between the preoperative anxiety associated with dental procedures and the interferences caused in the daily life of the people with a strong dental anxiety. Dental anxiety correlated significantly and negatively with the quality of life. In the studies conducted by Tellez et al. [8], dental anxiety correlated significantly and positively with the pain experienced during the last dental appointment and the fear of revealing one's own looks. Similarly, results of the studies carried out by Hoogstraten [18], Cohen et al. [28], and Kent [29] report a significant positive correlation between dental anxiety and the state of fear and pain related to dental procedures.

Kyle et al. [30] showed that dental patients remember stronger pain, i.e. stronger than than the one actually experienced, and this correlation is stronger in the case of patients with a more severe dental anxiety [31, 32]. Study results published by van Wijk and Hoogstraten [18] show that people have a tendency to experience exaggerated fear of dental pain if they have not felt this type of pain personally. People generally expect stronger pain than the one they will be actually experiencing. The said dependency is stronger in persons with more dental anxiety [18, 33].

Moreover, study results show significant positive correlations between dental anxiety and anxiety disorders, such as Generalized Anxiety Disorder (GAD), agoraphobia, fear of injections and blood, and Social Anxiety Disorder (SAD) [17, 34, 35]. In the studies conducted by McNeil and Berryman [4], dental anxiety co-existed with the fear of pain, fear of closed spaces and of being hurt.

Models of dental anxiety

Professional literature defines several types of dental anxiety models: the ones based on the theory of learning, the ones based on cognitive theories, the ones associated with the concept of social functioning and the systemic and functional ones. Among the most popular dental anxiety and fear models, there are models understood as a variable conditioned by an aversive dental experience [8, 20]. These models assume that a negative, often painful dental experience is a decisive factor in the development of dental anxiety. As a result of a negative dental experience, the patient associates dental appointment stimuli, such as the sound of drilling, smell of disinfectants, etc. with discomfort and pain. An example here is the model of three pathways proposed by Rachaman [36]. Rachaman [36] assumes that people acquire pain before dental appointments, because they have either experienced an aversive dental appointment themselves, witnessed someone else experience an aversive dental appointment or heard of an incident of an unpleasant dental appointment.

An example of the Cognitive Vulnerability Model is the model proposed by Armfield [6, 20]. The model is primarily based on a cognitive evaluation of the situation. According to Armfield [6, 20], a person's perceptions of a dental experience are crucial in the development of fear, i.e. the perceptions of an uncontrollable, unpredictab-

le, dangerous and disgusting experience. How a dental experience is perceived stems from the combination of personal qualities and life experiences.

The cognitive nature of dental anxiety is stressed out by other researchers, too. Litt [37] for example, points out that the phenomenon of dental anxiety is a perfect illustration of a certain regularity, namely that characterisation of and experiences associated with a specific situation matter more in the evaluation of how aversive a situation is than the situation's objective properties.

The model of anxiety proposed by Berggren [5] is an example of a social model. This is a model of a vicious circle. It presents correlations between dental anxiety, avoidance of dental care, deterioration of dentition and feelings of guilt, shame and inferiority. In this model, it is of significance that people are social beings, and interpersonal relations constitute a very important aspect of human life. In interpersonal relations, external appearance plays a significant role which is why oral health issues so strongly and negatively influence the quality of social functioning.

Dental anxiety may be also interpreted in terms of systemic and functional models, such as by the Function-Action Model of Psychological Defense proposed by Senejko [38]. From the perspective of this model, dental anxiety may be treated as an indicator of blocking (hindering or preventing) the realization of the most important needs, constituting the basis of the human motivational system. One may assume that dental anxiety is a sign indicating that the satisfaction of basic needs has been disturbed, i.e. of the need for safety, control, identity, emotional contact, etc. From this perspective, it is the specificity of the blocked standards of regulation and their degree of importance for the subject which have a decisive impact on both how a dental appointment is treated in terms of danger and/or challenge, and what the subjective feeling of dental anxiety is, as well as how to deal with it.

What are dental patients afraid of?

Based on their clinical trial, Milgrom et al. [39] proposed a classification system (known as the Seattle system), reflecting the main categories of dental anxiety. In the system, four diagnostic types are proposed: 1. fear of specific dental stimulus; 2. anxiety about somatic reactions during treatment; 3. generalized anxiety or multiphobic symptoms; 4. distrust of dental personnel [34].

In the research conducted by Armfield [20], dental anxiety significantly and positively correlated with the following aversive dental experiences: feeling of being gagged, fainting and personal issues with the dentist. Of note, most of the subjects who claimed to have experienced strong pain during a past dental appointment reported a low or moderate degree of dental anxiety.

Measuring the degree of dental anxiety

To measure dental anxiety, physiological, behavioural and psychological methods are used [40]. Physiological methods assess physiological reactions of the body, such as the pulse, heart rate, blood pressure, tension of the muscles. Another technique used is measuring the Cortisol level in saliva [40, 41]. In the case of behavioural methods, a dentist evaluates patient's behaviour. The evaluation is carried out on an appropriate numerical scale.

In the psychological evaluation of the level of dental anxiety psychological questionnaires are used. The questionnaires mainly involve self-assessment, which means that the patient subjectively evaluates the level of experienced dental anxiety. **Table 1** presents the most popular psychological methods used to evaluate dental anxiety.

Kaczmarek et al. [40] point out that on a daily basis, dentists extremely rarely use scales to measure dental anxiety [see 50]. Some dentists believe that the use of such questionnaires before a dental procedure can worsen the relationship between the patient and the dentist, because the patient concentrates on unpleasant events. Study findings, however, contradict such a dependence. The study carried out by Kent [29] shows that the measurement of fear and pain before a dental procedure did not affect the level of anxiety and discomfort of patients after the procedure. What is more, this type of measurement can have a beneficial influence on the general condition of the patient. Research results by Carlsen et al. [51] showed that the measurement of fear and pain in children before the dental procedure related to a decrease in the level of anxiety associated with

Table 1. Short characteristics of popular psychological methods used to evaluate dental anxiety

Questionnaire	Author(s)	Number of items	What does it measure?
Corah's Dental Anxiety Scale (CDAS)	Corah [42]	4	General dental anxiety
Modified Dental Anxiety Scale (MDAS)	Humphris, Morrison, Lindsay [43]	5	Anxiety about dental treatment with an additional question concerning anxiety about local anaesthesia
Gatchel's 10-Point Dental Fear Scale	Gatchel [44]	1	General dental anxiety
Dental Hygiene Fears Survey (DHFS)	Gadbury-Amyot and Wiliams [45]	16	Anxiety associated with dental and hygienic procedures
Fear of Dental Pain Questionnaire (FDPQ)	van Wijk and Hoogstraten [18]	18	Fear for dental pain
Dental Anxiety Inventory (DAI)	Stouthard and Hoogstraten [46]	36	General dental anxiety
Index of Dental Anxiety and Fear (IDAF-4C+)	Armfield [20]	23	Dental fear and anxiety, dental phobia, fear of specific dental stimuli
Photo Anxiety Questionnaire	Stouthard, De Jongh, Hoogstraten [47]	10	Dental anxiety. The questionnaire contains non-verbal response scales
Kleinknecht's Dental Fear Survey (DFS)	Kleinknecht, Klepac, Alexander [48]	20	Fear about various situations and objects related to dental procedures
Gale's Ranking Questionnaire (RQ)	Gale [49]	29	Fear of specific dental situations

the dental procedure. According to Dailey et al. [50], the measurement of dental anxiety by questionnaires provides important information for the dentist and can also provide psychological benefits to the patient. Dailey et al. [50] checked whether informing the dentist about the level of dental anxiety before starting treatment reduces the level of state anxiety in the patient. The results showed that patients who informed the dentist about their level of anxiety before the treatment began were characterized by a lower level of anxiety compared to the patients who did not inform the dentist about the level of their anxiety.

The use of psychological methods to assess dental anxiety allows for a more reliable assessment of the patient's mental state before treatment and adjustment of dental procedures to the patient's needs [40]. As study results show, using the above methods is beneficial also due to the fact that subjective assessment of patient's emotional state by the dentist during a dental appointment is often not consistent with the actual condition of the patient [43]. Dailey et al. [50] emphasize that very few dentists use questionnaires to diagnose dental anxiety in their daily practice [50].

Dental anxiety therapy

What dentists can offer patients with severe dental anxiety is usually sedatives or general anaesthesia [5]. In the case of severe dental anxiety, psychological therapy is recommended, which may be accompanied by pharmacological therapy. Therapeutic procedures used in the treatment of dental anxiety usually contain elements of psycho-education [9]. Psychotherapy is usually cognitive, behavioural or cognitive-behavioural. Hypnosis is also used [25].

Most of the techniques involving psychological work with patients with a dental phobia are based on regular desensitisation [52]. Regular desensitization, in relation to dental stimuli, consists in displaying more and more fearful stimuli associated with dental appointments to the patient, who has been put into the state of relaxation. The patient is usually asked to visualize certain dental stimuli [53], which are also presented on the screen [54], or dental devices and dental office equipment are used [9].

An interesting therapeutic procedure, based on a regular desensitization, is described by Carlsson et al. [5]. As some patients may have problems with the visualization of dental devices or dental surgery, presentations of video recordings are used in this procedure. Such recordings contain various filmed dental treatments. Therapeutic sessions take place in a special room, arranged in a similar way to a dental office. During a therapy session, patients sit in a dental chair and watch films presenting dental procedures. Patients have the possibility to stop the film

when they begin to feel too tense. They stop the film by means of remote devices. Before starting therapy sessions, patients receive instructions to relaxation exercises to be be done at home. To strengthen patients' relaxation skills, the biofeedback method is used. Thanks to this method, continuous monitoring of the patient's tension status is ensured. In this therapeutic procedure, emphasis is placed also on the cognitive aspects of dental anxiety, i.e. exploring and reformulating patient's thoughts and beliefs about dental procedures and the dental phobia.

Cognitive therapy of dental anxiety intends to change and restructure negative thoughts and increase control over such thoughts. Short-term behavioural-cognitive therapies are popular. The results of meta-analyses and review analyses of studies on the effectiveness of cognitive-behavioural therapy [9] confirm the effectiveness of this type of therapy in reducing dental anxiety, even in the case when these therapies are short--term and involve only several meetings with the patient. Working with such a type of patients involves concentrating on a specific symptom, and changing the way they think about dental treatment. It is very important to understand what exactly the patient is afraid of and why he or she is afraid of it, and also what the sources of dental anxiety are.

One of the latest forms of this kind of therapy is computer cognitive-behavioural therapy (C-CBT) [54]. It is a therapy that is easy to use in dental surgeries. It is based on psycho-education, exposure to anxious dental stimuli and cognitive restructuring. C-CBT therapy takes the form of a one-hour session based on a computer intervention supporting the patient in their coping with dental anxiety. The patient is sitting at the computer, with headphones on, but he or she can ask for help or ask the person conducting therapy a question at any time. C-CBT begins with a psycho-educational module, which gives the patient basic knowledge about dental anxiety. Then, the patient is guided through a short motivational interview, which helps analyse the benefits and loses related to the work undertaken over their dental anxiety issues. Later on, the patient does exercises based on the exposition to dental anxiety stimuli, during which they may practise dealing with their own dental anxiety. At the initial stage of exercises, the patient is asked to chose three most frightening procedures out of the six given medical procedures, and arrange them from the least to the most frightening. The list of procedures includes: drilling and filling the cavity, teeth cleaning, injecting anaesthesia, root canal treatment, x-ray of the oral cavity and tooth extraction. Having arranged the procedures, the patient watches a film in which the procedures they chose – they are shown from the least to the most frightening one. For each procedure, there are three films. The first film shows how the procedure is done by a dentist and is accompanied by some basic explanations about what it involves. To aid the process of explanation, animations are occasionally played, containing details about the procedure. The second film also shows how the procedure is done, but the contents focus on the emotions experienced by the patient. The narrator provides basic information about the nature of emotions experienced by the patient and the ways in which they can handle these emotions. The third film presents a chosen procedure from the perspective of a patient sitting in the dental chair. The film exposes the elements of the frightening procedure more intensively. In this film, the narrator talks to the patient undergoing the given procedure about the effective ways of dealing with anxiety and the emotions experienced during the procedure [54].

Doering et al. [55] propose another approach to dental anxiety treatment, consisting in focusing on a traumatic dental experience from the past. They note that exposing patients who have a negative dental experience to aversive stimuli, as part of regular desensitization, may occur ineffective, or even aggravate dental anxiety by activating traumatic memories. Such patients could benefit more from the therapy involving concentrating on trauma. In the case of patients who work on their dental anxiety in the context of the post-traumatic stress, Doering et al [55] suggest the desensitization therapy which involves the eye-movement technique (EMDR) [56]. Dental anxiety therapy using EMDR was also described by De Jongh, Van Den Oord [57]. Work with patients suffering from severe dental anxiety which was carried out by Doering et al. [55] included several sessions. During the first session, negative memories about an aversive dental appointment were activated and distancing techniques used. Then, on a 10-digit scale, subjective

discomfort was assessed which accompanied the memories. Further on, eye movement exercises were introduced – series of 25–30 horizontal eye movements, repeated until the level of the subjective discomfort related to the negative memories reached 0. At the end of the session, the patient was instructed to conduct daily observations and note down new memories associated with trauma, as well as the dreams related to the trauma. The second and third session resembled the first one, and possibly included new memories about the traumatic dental experience. During the third session, the patient was prepared to confront future dental appointments by positively imagining himself or herself dealing with successful dental treatment. Studies conducted by Doering et al. [55], involving dental anxiety therapy given in such a form have proven highly effective.

Summary

The aim of the presented article was to analyse dental anxiety studies published so far, as well as review models attempting to explain the sources of this anxiety. The article also presented the most popular methods of anxiety measurement and therapeutic techniques used in the treatment of dental anxiety. Anxiety related to dental appointments constitutes a problem for both the patient and the dentist or dental assistant. It is a barrier to regular dental appointments, affecting oral and whole body health. Coping with dental anxiety requires knowledge about the nature and conditions of dental anxiety. Tellez et al. [8] point to the fact that little is still known about the factors which may affect the level of dental anxiety, including the factors contributing to the development and maintenance of various forms of anxiety disorders. For theoretical and application reasons, this complex phenomenon needs further empirical research, as well as organizing the knowledge obtained in the field of dental anxiety so far.

It is also worth noting that a moderate level of dental anxiety can be a kind of a warning signal, urging to take actions aiming to provide safety to the body, which means that a moderate level of anxiety can also bring some benefits and perform adaptive functions. In her classical concept of "work of worrying", Janis [58] underlines positive aspects of moderate anxiety felt in the process of medical procedures. In her research, Janis reports that patients with moderate preoperative anxiety are more prepared to cope in post-operative circumstances than the patients with a high or low level of preoperative anxiety. According to Janis, this is so because patients with a moderate level of anxiety develop better tolerance to stress, based on a realistic assessment of the situation. The same situation may occur in the case of dental anxiety. It might be the case that moderately anxious patients feel more motivated to take a better care of their oral hygiene. Presumably, also patients with a moderate dental anxiety – in comparison to the patients with a weak and severe dental anxiety – cope better when waiting for an imminent dental appointment, during the appointment and after it, thanks to a more rational approach to waiting and a better mental preparation for a certain level of a discomfort, inseparably associated with dental treatment. The above constitutes an interesting subject of empirical studies.

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REVIEW PAPER

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Ketogenic diet as possible therapy of autism spectrum disorder — review and implication

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ABSTRACT

Autism spectrum disorder (ASD) has become widespread neurodevelopmental disorder, which currently can be treated with only few therapeutic options. Furthermore, their effectiveness is limited therefore novel treatment strategies for ASD are needed. This review seeks to address this need by discussing a ketogenic diet (KD) in the context of ASD therapy. KD effects have been examined in animal and human studies. They indicate effectiveness of KD by improving autistic features. Moreover, animal studies have revealed clinically useful information about caloric restriction component of KD, which is not necessary to achieve therapeutic effects. Significantly administration of KD but not β-hydroxybutyrate or acetone has a therapeutic effect on social interactions. Human studies are scarce, however previous researches imply KD as an effective treatment at least in certain types of autism. KD in an altered form as: modified Atkins diet (MAD), ketogenic gluten-free diet with supplemental medium-chain triglyceride (MCT), and John Radcliffe ketogenic diet is an alternative to classic KD. These variants provide better quality of nutrition and are less strict, thus less difficult to maintain. KD is described as safe with limited, easily manageable adverse effects. Taken together human and animal studies would seem to suggest that KD will become part of ASD treatment. However, in order to determine accurate recommendations for all ASD patients, further studies are required.

Keywords: autism spectrum disorder, ketogenic diet, autism, dietary approach.

Introduction

ASD is a neurodevelopmental disorder of unknown etiology, which is characterized by impairment in reciprocal social interactions and behavior [1]. The term "spectrum disorder" refers to the conditions and their specific symptoms that differ among affected individuals, ranging from severely impaired, low-functioning to mildly affected patients [1]. ASD is typically diagnosed during early childhood by behavioral abnormalities such as hyperactivity, aggression, repetitive behaviors and lack of social communication. The etiology is still not understood, however some risk factors have been implicated in the pathogenesis, including genetics, inborn error of metabolism (IEM), pre/peri/post-natal factors, and interactions between them [2]. These factors affect brain maturation by changing neuroanatomy, synaptogenesis, axon motility and functioning, which, in turn, results in dysfunctional neural networks engaged in socioemotional processing [2]. As shown in neuroimaging studies, the pathophysiology of ASD can generate micro- and macro-effects with disorganized cortical layers, different than normal ratio of short- to long-diameter axons, and overgrowth of grey matter in cortical and subcortical regions in early develop-

ment of the brain [2]. It has been proposed that the disease-associated lesions in amygdala and nucleus accumbens play crucial role in the development of behavioral symptoms of ASD [3].

While there is no cure currently available for patients with ASD, the right support can make an enormous difference in patients' quality of life and ability to function in the society [1, 4]. At present, there is a growing interest in possible dietary intervention as a potential management for ASD. KD appears to be one of the promising therapeutic options for this disorder, however, prospective controlled trials with large sample size are needed for establishing an official recommendations. The "classic" ketogenic diet, originally developed by Wilder in 1921, is a special high-fat, low-carbohydrate diet described by ratio 4:1 (energy from fat: energy from carbohydrate and protein) [5], which has been used successfully to treat drug-resistant epilepsy [6, 7]. Today, several variations of KD have been introduced for treatment purpose, including Radcliffe Infirmary diet, which represents a combination of the traditional and MCT diets, or MAD characterized by fewer protein and caloric restriction [8, 9].

Although the KD is linked with a long list of possible side effects (such as metabolic abnormalities, gastrointestinal symptoms, carnitine deficiency, hypercholesterolemia, renal calculi, cardiac abnormalities, higher risk of bone fractures, kidney stones, and decreased rate of growth), the risk of severe adverse effects is not high [10]. Regarding the clinical management of the KD, it is recommended to consider all pros and cons individually. Since the KD sets up lipids as the major energy source, the absolute contraindications are associated with fat metabolism disorders and include, among others, primary carnitine deficiency, carnitine palmitoyltransferase I or II deficiency, carnitine translocase deficiency, β-oxidation defects, medium-chain acyl dehydrogenase deficiency, long-chain acyl dehydrogenase deficiency, short-chain acyl dehydrogenase deficiency, long-chain 3-hydroxyacyl-CoA deficiency, medium-chain 3-hydroxyacyl-CoA deficiency, pyruvate carboxylase deficiency, porphyria. The list of relative contraindications is brief and comprises: inability to maintain adequate nutrition, surgical focus identified by neuroimaging and video EEG monitoring, parent or caregiver noncompliance [10]. KD works through several combined mechanisms that reduce neuronal excitability. Increased ketones production and restriction of glucose affect ion channels, enzymes, and variety of receptors in the central nervous system. KD additionally enhances adenosine level with concomitant inhibition of DNA methylation. These mechanisms, working together, improve mitochondrial function, alleviate oxidative stress, affect circadian activities and improve synaptic vesicle recycling. The final effects include anti-seizure, neuroprotective, and anti-inflammatory influence of KD [11]. More recently, the therapeutic use of KD in human and animal models of ASD has been studied with positive results. This paper presents selected important findings on the ketogenic diet effects and possible mechanistic insights in ASD affected individuals.

Animal studies – different models, similar behavioral effects

Although large body of evidence indicates beneficial effects of KD for animal ASD treatment, there is much about mechanistic insights that remain unclear, however, some published reports can provide useful suggestions. Obviously, no single pathway is likely to explain the clinical effects of KD. Key mechanisms may include: improved mitochondrial function [12], regulation of neuronal membrane excitability [13], reduced inflammation [14], increased total quantity of bioenergetic substrates [15], or neuroprotection by sparing glucose [16].

Given that different environmental factors during pregnancy are associated with development of ASD in children, prenatal exposure to valproic acid (VPA) is widely used as a reliable animal model of ASD. The VPA-treated mice present, among others, abnormalities in play behavior (decreased number of play initiations/attacks), repetitive behavior, higher nociceptive threshold and bioenergetic dysfunction in mitochondria. It has been found that these abnormalities could be reversed, to some degree, with the KD [17]. KD treatment in prenatal VPA exposed rodents normalized dysfunctions in mitochondrial respiration and significantly improved social impairment [18]. Dai et al. tested protective effect of a ketogenic diet on ultrasonic vocalization, sociability, spatial learning and memory, and electroencephalogram seizures

in glut3 heterozygous null (glut3+/–) mice exhibiting features relevant to ASD. They observed KD-related partial restoration of social features and alleviation of seizure events in male subjects without affecting perturbed vocalization, spatial learning and memory. They have also found that neuroprotection of females results from higher circulating and cerebrospinal fluid ketone concentrations and/or lower brain Glut3 concentrations [19].

Testing mutant EL mice with comorbid epilepsy and ASD symptoms, Ruskin et al. have found a clear sex-related difference in response to the beneficial effects of KD. They used two ketogenic diet formulas: with a 6,6:1 and 3:1 ratio of fat: (carbohydrate + protein) and found that caloric restriction component of KD is not necessary to achieve therapeutic effects in this model. Feeding with both types of KD improved multiple measures of sociability and reduced repetitive behavior in female mice, with limited effects in males [20].

Complete understanding of sex-specific changes may provide an insight into unique factors that may contribute to the partial protection and lower prevalence of ASD in females [21].

A high fat, moderate protein, and low net-carb diet was also used in the experiments with BTBRT+Tf/J mice. These animals display behaviors consistent with diagnostic features for ASD (impaired social interaction and communication and increased repetitive behaviors). Rutskin et al. described improvement in behavioral symptoms of ASD, expressed by decreased self-directed repetitive behavior and better social communication, in ketogenic diet-fed BTBR mice [22]. Mychasiuk et al. observed positive effects of KD administration on ASD deficits associated with myelin formation and white matter development in BTBRT+Tf/J mice [23]. Additionaly, based on spontaneous intrahippocampal EEGs and tests of seizure susceptibility, they found that behavioral improvements are dissociable from any antiseizure effect of KD.

Based on high-resolution intracortical microstimulation, findings from BTBR mouse model of ASD have documented imbalance in excitation to inhibition and aberration in cortical motor maps. Importantly, the KD appeared to be effective in reversing both of these abnormalities [24].

The two other studies have shown no significant effect of KD on tested brain parameters in BTBR model of ASD [25, 26]. Because abnormal mitochondrial function of neurons plays crucial role in the pathophysiology of ASD and mitochondria itself represent the metabolic endpoint for dietary foodstuffs, these studies focused on examination of mitochondrial dynamics in BTBR mice after administration of KD. The first study was scheduled to determine whether KD induces changes in brain and liver protein O-linked-β-N-acetyl glucosamine (O-GlcNAc), which patterning is usually abnormal in ASD epilepsy [25]. The second one, analyzed impact of KD on mitochondrial gene expression and proteins levels in the brain and liver [26]. Both experiments have shown tissue-specific effects of KD with evident changes (reduced global O-GlcNAc and increased mitochondrial turnover) in the livers and no disturbances in brain dynamics. This suggests that other than tested mechanisms are involved in beneficial activity of KD for BTBR mice.

Recently, it has been shown that ASD coexists with altered gut microbiota in a BTBR murine model of ASD [27, 28]. Although the exact mechanisms remain unknown, the therapeutic effectiveness of KD may partially result from the restoration of the correct gut microbial composition. This observation allows researchers to consider gut microbial abundance and diversity as a possible factor capable to mitigate some of the ASD symptoms in humans. However, further research investigating the microbiota in the context of dietary intake and severity of ASD is needed.

Another tested animal model of ASD is associated with mutations in the En genes. Mice with deletion of the En2 gene from birth demonstrate behavioral impairments typical for ASD due to the several anatomic changes in the cerebellum and hippocampal region of the brain and defects in monoamine system [29]. Verpeut et al. conducted experiments with En2 knockout mice exposed to KD from post-natal day 21 to 60. The early timing of dietary intervention was recognized as being important for the brain reorganization and maturation influenced by nutrition. Although 2 null mice (En2(-/-)) displayed no altered monoamine content in the forebrain regions, the increased frontal social contact and reduced grooming behavior were evident in response to KD intervention.

To weigh up the effects of KD and administration of an exogenous ketones, an interesting study was performed on wild type Long-Evans(LE) rat males, a model with behavioral characteris-

tics of autism spectrum disorder and comorbid epilepsy [30]. Authors compared behavioral outcomes following exposure to a ketogenic diet versus β-hydroxybutyrate or acetone administration and noted improvement in social interactions only in KD-fed animals. This suggest that therapeutic effect of the KD is more complex than simply raised β-hydroxybutyrate or acetone blood-levels.

A number of epidemiological studies have reported increased risk of ASD associated with maternal infection during pregnancy and maternal immune activation (MIA) hypothesis has been widely tested in animal models [31]. Using synthetic agents that induce MIA in mice, Ruskin et al. have demonstrated that male MIA offspring were significantly asocial in the three chamber sociability test, while female mice displayed normal and social behavior [32]. Within 3–4 weeks of KD treatment the lack of sociability in male offspring reversed completely and reduced MIA-elevated self-directed repetitive behavior was observed. This model seems to be of particular importance because it mimics clinically-common conditions whereby ASD incidence is increased by maternal infection during pregnancy in humans.

Human studies

Current data indicate that at least certain types of autism respond to KD treatments in humans. At this time, with few therapeutic options, new treatment strategies for ASD are needed, but considering the possible adverse effects of KD, the intervention requires high-quality scientific evidence about effectiveness and safety. Therefore, ongoing studies are looking for an optimal, safe and well tolerated dietary modifications.

Clinical benefits of ketogenic diets

El-Rashidy et al. designed a prospective clinical interventional study on 45 ASD children, aged 3–8 years, to compare the effect of MAD (n = 15) and gluten-free and casein-free diet (GFCF) [33]. MAD is similar to the classic KD but is less restrictive, has no limit on calories or protein, and the lower overall ketogenic ratio does not need to be maintained in all meals. At 6-month follow-up, the Childhood Autism Rating Scale (CARS) and Autism Treatment Evaluation Test questionnaire (ATEC) showed significant improvements in both, MAD and GFCF-fed patients including speech, social and cognition parameters. On the other hand, several systematic reviews focusing on pure GFCF in ASD reported inconclusive results and definitely further efforts must be made to identify the group of ASD patients who may be the best responders to this intervention [34,35]. In another clinical trial, Lee et al. used a modified ketogenic gluten-free diet with supplemental MCT in 15 patients aged 2 to 17 years [36]. After 3 months of observation, they reported significant improvements in CARS-2 and ADOS-2 (Autism Diagnostic Observation Schedule) items without restricted and repetitive behavior scores.

Beneficial effects of modified KD have been reported in a 1-year prospective uncontrolled study conducted on thirty children, aged 4–10 years, with autistic behavior [37]. Patients received the John Radcliffe ketogenic diet, which is a variation of the medium-chain triglyceride diet and, as reported by parents and caregivers, is less restrictive and easier to implement practically than classic KD. Moreover, to prevent adverse effects that may accompany KD administration, the diet was implemented in a 4-week intervals followed by 2 weeks break. Interestingly, such a regimen provided long lasting effects and the improvements persisted even after termination of the trial in 60% of study participants (18 out of 30 patients) with better response in mild cases of ASD.

Herbert and Buckley presented a case of 12 year old autistic girl with comorbid autism and epilepsy put on gluten-free casein-free KD [38]. In their remarkable case study, they described significant reduction in seizures and improved cognitive and behavioral function accompanied with successful management of morbid obesity subsequent to initiation of modified KD. The main rationale for using a casein-free, medium-chain-triglyceride-predominant ketogenic diet was to achieve ketosis with a much lower ratio than is typically needed and to provide better quality of nutrition (more calories were available for vegetable consumption) when compared with pure KD.

Tolerability and adverse effects of KD

The concept that the ketogenic diet may be neuroprotective has raised the possibility to use it as an additional or alternative therapy among children with autistic behavior, especially those with epileptic events. To achieve the benefits of this

dietary management, patients need to be adhered to the prescribed KD for at least 6 months to 1 year [39]. A medical consequences of the ketogenic diet, in terms of side effects, may include constipation, diarrhea, vomiting, dehydration, kidney stones, slow growth, osteomalacia, cardiomyopathies, gout, hypocalcemia, hypomagnesemia, acidosis, vitamin D deficiency, hypoproteinemia, hypoglyceamia, iron deficiency, hyperlipidemia, lack of energy and increased susceptibility of infections. The list is long, but the most of the complications are usually transient, easily manageable and limited [39, 40].

In a large Scandinavian retrospective study, 290 KD-fed children were investigated over two years follow up [41]. Side-effects were noted in 29 subjects and most of them were treatable. Only 4 patients needed to stop the therapy (due to hyperlipidaemia and to kidney-stones).

The relative effectiveness and tolerability of KD in ASD patients has been well documented in an online survey-based study that was conducted on large population (733 children with ASD and clinical seizures, subclinical epileptiform discharges or seizure-like activity and 290 controls) [42]. The questionnaire was validated and very detailed in documenting seizures and ASD core symptoms through parental reports while implementing anti-epileptic drug (AED) or non-AED treatment. Among non-antiepileptic drug (non-AED) treatments the KD was the third (after vitamin B6 and steroids) most commonly used intervention and, as compared with GFCF, was thought to decrease seizures significantly more. In addition, the survey provided an information about most common adverse effects regarding type of management. Ketogenic and Atkin's or modified Atkin's diet tended to result in drowsiness, tiredness, fatigue, constipation or diarrhea. 27% of responders declared one mild side effect that occured during therapy with KD, 13% declared two and only 4% declared 3. Interestingly, AED treatments, except for ethosuximide, were reported to have a higher rate of adverse effects as compared to non-AED treatments, especially with respect to severe adverse effects.

Summary

Although a number of clinical studies and reviews described KD as being relatively safe, this therapy requires careful complete physical and laboratory examination prior to the diet's initiation, and regular follow up visits. Moreover, to meet the specific medical needs of each patient an individualized dietary plan should be developed with caution by the patient's healthcare providers.

Based on the current scientific data, KD holds promise for ASD affected patients as an alternative treatment strategy. However, human studies in this field are scarce and establishing accurate recommendations for all ASD patients requires further large multicenter trials.

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CASE STUDY

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Bicompartmental locked bucket-handle tears of menisci concealing the concomitant anterior cruciate ligament injury for 2 years – a case report

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ABSTRACT

Locked bucket-handle tears of both medial and lateral menisci, called in literature "Jack and Jill lesion", with simultaneous anterior cruciate ligament (ACL) injury is an extremely unusual phenomenon. It was reported only in a few cases in literature, which were unfortunately highly differentiated in the field of trauma mechanism, treatment options and postoperative care. Authors presented a clinical case of patient with a locked knee joint, by the torn both menisci, what masked the simultaneous ACL rupture and supremely imitated a stable joint. The locked knee is a clinical case demanding the urgent intervention to prevent further damages to the joint structures. Surprisingly, the patient after locking of the menisci and migration of their inner fragments to the intercondylar notch, started to feel the affected joint stabile and dismissed from the further treatment for next 2 years. Complex trauma needs a complex treatment, and a single-stage or multi-stage surgical approach is performed. Authors chose a two-steps surgical procedure with primary meniscal repair and secondary, early ACL reconstruction.

Keywords: meniscal tear, ACL tear, locked knee.

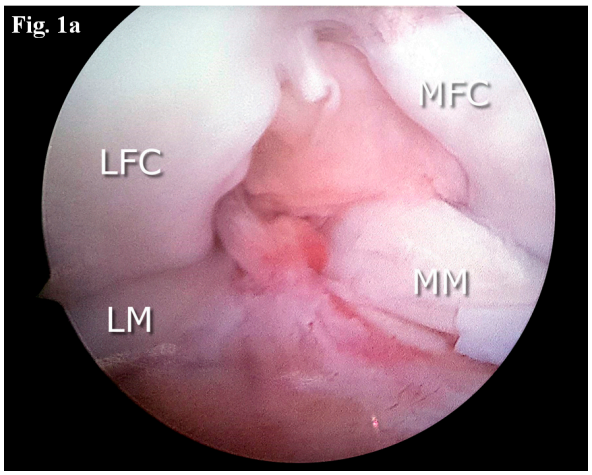
Introduction

Knee menisci are fibrocartilaginous structures, important in such actions as load transmission, absorption of the shocks and joint stabilization. Meniscal tear is a common result of the knee joint trauma, especially twisting, and may cause pain and persistent functional impairment of the lower limb [1]. Meniscal lesions are classified according to location, size, pattern of tear (horizontal, vertical, radial, oblique), etiopathology (degenerative or traumatic), dislocation of the fragments (bucket-handle tears and parrot-beak) [2, 3]. Locked bucket-handle tears are not frequent type and locked bucket-handle tears of both menisci is called in the literature "Jack and Jill lesion" [3]. Simultaneous ACL injury is an extremely rare phenomenon and were reported only few highly differentiated cases in the literature [4, 5, 6]. Moreover data regarding to the mechanism of this complex trauma, treatment and postoperative care have not been well explained and established yet.

We presented a clinical case of patient with torn both menisci that masked the simultaneous ACL rupture and supremely imitated the stability of the knee joint.

Case report

A 22 year-old male patient with a history of right knee joint trauma was admitted to the Orthopedic Department for a surgical treatment with a suspicion of ACL lesion and medial meniscus horizontal tear without dislocation. There was no previous history of the knee injury or pathologic symptoms in the affected limb. The trauma occurred during stepping off the stairs two years ago. According to recorded data from the Emergency Department, the injured knee was swollen, painful, range of movement was limited to 40° of flexion and there was tenderness in the medial part of joint line with no signs of instability during the clinical evaluation. The antero-posterior and profile X-rays were normal and did not show any signs of a bone injury or pathology. The Magnetic Resonance Imaging (MRI) performed two weeks after trauma revealed complete ACL rupture and longitudinal medial meniscus (MM) tear. Two years later patient decided for the arthroscopic treatment due to reported temporary, mild pain in the medial and lateral compartments, locking of the knee joint but negated symptoms of instability. Preoperative physical examination in the Orthopedic Department revealed moderate joint effusion, range of motion 10–120° of the affected knee joint, negative ACL stability tests. Meniscal tests were positive for MM and lateral meniscus (ML).



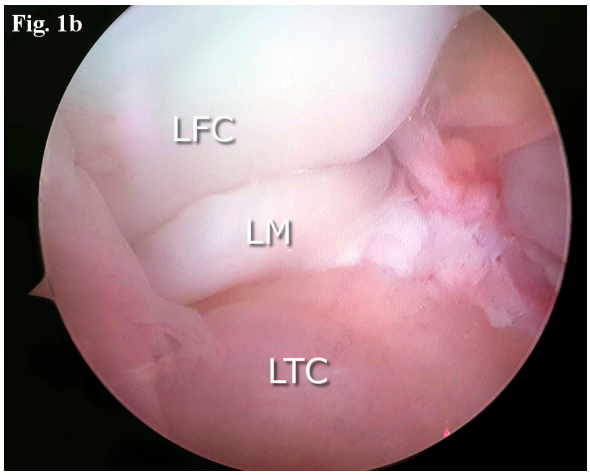
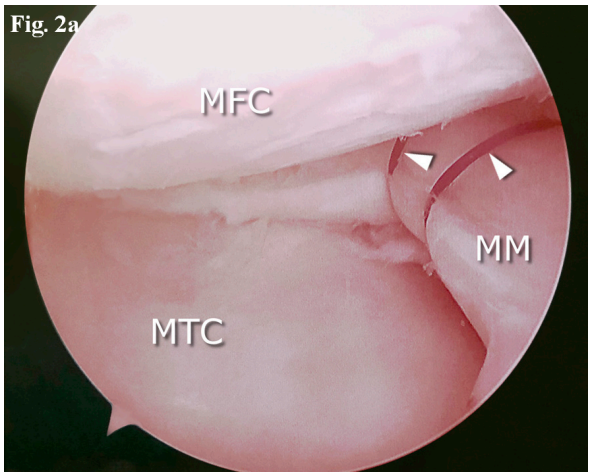


Figure 1. a. Arthroscopic picture illustrating both MM and ML locked. b. Arthroscopic picture illustrating locked ML (MM — medial meniscus, LM — lateral meniscus, LFC — lateral femoral condyle, MFC — medial femoral condyle, LTC — lateral tibial condyle)



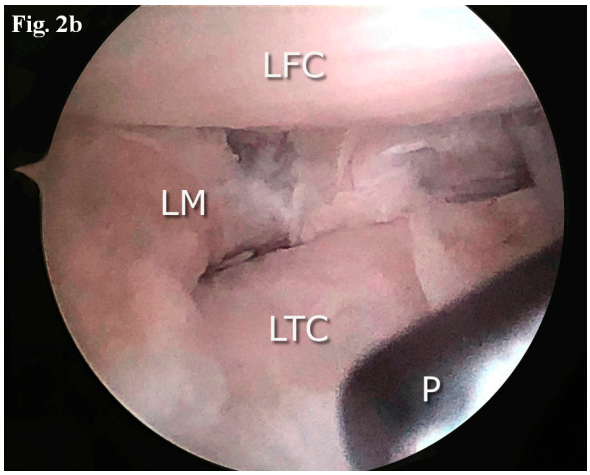


Figure 2. a. Arthroscopic picture illustrating sutured MM. b. Arthroscopic picture illustrating partially resected ML (MFC — medial femoral condyle, MTC — medial tibial condyle, MM — medial meniscus, arrowheads — sutures, LTC — lateral tibial condyle, LFC — lateral femoral condyle, LM — lateral meniscus, P- arthroscopic probe/hook)

Arthroscopic inspection was performed under spinal anesthesia. During knee joint arthroscopy bucket-handle tears of both MM and ML were found with the inner fragments displaced into the intercondylar notch. The ACL was completely ruptured with atrophy of the stumps and the PCL was intact. There were foci of chondromalacia 2° stage, according to The International Cartilage Repair Society classification on the both femur condyles. After reduction of both menisci by the arthroscopic hook and detailed investigation of the injury side, it was decided to perform partial meniscetomy of the torn and degenerated fragment of the ML. The MM was repaired using all-inside and outside-in technique, totally with 4 sutures. According to postoperative protocol brace and crutches were used for six weeks. The torn ACL treatment was planned as a second-stage arthroscopic reconstruction, in 6–8 weeks after the primary operation. Informed consent of the case report was obtained from the patient.

Discussion

Locked bucket-handle tears of both MM and ML with simultaneous ACL rupture is an extremely rare pattern of the knee joint injury, with individual approach to the treatment.

A majority of cases when both meniscus locking is associated with sport activities: skiing, volleyball, basketball, football [2, 7, 8, 9]. The mechanism of this injury is usually a result of high-energy valgus trauma or hyperextension trauma with rotation [2]. The bucket-handle tear of meniscus is commonly associated with anterior cruciate ligament rupture however it seems to be interesting that the moment of the meniscus locking sometimes does not occur together with the ACL rupture [9]. Koukoulias et al described locked bucket-handle tears of both MM and ML with simultaneous ACL and medial collateral ligaments injury in a male patient who had fallen from the height with the valgus mechanism of the trauma [5]. In our case the trauma was work-related, low-energy and probably meniscus locking appeared as a multi-stage process; the MRI performed 2 weeks after trauma did not reveal the "Jack and Jill lesion" in the beginning. We conclude that moment of meniscus locking occurred in a early few weeks after injury and prevented the sensation of joint instability in ACL deficient knee. Shepherd et al described a similar case, that appeared as a multi-stage process in a period of two years, however their patient had an unstable knee joint [9].

The locked knee is a clinical case when the urgent treatment is strongly advised. A choice of single-stage or multi-stage surgical approach depends on operator's experience, number and difficulty of the required procedures. Cetik et al made a single-stage partial meniscetomy and ACL reconstruction [6]. Shelbourne et al advised a two-stage procedure to reduce the complication rate [10]. We chose a two-stage procedure and in our opinion meniscus repair should be based on degeneration of its structure, however it is important to protect the operated limb in the brace in the period between surgical procedures.

The MRI is a highly sensitive and specific diagnostic technique for bucket-handle tears of menisci [3]. There are described various signs for this pathology which facilitate the proper identification [8]. Ultrasound is a standard imaging modality of the knee joint however it should be clearly stated that the role of the ultrasound in meniscal tears diagnosis is controversial, with sensitivity and specificity from as low as 60% and 21% to as high as 90% and 83%, respectively [11]. Our case presented that the locking of the bucket-handle tears of menisci, with the concurrent ACL rupture can appear surprisingly and how important is to suspect locking of the meniscus during physical examination.

Conclusion

Early and proper arthroscopic treatment allows to avoid progression of the knee structures damage and to restore knee stability and function.

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Conflict of interest statement
The authors declare no conflict of interest.

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