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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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ORIGINAL PAPER

JMS Journal of Medical Science

Birthweight for gestational age: standard growth charts for the Polish population of full-term infants

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ABSTRACT

Introduction. Birthweight is one of the most important factors determining neonatal well-being. From an epidemiological viewpoint, a neonatal reference chart provides a picture of the health status of a population. Global customized growth charts seem to be the most practical in multicultural settings, allowing adjustment for ethnicity. However, regional charts might be a valuable contribution to reliable growth assessment.
Aim. Our study aims to establish a reference tool for growth assessment and visualize the local potential, by creating standard charts based on the data from the tertiary center with the highest number of deliveries per year in Poland.
Material and Methods. We retrospectively analysed 31,353 records from the electronic database of singleton births from a five-year period from a tertiary hospital in Poznań, Poland. We excluded pre-term deliveries and high-risk pregnancies basing on well-known factors influencing fetal growth, bringing the number of records to 21,379. The data were processed separately by gender (girls n = 10,312, 48.2% and boys n = 11,067, 51.8%). Percentiles were calculated for each week of gestational age. Means and standard deviations were determined.
Results. Standard growth charts (including 3rd, 10th, 25th, 50th, 75th, 90th and 97th percentiles) are presented. Descriptive data of population distribution are shown.
Conclusions. In conclusion, obtaining standard growth charts for mature newborns has created the opportunity for a more actual and adequate assessment of the Polish neonatal population. It should allow for the implementation of new standards in future research on perinatal care.

Introduction

Birthweight is one of the most important factors determining neonatal well-being [1]. Either large for gestational age (LGA) or small for gestational (SGA) fetuses are predictors of adverse outcomes. Birthweight is strongly correlated with gestational age and sex of the neonate, maternal and paternal anthropometric parameters, and mother's comorbidities, i.e., diabetes, hypertension, or smoking status. Birthweight is also associated with ethnicity, access to modern healthcare, and proper nutrition, making this attribute strongly dependent on place of birth and susceptible to significant variability [2–4].

From an epidemiological viewpoint, neonatal reference charts provide a picture of the health status of a population. The comparison of charts referring to different and clearly defined populations living in the same country, or different countries, or to the same population in different periods is a way of measuring the extent of inequalities in health between populations. From a clinical viewpoint, a neonatal chart is an essential tool to detect neonates at higher risk of neonatal and postnatal morbidity and growth impairment. At present, further clinical studies are needed to reach a consensus on combining neonatal and prenatal information to discriminate neonates with growth derangements, such as intrauterine growth retardation or overgrowth [5–7].

Global customized growth charts seem to be the most practical in multicultural settings, allowing adjustment for ethnicity [8]. In Europe and in Poland, Fenton Growth Charts and Intergrowth project charts [6] are the most commonly used. However, it has been suggested that such a uniform attitude may result in a significant bias, with a risk of over- or under-estimation of LGA and SGA [9–12]. Therefore, regional charts might be a valuable contribution to reliable growth assessment. Updating neonatal charts has become necessary due to changes in parity, maternal age and weight, but also in socio-economical or environmental conditions, and obstetric or neonatal care.

Kajdy et al. recently analysed 39,032 electronic database records of singleton live births from one hospital in Warsaw between 2010–2016. Subsequently, the authors published reference growth charts for premature and mature newborns (from the 24th to the 41st week of gestation) [6]. The most recent regional growth charts were published in 1995 and 2003, respectively [1, 16]. However, Gadzinowski et al. [16] published reference growth charts, whereas Malewski et al. excluded major defects and developmental anomalies that could influence birthweight (hydrocephalus, anencephalus, hydrops fetalis, etc.). Our study aims to establish a reference tool for growth assessment and visualize the local potential, by creating standard charts based on the data from the tertiary center with the highest number of deliveries per year in Poland.

Material and Methods

This retrospective study is based on a five-year analysis period (from February 2017 to February 2022). A total of 31,353 computed records from the electronic database of singleton births at Poznan Obsterics and Gynecological University Hospital were considered. Approximately 99% of patients in the database were Caucasians.

Gestational age was verified and confirmed by the last menstrual period and ultrasound in the first trimester, both as described in the ACOG Committee Opinion [13]. The data were filtered depending on the mother's age (20–40 years) and gestational age at birth (36–42 weeks), bringing the number of analyzed neonates from 31,353 to 28,311. This count was further reduced to 21,379 by excluding pediatric and obstetric conditions with an important impact on development in utero (ICD-10): F17.2, O13, O14.0, O14.1, O24.0, O24.4, Q03.9, Q04.2, Q05.2, Q07.0, Q20.3, Q21.0, Q21.1, Q21.2, Q21.3, Q22.5, Q36.9, Q37.8, Q37.9, Q41.0, Q45.9, Q52.8, Q60.0, Q73.8, Q77.4, Q79.0, Q79.3, Q79.9, Q89.7, Q90.9, Q91.3, Q91.7, Q96.9.

The following records were excluded from the study: fetuses and neonates with abnormal karyotype, major congenital defects, infections, and stillbirth. Minor congenital defects, as defined by the European Surveillance of Congenital Anomalies, were included. High-risk women had the following characteristics: aged < 20 and > 40 years, diabetes mellitus, gestational diabetes mellitus, pregnancy hypertension, pre-pregnancy hypertension, preeclampsia, haemolysis, elevated liver enzymes and low platelets (HELLP) syndrome, or cholestasis of pregnancy.

The data were processed separately depending on the sex of the neonate: females (n = 10,312, 48.2%) and males (n = 11,067, 51.8%). Percentiles were calculated 100 times for each week of gestational age through random sampling of 80% of the population without replacement (bootstrap). Locally estimated scatterplot smoothing (LOESS) was used to plot the data in R (tidyverse). The data are presented graphically and within nomograms for the 3rd, 10th, 25th, 50th, 75th, 90th, and 97th percentiles (from P3 to P97), as well as means and standard deviations. Percentiles for each week were also calculated without smoothing and presented in tables. To compare the currently investigated population with the population previously studied by Gadzinowski et al. [18] and Malewski et al. [1], we compared means and standard deviations. This was done using a 100-fold bootstrap procedure with distribution-preserving random sampling of 1000 values in both groups, and subsequent comparison using the Student's t-test (means) and the F-test (variance). This set-up enabled relative comparison of the importance of differences through the minimalization of the impact of sample size on p-values. The analyses were conducted in R 4.1.3 (R Foundation for Statistical Computing, Vienna, Austria).

Results

A total of 21,379 newborns were included in the final analysis. The sex and age distribution are presented in **Table 1**. Boys, on average, were heavier approx. by 5%. Significant differences were observed for all studied gestational age subgroups.

Growth charts of body weight (showing P3, P10, P25, P50, P75, P90, P97) for the term infants are shown in **Figures 1–2**, whereas the means and standard deviations are documented in **Table 2**. Percentiles calculated without smoothing are presented in **Table 3**.

Table 1. Number of births by gestational age and sex

Week of gestation*	Study subgroups [N/%]	
	Boys	Girls
37	866 (8.0)	693 (6.9)
38	2197 (20.4)	2020 (20.1)
39	3541 (32.9)	3320 (33.1)
40	2953 (27.5)	2829 (28.2)
41	1156 (10.8)	1148 (11.4)
42	39 (0.4)	30 (0.3)

* week "n" is defined as age from "n" weeks up to "n" weeks +6 days.

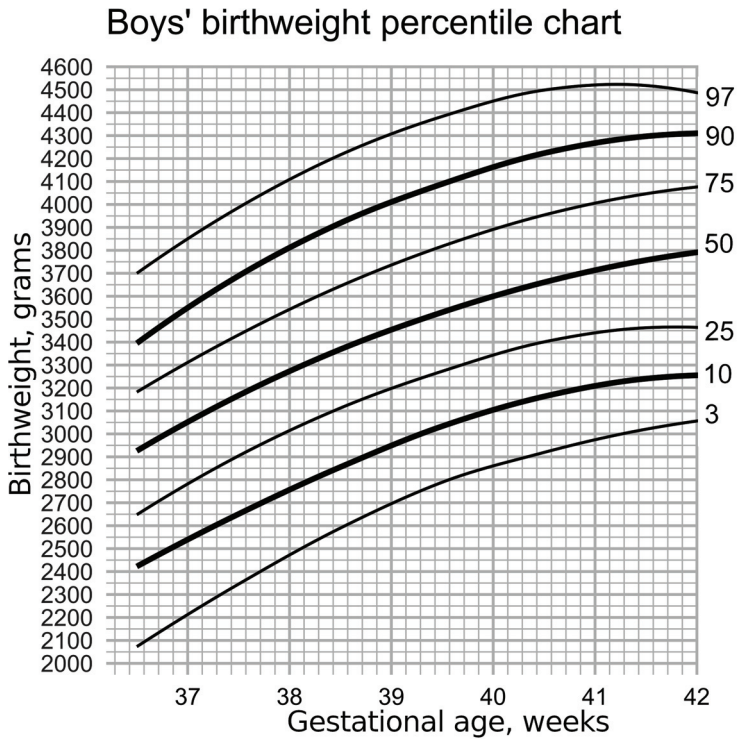

Figure 1. Growth charts of boys' body weight (showing P3, P10, P25, P50, P75, P90, P97) for the term infants

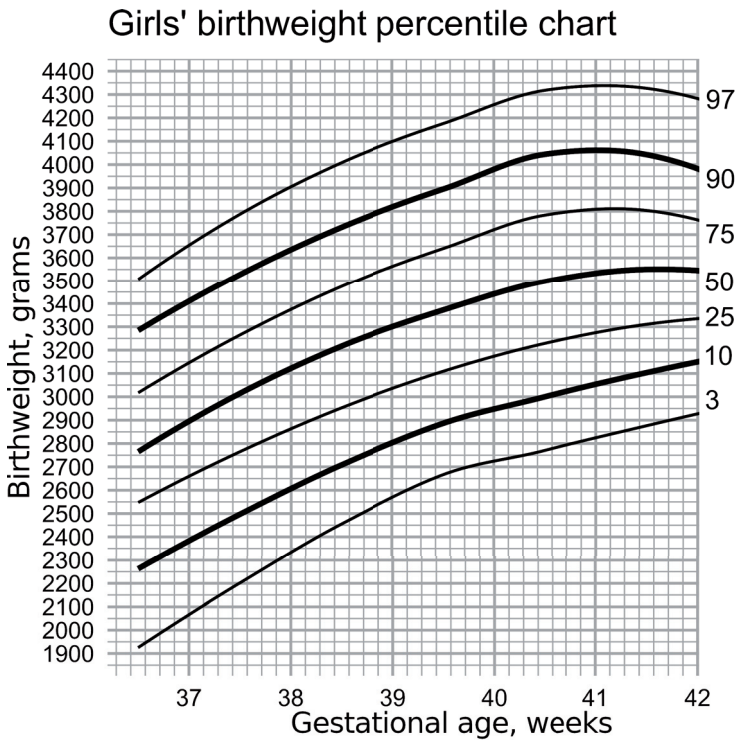

Figure 2. Growth charts of girls' body weight (showing P3, P10, P25, P50, P75, P90, P97) for the term infants

Table 2. Average birthweight of infants by gestational age and sex

Week of gestation*	Birthweight [grams] Mean ± SD		Sex difference p
	Boys	Girls	
36	2920 ± 429	~~2778 ± 415~~	5.34×10^{-5}
37	3157 ± 440	2998 ± 430	1.03×10^{-12}
38	3388 ± 421	3226 ± 398	4.42×10^{-37}
39	3545 ± 428	3383 ± 415	3.20×10^{-56}
40	3677 ± 424	3498 ± 408	8.29×10^{-59}
41	3775 ± 412	3598 ± 400	3.78×10^{-25}
42	3790 ± 434	3522 ± 326	0.00467
All neonates	3525 ± 467	3366 ± 447	1.56×10^{-141}

* week "n" is defined as age from "n" weeks up to "n" weeks +6 days.
SD – standard deviation

Table 3. Average birthweight of infants by gestational age and sex

Week of gestation*	Birthweight [grams] Mean ± SD		Sex difference p
	Boys	Girls	
36	2920 ± 429	~~2778 ± 415~~	5.34×10^{-5}
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39	3545 ± 428	3383 ± 415	3.20×10^{-56}
40	3677 ± 424	3498 ± 408	8.29×10^{-59}
41	3775 ± 412	3598 ± 400	3.78×10^{-25}
42	3790 ± 434	3522 ± 326	0.00467
All neonates	3543 ± 456	3382 ± 437	3.27×10^{-147}

* week "n" is defined as age from "n" weeks up to "n" weeks +6 days.
SD – standard deviation

Within the currently studied population older neonates significantly were heavier than in historical data from Gadzinowski et al. [16]; this was true for the majority of age subclasess in the case of Malewski et al. research [1]. Similarly, significantly less variance was observed. In the present study, babies born at 40th and 41st week of pregnancy had 1-2% higher body weight and up to 10% less variance in mass than those in the first mentioned study [16]. This trend was found both in male and female neonates. Differences in body weight were more pronounced (4-6%) and spanned from 38th to 41st week of pregnancy when comparison was made between the current data and parameters reported in the second study [1]. Furthermore, the variance in the population investigated by Malewski et al. was greater by up to 17%. The use of previous growth charts would result in improper classification of significant part (even up to one third for the second study) of 10 top and down percent of newborn population.

Discussion

We analysed a large and up-to-date cohort of term newborns from low-risk pregnancies, and created standard growth charts that reflect ideal (normal) growth. Such an attitude seems to be most reasonable for babies born at term. Therefore, in the present study we assessd exclusively children born between 37th and 42nd week of gestation. In contrast, reference growth charts (including both low- and high-risk pregnancies) could be considered more appropriate for preterm neonates and better reflect overall population. Since in the recently studied cohort by Kajdy et al. in another Polish region [6] and investigated in the past in our region by Gadzinowski et al. [16] and Malewski et al. [1] high-risk (all for the latter study significant part of high-risk) pregnancies were included it is difficult to directly compare our results to those data. It is worth underlining that our study was carried out in an ethnically homogenous population, in a region with good access to medical care.

Neonatal growth charts help in identifying values that best discriminate between infants at high and low risk of complications later in life [14]. Historically, studies on fetal growth were primarily associated with low birth weight Birthweight, but the problem of fetal overgrowth increased in the last decades. Detecting LGA fetuses is important to prevent shoulder dystocia, peripartum hemorrhage, or cesarean section, and prevent the risk of maternal diabetes, and metabolic syndrome in childhood [15] SGA is a condition in which the fetus is smaller than expected in the absence of any pathological conditions or toxic factors. SGA fetuses may be compromised, and thus need to be prematurely delivered. Such newborns are at risk of hypoglycemia, hypoxic-ischemic encephalopathy, gastrointestinal bleeding, polycythemia, pulmonary hemorrhage, apnoea, disseminated intravascular coagulation, and prolonged hospitalization [16].

Neonatal growth standards (reflected by growth charts) may differ due to essential differences in parity, maternal age, and prevalence of malnutrition/obesity. The availability and quality of obstetric or neonatal care may also exert a significant impact. All these factors create the basis for potential country/regional differences in growth references (charts). Moreover, growth charts should be updated in conformity with the intensity of the "secular trend of growth" and observed socio-economic changes in the population (e.g., every 10–20 years) [17–18]. The comparison of results obtained in the present study with previous data [1, 16], even considering differences in populations included and methodological issues, suggest the existence of a mentioned secular trend. Consequently, the sensitivity of older norms to pathology would be reduced, resulting in improper classification of up to a third of children in the extreme 20 percent (including both SGA and LGA) of the current newborn population, where high precision is required.

In conclusion, obtaining standard growth charts for mature newborns has created the opportunity for a more actual and adequate assessment of the Polish neonatal population. It should allow for the implementation of new standards in future research on perinatal care.

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

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ORIGINAL PAPER

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Cytokine profile in childhood asthma

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ABSTRACT

Childhood asthma is a chronic airway disease, which pathogenesis is markedly heterogeneous–with multiple phenotypes defining visible characteristics and endotypes defining molecular mechanisms. Cytokines and chemokines released during inflammatory responses are key immune mediators. The cytokine response can largely determine the susceptibility to childhood asthma and its severity. The purpose of this study was to characterize the immune profile of childhood asthma. The study involved 26 children (3–18 years old), who were divided into 2 groups: study–with childhood asthma; control–without asthma. The innovative Bio-Plex method was used to determine the serum concentration of 37 inflammatory proteins in one experiment. The results were analyzed using univariate statistical tests. In the study group, the level of the 10 tested markers increased, while the level of the remaining 9 decreased compared to the control; a statistically significant reduction in concentration was obtained only for the MMP-1 ($p < 0.05$). According to the ROC curve, MMP-1 can be considered an effective discriminator of childhood asthma ($p < 0.05$; AUC = 0.752). Cytokines/chemokines may be useful in the diagnosis of childhood asthma and may also become a prognostic target in determining the phenotype/endotype of this condition. This study should be a prelude to and an incentive for more complex proteomic analyzes.

Introduction

Childhood asthma has become a public health problem worldwide [1]. The global prevalence of asthmatic symptoms among 6-7-year-olds is 11.5% and 13-14-year-olds 14.1% [2]. Moreover, the number of children suffering from asthma continues to grow [3]. In the face of this situation, the most important thing is to improve and optimize diagnostic and therapeutic procedures. Currently, the correct, early diagnosis of asthma in children constitutes a huge challenge. Symptoms of childhood asthma may be atypical or overlap with other respiratory diseases characterized by obstruction of the airways [4]. The criteria used for diagnosis may also vary according to different guidelines, studies, and among clinicians. Furthermore there is difficult to perform objective pulmonary function tests at such an early age [4]. Diagnosis, as well as predicting response to treatment, may be made easier by recognizing childhood asthma phenotypes/endotypes, that is, the underlying pathophysiological and/or molecular mechanisms [5]. There are significant differences between childhood and adult asthma regarding immunology, histopathology, and clinical symptoms. Many researchers are still trying to identify specific biological markers of childhood asthma that would assist in the diagnosis of this disorder. Finding these compounds is extremely important as accurate diagnosis and optimal treatment play a key role in the proper functioning of children with asthma and can significantly improve their quality of life [6]. Currently, there is still a need to search for new, more precise, and accurate diagnostic methods, differentiating phenotypes and endotypes of asthma so that a complete, personalized approach to the patient will be possible.

Asthma is characterized by immune system activation, airway hyperresponsiveness (AHR), epithelial cell activation, mucus overproduction and airway remodeling. The immune system is thought to be a regulator of asthma and airways inflammation. Both innate and adaptive immunity play roles in immunologic mechanisms of asthma [7]. Important mediators of immunity are cytokines and chemokines [8]. They are secreted during inflammatory response [9]. Cytokines regulate the production of other inflammatory mediators and chemokines attract leucocytes to the site of inflammation [9]. The cytokine response due to an imbalance or deficiency in the cytokine network can largely determine the susceptibility to childhood asthma and its severity. Inflammatory factors may be useful in identifying chronic airway inflammation in childhood asthma and could be a promising prognostic target in phenotype/ endotype differentiation [9]. Many cytokines are involved in asthma pathogenesis, including: interleukin-1β (IL-1β), interleukin-2 (IL-2), interleukin-4 (IL-4), interleukin-5 (IL-5), interleukin-6 (IL-6), interleukin-8 (IL-8; CXCL8), interleukin-18 (IL-18), C-C motif chemokine ligand 3 (CCL3), macrophage migration inhibitory factor (MIF), C-C motif chemokine ligand 5 (CCL5; RANTES), interferon gamma-induced protein 10 (IP10; CXCL10), tumor necrosis factor α (TNF-α) and interferon gamma (IFN-g) [9–11]. Furthermore circulating levels of proteins are dynamic and modifiable and therefore amenable to therapeutic targeting [8].

Aim

The aim of this study is to characterize the cytokine profile of childhood asthma by determining the serum concentrations of 37 circulating inflammatory factors, which are probably involved in the pathogenesis of this condition. Our research findings could be helpful in the search for new biomarkers and therapeutic targets for childhood asthma. By learning more biomarkers, it will be possible to diagnose, monitor and adjust treatment of asthma. The use of the Bio-Plex system enabled the study of thirty-seven inflammatory factors in one experiment. This method quantified a biologically relevant target using fluorescently dyed magnetic beads [12].

Material and Methods

Patients and Serum Samples

Twenty-six participants aged 3-18 were qualified for the experiment and divided into two groups. The study group consisted of children with asthma (n = 11) and the control group of children without asthma and allergies (n = 15). The control group was appropriately matched to the study group in terms of gender, age, and ethnicity. **Table 1** presents the characteristics of patients from the asthma group and the control

Table 1. Characteristics of patients with asthma (study group) and without asthma and allergies (control group)

Characteristics of the Participants *(n = 26)*		Asthma (Study Group) *(n = 11)* No (%)	No Asthma (Control Group) *(n = 15)* No (%)
Age			
– Median – Range		12 4-16	10 3-18
Sex			
– Female – Male		5 (45.5) 6 (54.5)	6 (40) 9 (60)
Asthma Severity			
– Mild – Moderate – Severe		5 (45.5) 4 (36.3) 2 (18.2)	
Budesonide or Equivalent (The daily dose of GCs)			
– 100 – 200 µg – 250 – 350 µg – 400 – 500 µg – >500 µg – No information		4 (36.3) 2 (18.2) 3 (27.3) 1 (9.1) 1 (9.1)	
Comorbidities			
– Atopic Dermatitis – Hypoacusia – Allergic Rhinitis – Cholecystitis – Coeliac Disease		1 1 3 1 1	
Total IgE (kU/l)			
– Mean – Range		278.61 15.5-1035	96.29 12.2-797
Lung Function (%)			
– FEV1/VC	Mean Range	85.25 67-94	
– FEV1	Mean Range	82.5 40-97	
– FVC EX	Mean Range	87-33 49-95	

group. The recruitment of children was carried out through the Department of Pediatric Pneumonology, Allergology and Clinical Immunology, K. Jonscher Clinical Hospital of the Medical University in Poznan, after prior written consent from the guardians of the children. The material for the research was obtained by collecting blood from patients into appropriate test tubes. The blood was centrifuged to obtain serum samples. The test material was properly secured and stored at -80°C until analysis. The study was approved by the Local Ethical Committee of the Medical University of Poznan, Poland (Decision No. 530/12), in accordance with the requirements of the Helsinki Declaration.

Inflammatory Marker Panel Measurement

The experiment involved the simultaneous determination of thirty-seven proteins of the inflammatory response (a proliferation-inducing ligand/ tumor necrosis factor ligand superfamily member 13 (APRIL/TNFSF13), B-cell activating factor/ tumor necrosis factor ligand superfamily member 13B (BAFF/TNFSF13B), soluble form of CD30/ tumor necrosis factor receptor superfamily member 8 (sCD30/TNFRSF8), the macrophage activation marker-soluble CD163 (sCD163), Chitinase-3-like 1, gp130/sIL-6Rβ, interferon alpha-2 (IFN-α2), interferon β (IFN-β), interferon γ (IFN-γ), IL-2, sIL-6Rα, IL-8, IL-10, IL-11, IL-12 (p40), IL-12 (p70), IL-19, IL-20, IL-22, IL-26, IL-27 (p28), IL-28A/

IFN-λ2, IL-29/IFN-λ1, IL-32, IL-34, IL-35, tumor necrosis factor superfamily member 14 (LIGHT/TNFSF14), matrix metalloproteinase-1 (MMP-1), MMP-2, MMP-3, Osteocalcin, Osteopontin, Pentraxin-3, sTNF-R1, sTNF-R2, thymic stromal lymphopoietin (TSLP), TNF-related weak inducer of apoptosis/tumor necrosis factor ligand superfamily member 12 (TWEAK/TNFSF12)) using the Bio-Plex Pro Human Inflammation Panel 1 assay (Bio-Rad, Hercules, CA, USA), in accordance with the instructions included in the manufacturer's leaflet. The inflammatory response protein profile was determined in serum using the following methods: flow cytometry, magnetic separation. The kit contains reagents, including standards and quality controls, and a reaction site, i.e., a 96-well plate. The principle of the Bio-Plex method is based on the use of primary antibodies conjugated with fluorescent magnetic beads, which have different colors and are directed against targeted markers. Briefly, 50 microliters of the test material, reagents (standards and quality controls) were added to each well of the plate containing the primary antibodies attached to the beads, and then prepared mixture was incubated for one hour at room temperature. Upon completion of incubation and subsequent sample washing cycles, antibody-biotin reporters for detection were inserted into the wells. Preparation of the final reaction mixture was accomplished by adding the streptavidin-phycoerythrin fluorescent conjugate. The concentration of inflammatory response proteins was measured by flow cytometry using a Bio-Plex array reader (Bio-Plex MAGPIX, Bio-Rad, Hercules, CA, USA). The reader is equipped with two LEDs, one of which emits red light with a wavelength of 635 nm and the other, which in turn emits green light with a wavelength of 532 nm. The obtained data was pre-processed with Bio-Plex Manager 6.0 software (Bio Rad, Hercules, CA, USA). Before the analysis, the software was completely calibrated and verified. The standard curve was created based on the standards provided by the manufacturer. The concentrations of the determined markers were presented in picograms per milliliter (pg/ml) analogously to the standard curves. Two of the 96 wells of the plate were filled with Bio-Rad diluents only, which were interpreted as blank. Negative and positive quality controls were used to verify that the procedure was correctly performed.

Data Analysis

Statistica 13.0 (StatSoft Inc., Tulsa, OK, USA) and MedCalc (MedCalc Software Ltd, Ostend, Belgium) were used for statistical analysis. Data with $p < 0.05$ were considered statistically significant. The obtained values were analyzed using univariate statistical tests. Depending on the type of data distribution, a detailed comparison of the control group with the study group was carried out using the t-test or Mann-Whitney test. The Shapiro-Wilk test was used to check the normality of the data distribution. Variables without a normal distribution were compared with the Mann-Whitney U test, and the equality of variance for normally distributed variables was tested with Levene's test. If the result obtained with Levene's test was statistically insignificant ($p > 0.05$), the variance between the groups was homogeneous and the Student's t-test was performed successively. If the Levene's test result was statistically significant ($p < 0.05$), the Welch t-test was performed. The univariate receiver operational characteristic (ROC) curve was determined by MedCalc (MedCalc Software Ltd, Ostend, Belgium). The visual correlation between sensitivity and specificity is presented on the ROC curve for each of the analyzed analytes. The ROC curve allows the specificity and sensitivity of the discriminator to be assessed.

Results

Serum Profile of Inflammatory Proteins in Childhood Asthma.

The Bio-Plex system used in the experiment allowed for the simultaneous measurement of the concentration of multiple cytokines and chemokines of the inflammatory response in the serum during one experiment. Successful measurement was performed in the serum of nineteen out of 37 (APRIL/TNFSF13, BAFF/TNFSF13B, sCD30/TNFRSF8, sCD163, Chitinase-3-like 1, gp130/sIL-6Rβ, sIL-6Rα, IL-19, MMP-1, MMP-2, MMP-3, Osteocalcin, Osteopontin, Pentraxin-3, sTNF-R1, sTNF-R2, TSLP, and TWEAK/TNFSF12) analyzed inflammatory factors. The results obtained in both groups subjected to the study are presented in **Table 2** and **Figure 1**. The remaining eighteen inflammatory markers were eliminated from further data analysis because the obtained concentrations were below the quantification limit or

Table 2. The values of the concentrations of 19 proteins of the inflammatory response in the study and control groups. The results are given in pg/ml

Inflammation Marker	Asthma (Study Group)					No Asthma (Control Group)				
	Median	Q1	Q3	Mean	SD	Median	Q1	Q3	Mean	SD
APRIL/TNFSF13	12762,1	7915,8	32639,5	19870,8	17420,8	17734,5	14481,4	21877,5	19149,4	10960,5
BAFF/TNFSF13B	9609,6	7247,1	10911,4	9215,0	2279,8	8732,1	7448,5	9740,4	9459,0	3673,5
sCD30/TNFRSF8	1448,1	1172,8	1727,3	1410,5	408,0	1475,4	1000,0	1892,4	1507,8	721,1
sCD163	146695,1	142626,0	221083,1	169391,4	57001,1	138355,9	103637,3	187489,5	156009,9	72201,3
Chitinase 3-like 1	6037,7	3729,6	9393,0	6685,6	3103,5	6790,3	4652,3	10186,1	7193,0	2805,4
gp130/sIL-6Rbeta	60735,9	56092,1	70486,7	67854,5	16322,3	62216,7	40731,1	73583,0	61963,6	22688,7
sIL-6Ralfa	15817,9	11713,8	17580,4	15141,8	3444,9	12329,2	10746,9	16614,7	12776,1	4444,6
IL-19	70,9	61,5	79,9	70,0	11,8	68,0	57,9	75,4	66,5	18,2
IL-26	92,5	83,1	107,1	94,2	20,1	92,5	61,6	133,5	96,6	36,7
MMP-1	901,7	695,7	1160,8	1056,5	583,5	1686,9	1105,1	2373,0	2585,6	3402,7
MMP-2	36789,1	27049,2	50823,7	40098,6	17277,9	29532,6	19322,0	43173,5	37722,8	26801,2
MMP-3	2169,8	726,2	3381,3	3675,0	5911,6	2045,3	1444,2	2134,9	2097,5	1099,2
Osteocalcin	17120,7	11015,3	20686,4	16445,1	5195,2	12019,2	7979,1	19130,9	14598,1	9154,6
Osteopontin (OPN)	33548,8	30052,6	36918,0	33919,2	6745,6	41168,1	16608,5	46928,8	36610,2	16446,9
Pentraxin-3	1099,8	612,4	1652,1	1167,5	648,2	866,2	659,7	1646,9	1257,0	877,3
sTNF-R1	3227,8	2812,9	4835,3	3682,8	1110,9	3479,1	2619,0	4635,8	3849,9	1619,5
sTNF-R2	5635,6	3823,4	7457,1	6021,3	2564,4	5294,8	2780,5	6963,5	5433,9	3405,1
TSLP	21,3	17,3	26,5	21,9	6,4	22,5	20,9	22,5	22,7	4,5
TWEAK/TNFSF12	678,4	535,6	757,4	642,7	139,8	535,6	421,2	749,5	590,2	212,8

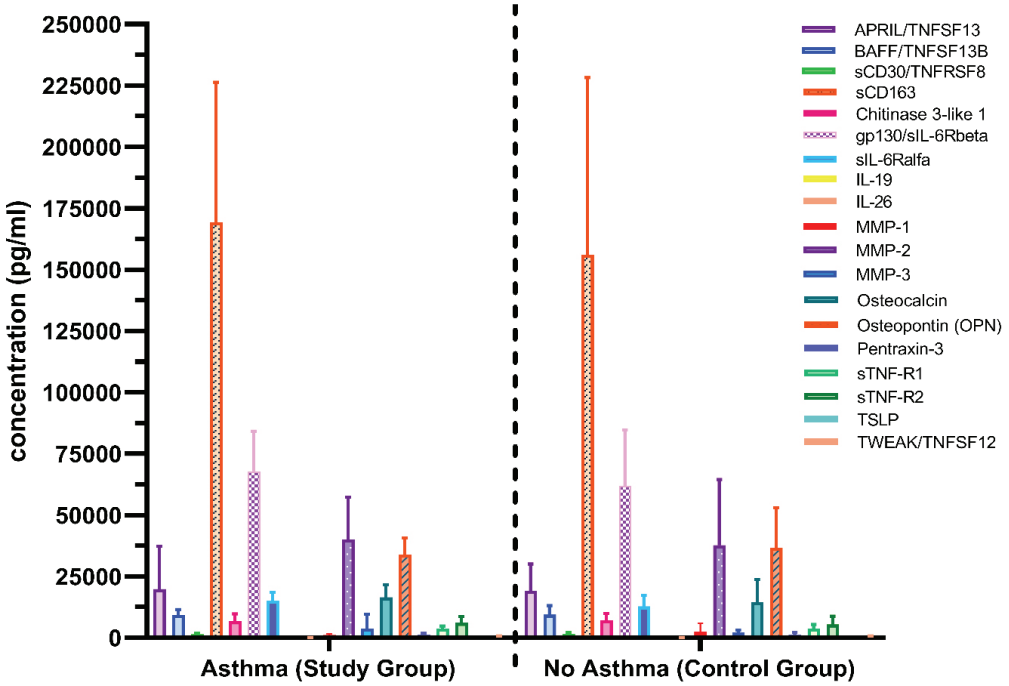

Figure 1. Comparison of the concentration of inflammatory proteins in the serum between the two groups: childhood asthma (study group) and control group

were incomplete. Ultimately, our statistical analyzes included 19 inflammatory response proteins for which the data were complete.

The inflammatory marker profile of patients with childhood asthma (study group) was compared to non-asthma and allergy-free volunteers (control group) using the Student's t-test, Mann-Whitney or Welch test. The most important data from these comparisons are presented in **Table 3**. After performing univariate statis-

Table 3. Univariate statistical analysis of serum inflammatory response proteins in children with and without asthma. The numerical values of "p" indicate statistical significance

Inflammation Marker	p value
	Mann-Whitney U Test
APRIL/TNFSF13	0,436
BAFF/TNFSF13B	0,568
sCD30/TNFRSF8	0,876
sCD163	0,253
Chitinase 3-like 1	0,678
gp130/sIL-6Rbeta	0,436
sIL-6Ralfa	0,194
IL-19	0,775
IL-26	0,815
MMP-1	0,033
MMP-2	0,233
MMP-3	0,979
Osteocalcin	0,324
Osteopontin (OPN)	0,35
Pentraxin-3	0,876
sTNF-R1	0,917
sTNF-R2	0,467
TSLP	0,484
TWEAK/TNFSF12	0,311

tical tests, a statistically significant difference was obtained between the studied groups in the concentration of one inflammatory factor. In the group of children with asthma, the circulating level of MMP-1 was significantly decreased ($p < 0.05$) compared to the control group. Other inflammatory factors were also characterized by differences in concentrations between the study and control groups, but the observed differences were not statistically significant. There was a slight increase in the concentration of 10 inflammatory factors (APRIL/TNFSF13, sCD163, gp130/sIL-6Rbeta, sIL-6Ralfa, IL-19, MMP-2, MMP-3, Osteocalcin, sTNF-R2, TWEAK/TNFSF12) in the group of children with asthma compared to control. On the other hand, the concentration of the remaining 8 inflammatory factors (BAFF/TNFSF13B, sCD30/TNFRSF8, Chitinase 3-like 1, IL-26, Osteopontin (OPN), Pentraxin-3, sTNF-R1, TSLP) was statistically insignificantly decreased in the group of children with asthma (**Figure 1**).

Usefulness of the Inflammation Markers in the Differentiation of Childhood Asthma

As statistically significant values were obtained only for the MMP-1 marker, its discriminant ability was additionally determined by calculating the ROC curve. A graphical summary of the sensitivity and specificity of the inflammatory marker MMP-1 is presented in **Figure 2**, while the numerical data is shown in **Table 4**. We found the value of the area under the curve close to 0.7 as a satisfactory discriminant factor. The results obtained

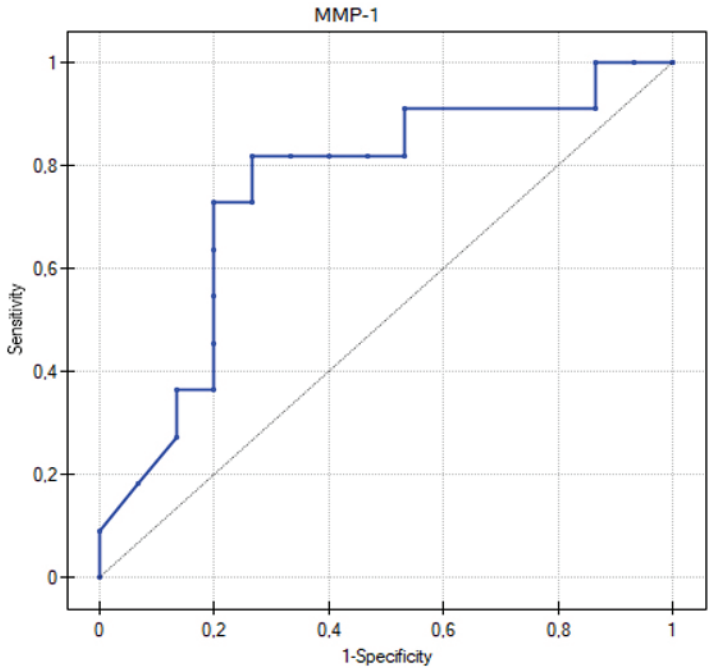

Figure 2. Univariate receiver operating characteristic (ROC) curve presents the relationship between the concentration of MMP-1 in the serum in patients with childhood asthma and the participants from the control group

Table 4. The discriminant value of expression in serum of the marker MMP-1 showing a significant "p" value and the area under the receiver operating characteristic (ROC) curve (AUC) between the two study groups (with childhood asthma vs. without asthma)

ROC curve analysis	Inflammatory Factor
	MMP-1
AUC	0,752
SE(AUC)	0,103
-95%CI	0,549
+95% CI	0,954
p value	0,0313

in this study allow to classify the MMP-1 protein as a satisfactory discriminant factor which allows to distinguish childhood asthma from children without asthma and allergies. The AUC (area under the curve) result for the MMP-1 protein (0.752) indicated 73.3% specificity and 81.8% sensitivity with a cut-off of 1160.83 pg/ml for this marker. The differences in the concentrations of other inflammatory proteins between the groups were statistically insignificant, therefore they were not analyzed for the ROC curves.

Discussion

In view of the high prevalence of asthma among children and the need for prompt treatment, it is important to have measurable indicators that correlate with the development of the disease. The aim of this research was to characterize the cytokine profile of children suffers from asthma using Bio-Plex system. The Bio-Plex assay is similar to the ELISA, both methods detect proteins and use antibodies for this purpose. The Bio-Plex system utilizes xMAP (the multiplex analyte profiling) technology to enable the multiplexing of up to 100 different analytes [13]. Multiplexed analysis has the advantage of simultaneously detecting multiple analytes in a single reaction vessel reducing time, labor, and cost when compared to single-reaction-based detection methods. For example, the determination of the concentration of 37 inflammatory cytokines with the Bio-Plex method takes only 3 hours, while the ELISA test for the same number of samples takes more than 60 hours. In addition, we used one 96-well plate in this study, an ELISA test would need 37 such reaction vessels. It should also be noted that there is a significant difference in the amount of biological material needed to perform the analysis. The Bio-Plex test requires a maximum of 12.5 µl of serum or plasma, and the ELISA test requires a minimum of 1 ml. Furthermore, the Luminex/xMAP system offers high-throughput detection of target proteins. Due to these properties, the Bio-Plex is an increasingly used method. Previously, the Bio-Plex system was used in research on mucopolysaccharidosis IVA, respiratory and reproductive pathogens in swine, colorectal cancer, achalasia, rhinosinusitis, nephropathy and many other diseases [14–19].

We successfully measured the concentrations of 19 inflammatory factors from the TNF superfamily proteins, IFN family proteins, Treg cytokines, and MMPs (APRIL/TNFSF13, BAFF/TNFSF13B, sCD30/TNFRSF8, sCD163, Chitinase-3-like 1, gp130/sIL-6Rβ, sIL-6Rα, IL-19, IL-26, MMP-1, MMP-2, MMP-3, Osteocalcin, Osteopontin, Pentraxin-3, sTNF-R1, sTNF-R2, TSLP and TWEAK/TNFSF12). According to our results, the serum concentrations of APRIL/TNFSF13, sCD163, gp130/sIL-6Rbeta, sIL-6Ralfa, IL-19, MMP-2, MMP-3, Osteocalcin, sTNF-R2, TWEAK/TNFSF12 were increased in asthmatic group, when compared to healthy participants. In turn, the circulating levels of BAFF/TNFSF13B, sCD30/TNFRSF8, Chitinase 3-like 1, IL-26, Osteopontin (OPN), Pentraxin-3, sTNF-R1, TSLP, were decreased in children suffering from asthma comparing to control group. Although, a statistically significant difference was only obtained for MMP-1 ($p < 0.05$). Based on the univariate ROC curve, that graphically presents the sensitivity and specificity of the analyzed compound, MMP-1 may be considered a prognostic factor for childhood asthma.

Matrix metalloproteinases (MMPs) belong to the family of endopeptidases, which are zinc

dependent and involved in the breakdown of a wide variety of extracellular components. MMPs play a role in physiological as well as pathological processes of structural remodeling including cell migration, tissue repair and tumor necrosis. Interestingly, the expression process of MMP genes is tightly controlled by various cytokines that can activate (e.g. TNF-alpha, IL-1, TGF) or inhibit transcription (e.g. IL-4) [20]. MMPs are synthesized and released in allergic diseases such as asthma by several types of cells, including leukocytes and macrophages, and cells constituting the structure of the airways, such as epithelial cells, fibroblasts and smooth muscle cells [21, 22]. Matrix metalloproteinase 1 is responsible for the degradation of the most abundant proteins in the human body – collagen types I, II, III, V, IX and X [21]. For this reason, MMP-1 is essential for the modeling and remodeling of the extracellular matrix [21]. Under physiological conditions, a low level of this protein is noticeable, but the level increases in pathological conditions. Elevated levels of MMP-1 level have been detected in many types of tumors, implant failure, occlusive peripheral arterial, coronary artery disease, osteoporosis and gingivitis or periodontitis [21, 23, 24]. In this study the concentration of MMP-1 in the serum of children suffering from asthma was reduced when compared to healthy subjects. Contrary to our results, Rogers et al. and Naveed et al. detected increased levels of MMP-1 in adult asthma patients [25, 26]. However, to our knowledge, this study for the first time shows this phenomenon in childhood asthma. All differences in the expression of inflammatory factors and mediators of immunity between childhood and adult asthma may be due to the immaturity of the children's immune system [27, 28]. Moreover, these scientists did not use the Bio-Plex in their research. The Bio-Plex system was only used by Sugai et al. to study cytokines in childhood asthma, but MMP-1 levels were not measured [29]. Previously, we detected the serum concentration of MMP-1 and MMP-3 in patients suffering from Hymenoptera venom allergy, but we did not observe significant differences between studied groups [12]. Dahlen et al. showed that MMP-3 is present in both mast cells and eosinophils, which are key effector of the asthmatic inflammatory response [30]. Furthermore, MMP-3 has been reported in vitro to be involved in the bioprocessing of pro-TNF alpha. Since TNF alpha is localized in human mast cells, this creates the possibility for MMP-3 to act potentially as an activator of TNF alpha release, thereby enhancing the profibrotic course and influencing endothelial cell activation and the recruitment of infiltrating leucocytes in asthma [31]. There is evidence that MMP-1 may also be involved in the pathogenesis of asthma. According to Ingram et al. the concentration of MMP-1 is related to the IL-13 activity [32]. They observed that IL-13 stimulates the secretion of matrix metalloproteinase 1 [31, 32]. Their research also showed that MMP-1 leads to the proteolytic cleavage of membrane-tethered heparin-binding epidermal growth factor (HB-EGF) on the cell surface, which is essential for epithelial repair [31, 33]. Damage to the bronchial epithelium can lead to many diseases, including childhood asthma. Moreover, Cataldo et al. reported in their studies that patients with asthma had an elevated level of matrix metalloproteinase 1 gene expression [35]. They explain that bronchial remodeling may be related to this condition and may lead to the development of asthma. The authors also point to a relationship between MMP-1 activity and inflammatory processes in the respiratory tract [35]. Ohta et al. showed the connection between the presence of IL-13 and increased secretion of matrix metalloproteinase 1. Additionally, they proved that the presence of IL-13 causes an increase in the expression level of MMP-1 mRNA and pointed that IL-4 has the same effect as IL-13 [36]. Unfortunately, in our study, we did not measure the levels of IL-4 and IL-13 in children with asthma.

In the development of asthma, a special role is ascribed to immunological processes. In the future, the inhibition/ activation of pro-inflammatory/ anti-inflammatory factors may be helpful in treating children with asthma. Matrix metalloproteinases can be considered as potential therapeutic target. Scientists are eager to look for agents that inhibit overexpression or block the action of the MMP-1 protein. Kim et al. showed that treatment with scopoletin decreases MMP-1 expression by reducing p38 MAPK phosphorylation, which can be used to combat inflammation in keratinocytes [37]. A study conducted on the mouse chondrogenic cell line ATDC5 by Liu et al. identified soya-cerebroside as a potential therapeutic agent for the treatment of osteoar-

thritis due to its properties that inhibit MMP-1 expression [38]. Additionally, the inhibitory effect of triflorethol-A on MMP-1 is investigated in the context of aging processes in human keratinocytes [39]. However, the studies mentioned above require further verification to confirm the effective and purposeful use of these substances in the indicated disease entities. The inhibition of MMP-1 synthesis and secretion should also be investigated as a potential asthma treatment to prevent bronchial remodeling.

In this study, we observed alterations in the serum profile of the remaining 18 inflammatory factors (APRIL/TNFSF13, BAFF/TNFSF13B, sCD30/TNFRSF8, sCD163, Chitinase-3-like 1, gp130/sIL-6Rβ, sIL-6Rα, IL-19, IL-26, MMP-2, MMP-3, Osteocalcin, Osteopontin, Pentraxin-3, sTNF-R1, sTNF-R2, TSLP and TWEAK/TNFSF12) in asthmatic patients, however, the obtained differences were not statistically significant. According to literature, most of these inflammatory factors may be involved in the course of asthma [33]. TNF-R1 and sCD30/TNFRSF8 belong to the tumor necrosis factor receptor (TNFR) superfamily [40]. An elevated level of sCD30 was observed in atopic dermatitis, and it was recognized as a potential marker of clinical severity in this condition. The study proved that sCD30 level was statistically higher in the exacerbation and remission phase of the disease compared to the control group. It was also observed a statistically significant positive correlation between the observed concentration of soluble CD30 receptor and the clinical status of patients in both periods [41]. There are also reports that sCD30 level is increased in allergic asthma [42]. In turn, Boonpiyathad et al. investigated the role of IL-2 in asthma and concluded that the level of IL-2 is higher in patients with severe asthma than in patients with mild asthma [43]. Raundhal et al. report that IL-17 secretion is inhibited, but INF-γ secretion is stimulated in asthma [44]. Charrad et al. revealed increased level of IL-8 and Kim et al. elevated level of TWEAK [44,45]. Our study had some limitations. The lack of statistical significance in our study may be due to the insufficient number of patients due to the pilot nature of the study. Future research should expand the patient population for more reliable results on childhood asthma. Another limitation of our study was the high heterogeneity of the study group in terms of the severity of asthma, comorbidities, and therapy. The obtained results are of a cognitive nature and broaden the understanding of the role of cytokines in the pathogenesis of childhood asthma, however currently they cannot be useful in clinical practice.

Conclusion

In conclusion, our study showed that the inflammatory protein MMP-1 is an important marker that can be used to recognize childhood asthma endotypes. As chronic inflammation of the airways is crucial for the development of asthma, monitoring the profile of its components should be included in the routine course of patient management. Treatment of asthma is aimed at gaining clinical control and minimizing the risk for the patient in the future. Continuous monitoring is essential to achieve this goal in childhood asthma. Cytokines carry a burden of drivers of immune responses and are therefore key coordinators and fixers of airway inflammation. This makes them an attractive target for treatment but may be at the fore in the diagnosis of asthma in the near future. This topic requires in-depth research to discover as many of the predisposing factors as possible to the development of asthma. Perhaps some immune factors change their levels much earlier than biochemical factors. This state of knowledge would allow for a much faster diagnosis and implementation of appropriate treatment, which is very important in the case of childhood asthma. Future research should also indicate to which endotype matrix metalloproteinase 1 is classified. Due to this fact, this study is only the beginning of more complex proteomic analyzes.

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

Funding sources
There are no sources of funding to declare.

Institutional Review Board Statement
The study was approved by the Local Ethical Committee of the Medical University of Poznan, Poland (Decision No. 530/12), in accordance with the requirements of the Helsinki Declaration.

Informed Consent Statement
Informed consent was obtained from the guardians of all children involved in the study.

Data Availability Statement
The data presented in this study are contained within the article.

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ORIGINAL PAPER

JMS Journal of Medical Science

Vitamin D3 in acute respiratory infections in patients under age five in a health institution in Colombia

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ABSTRACT

Aim. describe the clinical behavior in acute respiratory infections in patients under age five in a Colombian health institution after VD3 administration. Clinical trials are required to determine this potential benefit.
Material and Methods. A performed series of 38 patients of both genders, aged 0-60 months to whom 50,000 units of VD3 were orrally administered per month for three months is described. The number of episodes, visits to the emergency room, and hospitalizations due to acute respiratory infections (ARI) before and after VD3 administration were described.
Results. The average age of the participants was 25.81 ± 17.50 months. The average number of ARI clinical events per month was 4.02 (95% CI 3.64-4.40) prior to VD3 administration. The number of episodes reduced at the end of the three cycles was 2.23/month (95% CI 1.81-2.65; p = 0.0230). The average number of emergency room visits during three months prior to the VD3 administration was 2.15 (95% CI 1.77-2.53). After three months of treatment, the average number of emergency room visits decreased to 0.52 (95% CI 0.32-0.72; p = 0.0180). Prior to VD3 administration, 31.58% required hospitalization. After the administration of three VD3 doses, only one patient required hospitalization (2.63%; $\overline{X}$:0.026 (95% CI 0.02-0.03; p = 0.0368).
Conclusions. Vitamin D3 administration could have a benefit in reducing the number of ARI episodes, emergency room visits, and hospitalalizations in children under age five.

Introduction

Acute respiratory infection (ARI) is among the most important causes of morbidity and mortality in children under age 5, ranking among the three leading causes of death worldwide in this age group [1, 2]. It represents one of the main causes of medical appointments and hospitalization in

this population group, originating between 40% and 60% of pediatric appointments in low income countries, and 20% to 40% of pediatric hospitalizations in most countries [3, 4].

The incidence and mortality due to acute respiratory infection is higher in low-income countries. There are several circumstances by which this predisposition can be increased in this group of countries where children under age 5 are the most affected [5, 6]. Among this circumstances, exists the difficulty in diagnosing lower ARI in young infants and the lack of education in caregivers to recognize the signs and symptoms of major disease [3, 7, 8]. Immunological immaturity of children under 5 makes them susceptible to an increase in morbidity and mortality from this group of diseases [3, 9, 10]. Given this, it is necessary to improve sociodemographic and nutritional conditions, as well as improving the response of the immune system to these diseases [10, 12]. The impact of micronutrient levels on respiratory infections has been described [13–15], among which is the closest association of vitamin D3 deficiency (VD3) with ARI risk [16–22]. Besides enhancing chemotaxis and phagocytic capabilities of innate immune cells, VD3 activates the transcription of antimicrobial peptides such as defensin β2 (DEFB) and cathelicidin antimicrobial peptide [23–28].

There is no consensus on the levels to conceptualize VD3 insufficiency or deficiency, although levels of at least 10 ng/ml are estimated to promote calcium mineralization and homeostasis, and a concentration between 20 to 50 ng/ml, for the immunomodulator effect [29, 30].

Aim

The objective of this work is to describe the clinical behavior in respiratory infections in a series of patients under age 5 in a Colombian health institution after VD3 administration

Material and Methods

A descriptive study was carried out in 38 patients of both genders, aged less than or equal to 5 years, who attended the pediatric outpatient clinic of the Policia Departmental of Bolívar for ARI attributed reasons, from February 1st to May 1st, 2018. The study was carried out in Cartagena de Indias, a warm-tropical climate city (ambient temperature 24° and 34°C) located in the Caribbean region of Colombia.

Exclusion Criteria

Patients who did not have informed consent from their parents, or guardians, and those with a history of hypersensitivity to VD3, or with pathologies in which VD3 administration is contraindicated were excluded from the study.

Definition of Terms and Classification Criteria

› The determination as an ARI case was made according to the definition proposed by the Atención Integrada a las Enfermedades Prevalentes en la Infancia (AIEPI) strategy (for its acronym Integrated Management of Childhood Illnesses-IMCI) of the World Health Organization (WHO) (**Table 1**). Classification according

Table 1. Definition and Classification According to Symptoms of Cough and/or Respiratory Difficulty of ARI

	BF (breaths/minute)	Age in months
No pneumonia	< 50	(2–11)
	< 40	(12–59)
	Without CR	
Nonsevere pneumonia	>50	(2–11)
	>40	(12–59)
	Without CR	
Severe pneumonia	With CR with or without tachypnea	
Very serious disease	Difficulty drinking, seizures, drowsiness, stridor, cyanosis	

Abbreviations: ARI, acute respiratory infection, an infectious process that can affect the nose, ears, pharynx, epiglottis, larynx, trachea, bronchioles or lungs, in which symptoms can be found that include cough, fever, odynophagia, earache, rhinorrhea, respiratory distress of an average duration of 7 to 14 days (34); BF, breathing frequency; CR, chest retractions; WHO: World Health Organization.

to the clinical status by symptoms of cough and respiratory distress was taken conforming to the WHO proposal [31, 32] (**Table 1**).

- To categorize patient as a type of upper or lower ARI tract, the classifications of the AIEPI strategy and the Colombian Ministry of Health were used [1] (**Table 2**).
- Classification of the socioeconomic stratum (SE) was carried out according to the criteria established by the Departamento Administrativo Nacional de Estadísticas de Colombia (DANE). Classification categories were defined as SE 1 which is referred to: SE as low – low, SE 2 as low, SE 3 as medium – low, SE 4 as medium, SE 5 as medium – high, and SE 6 as high [33].
- The states criterion of VD3 serum levels were determined according to the Practice Guide of the Society of Endocrinology [34] considering deficiency when the levels were less than 20 ng/ml, insufficiency between 21–29 ng/ml, sufficiency greater than 30 ng/ml, and intoxication greater than 150 ng/ml.
- Exposure to environmental pollution, humidity and house dust was realized by the location and housing conditions, and the type of food of the participants.
- Nutritional classification was made according to parameters established by resolution 2465 of 2016, using the Anthro program version 3.22.

Vitamin D Levels

Determination of serum vitamin D was performed using the LIAISON XL kit, immunochemiluminiscence analyzer, and the *LIAISON-25 OH Vitamin D TOTAL* Kit. This kit evaluates the serum levels of 25-hydroxyvitamin D Total, which corresponds to the sum of the fractions 25-OH-D2 and 25-OH-D3.

Supply of VD3

Patients were provided orally once a month for 3 months with two VD3 vials of 25,000 units (50,000 units) under the trade name of Histotal®, in the presence of the legal guardian and the investigator who had knowledge of the provided supplements. The vials were provided to participants in their original form.

Statistical Analysis

Data were entered into a database in the Microsoft Excel 2010 program. For univariate statistical analysis, Epi Info version 7.2.2.6 statistical software (*Centers for Disease Control and Prevention, Atlanta, Georgia, USA*) was used. Bivariate analysis was performed using Stata program software version 12.0 (*StataCorp. 2011. Stata Statistical Software: Release 12. College Station, TX*). Nominal type variables were presented using a frequency distribution. Confidence intervals were calculated for the estimates. Comparison of proportion differences in nominal qualitative variables were made using Fischer's exact test. Statistical significance was given for $p < 0.05$.

Ethical considerations

Study was submitted for evaluation by the audit committee of the Cartagena de Indias Medical Unit and the scientific subdirectorate of the National Police under the standards set forth in chapter I and II of Resolution 8430 of 1993 and the commitment to Good Practices. Clinic, consigned in resolution 2378 of 2008 (Colombia).

Table 2. Main Clinical Manifestations of ARI

ARI Type	Clinical Manifestations
ARI of upper respiratory tract	
Acute rhinopharyngitis	Fever, cough, rhinorrhea without respiratory difficulty for less than 14 days
Acute sinusitis	Fever, purulent rhinorrhea, halitosis, hyposmia for less than 2 weeks
Acute otitis media	Fever, otalgia for less than 2 weeks. Erythematous tympanic membrane, opaque, prominent or retracted with decreased mobility
Acute pharyngotonsillitis	Fever, odynophagia, erythema and pharyngo-tonsillar exudate, hypertrophy of tonsils, painful cervical lymphadenopathy (bacterial etiology)
ARI of lower respiratory tract	
Acute bronchiolitis	Initial catarrhal phase: fever, rhinorrhea, cough. Additional manifestations can include tachypnea, cough with cyanosis, refusal to feed, respiratory dyspnea, wheezing and/or crackles
Pneumonia acquired in the community (NAC)	Fever, cough, rhinorrhea, tachypnea for less than 15 days. Additional findings can include crackles and hypoventilation on pulmonary auscultation

Results

Of 93 eligible patients, 55 consented to participate. Seventeen patients were lost to follow-up, and 38 patients had definitive participation, with a distribution of 50% for both genders. The ages of the participants ranged from 4 to 60 months, with an average age of 25.81 ± 17.50 months (95% CI 19.23–30.81 months). Most of the participants belonged to a medium-low socioeconomic stratification (SE 3), with 25 patients (65.79%) (**Table 3**).

Clinical Features

Participants were exposed to house dust (16/38; 42.11%; 95% CI 26.31–59.18%), humid environments (18.42%; 95% CI 8.84–34.03%), smoking

Table 3. General Data on the Participants in the Study

		Basal status of VD3 serum levels	
	Total	Sufficiency	Insufficiency
Population, n (%)	38 (100)	31 (81.6)	7 (18.4)
Basal serum VD3 levels (ng/ml), average (SD)	38.7 (8.17)	41.5 (6.13)	26.4 (2.57)
Serum VD3 levels (ng/ml) after VD3 administration, average (SD)	49.0 (16.6)	51.8 (8.11)	36.3 (5.84)
Sex, n (%)			
Man	19 (50.0)	14 (45.2)	5 (71.4)
Woman	19 (50.0)	17 (54.8)	2 (28.6)
Age in months, average 95%CI	25.8 (19.2–30.8)	24.3 (18.0–30.6)	28.27 (9.0–47.6)
Socioeconomic stratification, n (%)			
2	6 (15.8)	5 (16.1)	1 (14.3)
3	25 (65.8)	21 (67.7)	4 (57.1)
4	7 (18.4)	5 (16.1)	2 (28.6)
Personal background, n (% / 95%CI)			
None	30 (79.0 / 62.7–90.5)	25 (80.7 / 62.5–92.6)	5 (71.4 / 29.0–96.3)
Malnutrition	2 (5.3 / 1.7–18.7)	1 (3.2 / 0.1–16.7)	1 (14.3 / 0.36–57.9)
Urinary infection	2 (5.3 / 1.7–18.7)	2 (6.5 / 0.8–21.4)	0 (0)
Otitis media	2 (5.3 / 1.7–18.7)	2 (6.5 / 0.8–21.4)	0 (0)
Idiopathic epilepsy	1 (2.6 / 1.1–14.5)	0/31 (0)	1 (14.29 / 0.36–57.9)
Adenoid hypertrophy	1 (2.6 / 1.1–14.5)	1 (3.2 / 0.1–16.7)	0 (0)
Respiratory background			
Environmental and nutritional exposure, n (%/95%CI)			
House dust	16 (42.1 / 26.3–59.2)	12 (38.7 / 21.8–57.8)	4/7 (57.14 / 18.4–90.1)
Humid climate	7 (18.4 / 8.8–34.0)	6 (19.4 / 7.5–37.5)	1 (14.3 / 0.4–58,0)
Intake chemical preservatives	5 (13.2 / 4.41–26.1)	5 (16.1 / 5.45–33.7)	0 (0)
Smoke	2 (5.3 / 2.6–17.8)	2 (6.5 / 0.8–21.4)	0 (0)
None	8 (21.0 / 9.6–37.3)	6 (19.4 / 7.5–37.5)	2 (28.6 / 3.7–71.0)
Respiratory disease background, n (% /95%CI)			
Allergic rhinitis	9 (23.7 / 11.4–40.2)	7 (22.58 / 9.6–41.1)	2 (28.6 / 0.36–57.9)
Asthma	7 (18.4 / 7.7–34.3)	7 (22.85 / 9.6–41.1)	0 (0)
None			
Hospitalization, n (%)	22 (57.9)	17 (54.8)	5 (71.2)
Acute bronchiolitis	14 (36.9)	12 (38.7)	2 (28.5)
Acquired pneumonia in the community	8 (21.1)	5 (16.1)	3 (42.9)
Number of times requiring hospitalization, n (%)			
Once	12 (31.6)		
Twice	2 (5.3)		
Anthropometric measures			
Men, average (95%CI)			
Weight in kg	13.6 (11.5–15.8)	14.3 (12.1–16.5)	8 (7.0–9.0)
IMC	16.2 (15.3–17.0)	16.1 (15.1–17.1)	16.75 (15.9–17.6)
Size in cm	91.2 (83.6–98.9)	93.8 (86.3–101.3)	69 (66.6–71.4)

Table 3 continued

	Basal status of VD3 serum levels		
	Total	Sufficiency	Insufficiency
Women, average (95%CI)			
Weight (Kg)	12.1 (10.1–14–2)	11.3 (9.0–13.7)	14.34 (9.6–19.1)
IMC	15.3 (14.7–16.1)	16.0 (14.9–16.3)	14.64 (12.8–16.5)
Size in cm	87.0 (79.2–94.7)	82.9 (74.2–91.7)	98.2 (83.3–113.2)
General z score, average (minimum SD -maximum SD)			
Weight/Age	0.45 (-1 to 3)	0.54 (-1 to 3)	0 (-1 to 2)
Weight/Size	0.11 (-2 to 2)	0.03 (-2 to 2)	-0.7 (-2 to 0)
IMC	0.03 (-2 to 3)	0.12 (-2 to 3)	-0.7 (-2 to 0)
Muscle-nutritional condition, n (% / 95%CI)			
Normal	30 (79.0 / 62.7–90.5)	25 (80.7 / 62.5–92.6)	5 (71.4 / 29.0–96.3)
Weight deficit	5 (13.2 / 4.4–28.1)	3 (9.7 / 2.0–25.8)	2 (28.6 / 3.67–71.0)
Overweight	3 (7.9 / 1.7–21.4)	3 (9.7 / 2.0–25.8)	0 (0)
Fitzpatrick skin phototype[a], n (%)			
Type IV Moderate Brown	19 (50.0)	16 (51.6)	3 (42.9)
Type III Light Brown	16 (42.1)	13 (41.9)	3 (42.9)
Type II White	2 (5.3)	1 (3.2)	1 (14.3)
Type V Dark Brown	1 (2.6)	1 (3.2)	0 (0)
Diagnostic impression, n (%)			
Acute rhinopharyngitis	20 (52.6)	16 (51.6)	4 (57.1)
Acute tonsillitis	4 (10.5)	3 (9.7)	1 (14.3)
Acute otitis media	4 (10.5)	3 (9.7)	1 (14.3)
Acute sinusitis	4 (10.5)	4 (12.9)	0 (0)
Acute bronchiolitis	3 (7.9)	3 (9.7)	0 (0)
Acquired pneumonia in the community	3 (7.9)	2 (6.5)	1 (14.3)
Serum calcium (mg/dl), average (95%CI)	10.1 (10.0–10.2)	10.1 (10.0–10.2)	10.0 (9.0–10.2)
Secondary effects of VD3 administration, n (%)			
Nausea	3 (7.9)	3 (9.7)	0 (0)
None	35 (92.1)	28 (90.3)	7 (100)

[a]Fitzpatrick Phototype Classification: I, pink and/or very pale skin, red or blonde hair, light eyes; II, light skin, blonde, red or light brown hair, light or brown eyes; III, intermediate light skin, hair and eyes of any color; IV, light brown skin, brown hair, brown eyes; V: dark brown skin, dark brown or black eyes and hair; VI: dark skin, dark brown or black eyes and hair (Marín D, Del Pozo A. Farmacia práctica. 2005;24(5):136-7)

(5.26%; 95% CI 2.64–17.75%), and intake of chemical preservatives (13.16%; 95% CI 4.41–26.09%) (**Table 3**).

As a history of respiratory pathologies, 9/38 patients suffered allergic rhinitis (23.68%; 95% CI 11.44–40.24%), and 7/38 suffered asthma (18.42%; 95% CI 7.74–34.33%). Fourteen (14/38) patients had a history of hospitalization for bronchiolitis (36.84%) and 8/38 for community-acquired pneumonia (CAP) (21.05%). Among other pathologies and previous conditions present in patients that could influence the development of ARI, are malnutrition (2/38; 5.56%; 95% CI 1.68–18.66%), acute otitis media (2/38; 5.56% 95% CI 1.68–18.66%), and hypertrophy adenoid (1/38; 2.63% 95% CI 1.07–14.53%) (**Table 3**), as well as family history such as asthma (7/38; 18.42% 95% CI 7.74–34.33%), and allergic rhinitis (9/38; 23.68% 95% CI 1.44–40.24%).

Nutritional status was normal in 30/38 patients (78.95%; 95% CI 62.68–90.45%), 5/38 had weight deficit (13.16%; 95% CI 4.41–28.09%), and 3/38 patients were overweight (7.89%; 95% CI 1.66–21.38%) (**Table 3**). In 10/38(26.32%) participants (95% CI 13.40–43.10%) breast milk was not given.

The influence that phototype could have on VD3 serum levels was determined by characterization according to the Fitzpatrick classification. The highest phototype found was type IV (moderate brown skin color) in 19/38 patients (50%; 95% CI 33.38–66.62%) (**Table 3**).

Respiratory examination findings varied according to ARI type. Hyaline rhinorrhea was

found more frequently (44.74%), followed by oropharyngeal erythema (13.16%) in those who had rhinopharyngitis and acute sinusitis. Tonsil hypertrophy with purulent exudate was found (10.53%) in patients with acute tonsilitis. Acute otitis media was more manifested with erythema and unilateral tympanic membrane bulging (10.53%). In cases with lower ARI respiratory tract, inspiratory wheezing was found (13.16%), and a patient presented use of accessory muscles (2.63%) requiring hospitalization under the diagnostic of pneumonia acquired in the community.

Imaging studies were not routinely requested. Chest X-ray was performed in four patients. Three presented a reticulonodular pattern (7.89%) and normality was reported in one of them (2.63%).

ARI Type

Of the 38 patients, 32 patients (84.21%; 95% CI 68.75–93.98%) had upper ARI respiratory tract symptoms discriminated in rhinopharyngitis in 20/38 patients (52.63%), acute tonsillitis in 4/38 patients (10.53%), acute otitis media in 4/38 (10.53%), and acute sinusitis in 4/38 patients (10.53%). Only 6/38 patients (15.79; 95% CI 6.02–31.25%) performed a lower presentation suggestive of ARI respiratory tract, including acute bronchiolitis in 3/38 patients (7.89%), and community-acquired pneumonia in 3/38 children (7.89%) (**Table 3**).

Serum levels of calcium and VD3

A serum calcium determination was performed in patients, which was normal for their age, with an average of 10.10 mg/dl (95% CI 9.99–10.22 mg/dl) (**Table 3**).

The mean vitamin D level was 38.72 ng/ml (SD 8.17). After three dosages of VD3, the average concentration increased to 49.00 ng/ml (SD 9.82). Seven patients (18.42%) had insufficient levels (21–29 ng/ml) with an average serum basal level of 26.4 ng/ml (SD 2.57), which increased to 36.28 ng/ml (5.84) after administration of three doses of VD3. Thirty-one patients (81.57%) had sufficient levels with an average serum basal level of 41.50 ng/ml (SD 6.13), which increased after the administration of VD3 to 51.88 ng/ml (SD 8.11) (**Figure 1**).

Regarding VD3 serum levels and nutritional status, patients with ponderal deficit presented an average of 33.76 ng/ml (SD 6.75). Two-fifths of these patients (40%) had insuffiency baseline status of VD3 serum levels and 3/5 (60%) had

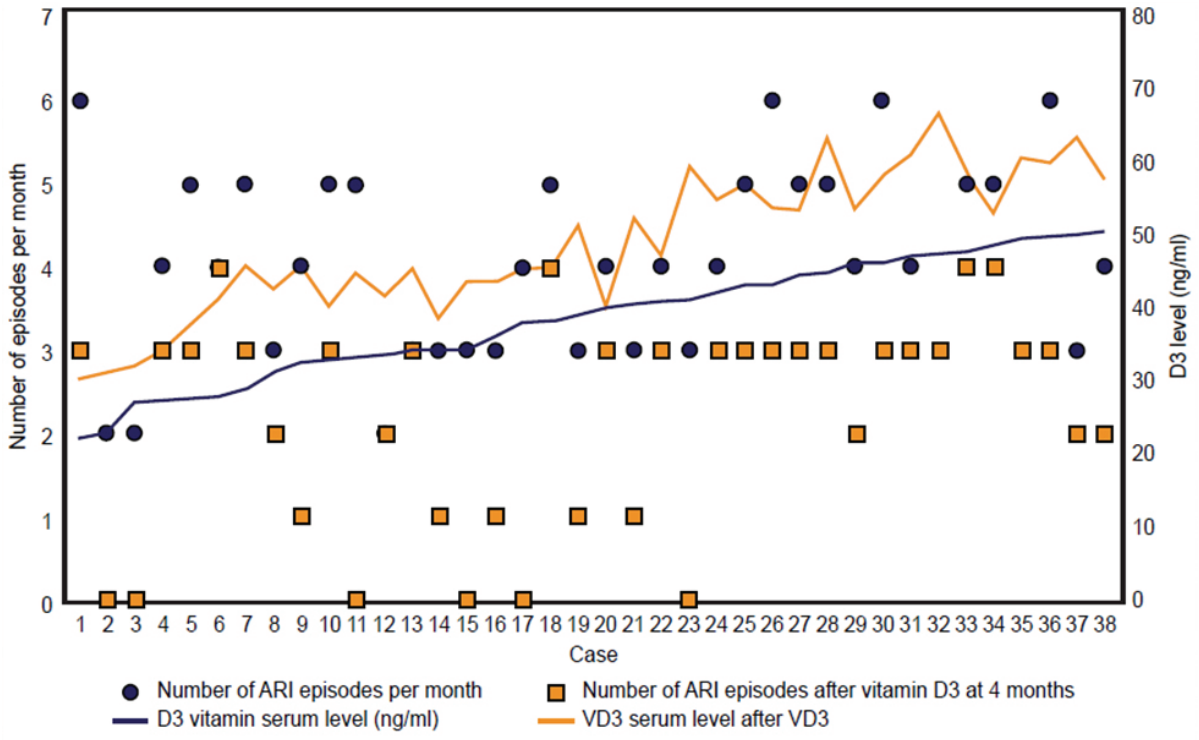

Figure 1. ARI episodes before and after VD3 supplementation. The Acute Respiratory Infection episode number before VDR (blue points) decreased (yellow squares) with increasing VD3 basal levels (blue line) after VD3 administration (yellow line)

a suffiency status. Patients with normal nutritional status had an average VD 3 baseline of 41.07 ng/ml (SD 5.97). A total of 5/30 patients in a normal nutrition state (16.67%) presented an insufficiency baseline status of VD3 levels, and 25/30 (83.33%) in a sufficiency status. In overweight patients, the average VD3 levels were 41.5 ng/ml (SD 9.0), all of them in a sufficiency baseline status of VD3 levels. Although, patients with weight deficit presented lower average baseline serum levels. In general, there was no evidence of significant difference regarding patients with adequate nutritional status and those who were overweight (p = 0.293).

Results of VD3 administration

We observed the number of ARI episodes, the times that the patients were taken to the emergency room and were hospitalized for this cause, before and after administering VD3.

We found that the average number of ARI clinical events in the general studied population prior to VD3 administration was 4.02 events per month (95% CI 3.64–4.40). The number of ARI episodes was reduced as the monthly doses were administered. The reduced number of episodes at the end of the three cycles was 2.23 times per month (95% CI 1.81–2.65; p = 0.0023) (**Table 4**).

In general, the average number of emergency room visits during three months prior to VD3 administration was 2.15 (95% CI 1.77–2.53). After three months of treatment, the average number of emergency room visits decreased to 0.52 (95% CI 0.32–0.72; p = 0.018).

Within the number of hospitalization events during the three months prior to VD3 administration, just once during this period, twelve patients (31.58%) required hospitalization, and two (5.26%) required hospitalization twice. After the administration of three VD3 doses, only one patient (2.63%) required hospitalization (**Table 1**).

VD3 Administration and ARI type

The average number of upper tract ARI episodes in three months of observation prior to administration of VD3 was 3.93 (95% CI 3.50–4.36). These patients were taken to the emergency room an average of 2.06 times (95% CI 1.63–

Table 4. Events Included Consultations for Emergencies and Hospitalization Before and After VD3 Administration

	Total	Basal Level		P Value
		Sufficient VD3	Insufficient VD3	
ARI average episodes (DE) (95% CI)				
Event/month before VD3 administration	4.02 (3.64–4.40)	4.03 (3.63–4.42)	4 (2.58–5.41)	0.0870
In ARI upper tract	3.93 (3.50–4.36)	3.96 (3.51–4.41)	3.8 (2.1–5.5)	
In ARI lower tract	4.50 (3.62–5.37)	4.4 (3.2–5.5)	2 (1.5–2.5)	
Decreased events after VD3 supply	2.23 (1.81–2.65)	2.22 (1.77–2.67)	2.28 (1.43–3.13)	0.0230
After first dosage	0.42 (0.23–0.60)			0.0010
After second dosage	0.47 (0.30–0.64)			0.0001
After third dosage	1.44 (1.17–1.71)			0.0032
For ARI upper tract	2.30 (1.93–2.75)	2.38 (1.97–2.79)	2.16 (1.35–2.97)	
For ARI lower tract	1.66 (0.28–3.629)	1.4 (1.02–3.82)	2 (1.5–2.5)	
Attendances to emergencies during 3 months				
Before VD3 administration				
Average (95% CI)	2.15 (1.77–2.53)	2.12 (1.70–2.55)	2.28 (1.43–3.13)	0.5825
For ARI upper tract	2.06 (1.63–2.49)	2.03 (1.54–2.52)	2.16 (1.93–3.39)	
For ARI lower tract	2.66 (1.80–3.52)	2.60 (1.48–3.71)	3.00 (2.5–3.5)	
After VD3 administration				
Average (95% CI)	0.52 (0.37–0.72)	0.38 (0.20–0.56)	1.14 (0.61–1.67)	0.0180
For ARI upper tract	0.59 (0.37–0.81)	0.45 (0.25–0.66)	1.16 (1.37–1.95)	
For ARI lower tract	0.16 (0.26–0.59)	0 (0)	1.10 (0.97–1.20)	
Hospitalization average (95% CI)				
Internment before VD3 administration	0.421 (0.22–0.61)	0.48 (0.25–0.71)	0.14 (0.1–0.43)	0.3340
Internment after VD3 administration	0.026 (0.02–0.03)	0.032 (0.030–0.097)	0 (0)	0.0368

The table shows the reduction in the number of events, attendance to emergency and hospitalizations after VD3 administration.
Abbreviations: VD3, vitamin D3; ARI, acute respiratory infection.

2.49). The number of episodes per month was reduced by 2.3 times the initial average (95% CI 1.92–2.75) with the VD3 administration, and the average number of visits to the emergency room decreased to 0.59 visits (95% CI 0.37–0.81).

For lower ARI respiratory tract, the average number of episodes per month prior to VD3 administration was 4.5 (95% CI 3.62–5.37), and the number of visits to the emergency room was 2.66 (95% CI 1.80–3.52). After VD3 administration, the average number of ARI episodes was reduced to 1.66 (95% CI 0.28–3.629), and the average number of emergency room visits were reduced to 0.16 (95% CI 0.26–0.59) (**Table 4**).

VD3 Administration and VD3 basal levels

The average number of ARI episodes per month found in patients with sufficiency baseline levels did not show significant differences compared to those with insufficiency levels (4.03; 95% CI 3.63–4.42 vs 4: 95% CI 2.58–5.41, p = 0.087). After administering three VD3 doses, a reduction in episodes was observed for both sufficiency and insufficiency patients (average: 2.22 times; 95% CI 1.77–2.67 and 2.28 times; 95% CI 1.43–3.13, respectively p = 0.047).

Patients with sufficiency and insufficiency were taken to the emergency room at least twice in the three months prior to VD3 administration (average 2.12; 95% CI 1.70–2.55 and 2.28; 95% CI 1.43–3.13 times, respectively; p = 0.5825). These episodes had a significant reduction after VD3 administration (average 0.38; 95% CI 0.20–0.56 and 1.14; 95% CI 0.51–1.60 times, respectively; p = 0.0180).

Of 22/38 patients (57.89%) with a history of hospitalization prior to VD3 administration, 14/22 (36.84%) were hospitalized for bronchiolitis, and 8/22 (21.05%) for CAP. After VD3 administration, only 1/38 (2.63%) was hospitalized (**Table 3**).

Three patients (7.89%) aged between 13 and 60 months, suffered nausea as a side effect to VD3. The other participants had no deleterious effects.

Discussion

In this study, 38 patients of both genders, under age 5 (25.81 ± 17.50 months), in whom an average VD3 baseline value was found to be 38.72 ng/ml (95% CI 36.03–41.40 ng/ml) with 18.42% insufficiency levels, were included. Insufficiency and deficiency status have usually been related to a deficit in intake, as might be expected to occur in patients with nutritional deficits [35–37].

However, no significant difference was found between VD3 baseline levels and nutritional status (p = 0.293), in patients from this survey.

Colombia has one of the highest solar radiation levels in the world due to its tropical location. Cartagena de Indias is located north of the equator (10º 25' 30" north latitude and 75º 32' 25" west longitude), with no seasonal variation. Despite this, in Colombia according to the results of the 2015 National Nutritional Survey, in a group of children aged 1 to 4 years, the total prevalence of vitamin D insufficiency was 35.2% and deficiency was 31.4%.

In a study carried out on 360 eutrophic children under age 10 in a similar Colombian Caribean region, an average value for 25-hydroxyvitamin D of 32.23 ± 8.25 ng/ml was found; 46.38% had levels considered insufficient (<30 ng/ml) and 3.05% showed deficiency (<20 ng/ml) [38]. In Uganda, an analysis was carried out on children between 6–24 months of age, in which no significant differences in VD3 levels and nutritional status were found on patients [39].

Some studies have shown that vitamin D has immunomodulator properties associated with protective effects against infectious diseases, including ARI, and has been proposed as a possible protective measure for these pathologies in pediatrics [30, 40–42]. VD3 benefits have been described in reducing episodes of pharyngotonsillitis [43], nasopharyngitis [44], otitis media, and lower respiratory tract episodes such as bronchiolitis and pneumonia [45]. In study patients, was found that after VD3 administration a reduction in the number of ARI episodes per month ($\overline{X}$ reduced episodes = 2.23 times 95% CI 1.81–2.65; p = 0.0230), visits to the emergency room ($\overline{X}$ = 0.52 consultations/month 95% CI 0.37–0.72; p = 0.0180), and the need to be hospitalized was observed. Taking into account that 57.89% of them were hospitalized for bronchiolitis and pneumonia, and that 5.26% needed to be hospitalized at least twice, prior to VD3 administration.

There is no consensus on needed levels for VD3 functions on the immune system. Although it is estimated that at least between 20 to 50 ng/ml

levels are necessary to achieve an immunomodulator effect [30]. In participants of this study, a VD3 bolus dose of 50,000 units was orally administered per month for 3 months, observing an increase in VD3 serum levels by an average of 49 ng/ml, with a decrease in ARI episodes, emergency room visits, and hospitalization.

In an analysis of 25 clinical trials (11,321 participants aged from 0 to 95 years) conducted in 14 countries, in which VD3 was orally administered, was found that seven studies administered VD3 in monthly boluses for three months, three studies performed it weekly, twelve studies performed it daily, and three more studies performed a daily dose and a combination of bolus. A redution of ARI risk in participants (adjusted OR 0.88; 95% CI 0.81–0.96 p for heterogeneity < 0.001) was found. In subgroups analysis, a protective effect in those who received bolus and daily doses (adjusted OR 0.81, 0.72–0.91) was found, but not in those who received one or more boluses (OR 0.97, 0.86–1.10; p for interaction = 0.05). Serious adverse effects with VD3 administration (adjusted OR 0.98, 0.80–1.20), were not found [42]. In patients described in our study, important secondary effects after VD3 administration, were not observed.

A descriptive study was carried out, in which no associations can be confirmed between exogenous VD3 administration and its influence on the favorable response in the reduction of the number of ARI episodes, visits to the emergency room, and hospitalization in children under age five. The described findings invite us to perform association tests that can sustain this relation.

Conclusions

Vitamin D3 administration could have a benefit in reducing the number of ARI episodes, emergency room visits, and hospital admissions in children under age five, although the supplementation régimen has not been defined yet. Clinical trials are required to determine this potential benefit.

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Conflict of interest statement
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ORIGINAL PAPER

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A research on the factors affecting the preference of medical specialization branches

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ABSTRACT

Aim. The aim of this study is to determine the factors affecting the preferences of specialization in the field of medicine.

Material and Methods. Mixed research and exploratory sequential research design were used. In the exploratory phase, data were collected from specialist physicians (n=14) and findings were analyzed by descriptive and content analysis. In the light of qualitative findings, a measurement tool was developed and applied to medical school students and the physicians who prepared specialty exams (n = 502).

Results. Qualitative findings were structured under 3 themes: individual, occupational and systemic factors. The measurement tool, which was named "Physicians' Preference Tendencies of Specialty Branch" was structured as 42 items and 7 dimensions: risk, comfort, health problems, status, emotional interest, gender, and marital status.

Conclusions. Although there are many factors that affect medical specialty choice preferences, it is concluded that personality traits and idealism of individuals and mortality rates associated with branch or field of medicine are the most significant professional factors, while the risks and the exposure to threatening behavior that poses a risk and the application of the additional payment based on the performance of candidates are the systemic factors that affect selections and preferences. Also, it was concluded that qualitative data obtained in the research were supported with quantitative data.

Key points

- The medical specialty choice was an important issue within the medical specialties, meriting a separate assessment domain.
- The study observed that specialist physicians and physician candidates consider professional factors in the selection of specialization.
- The study acknowledged threatening behavior that poses a risk and performance-based payment system impacted specialty choices.

Introduction

The density of knowledge emerging in medical sciences has increased continuously with the effect of developing technology, requiring it to be divided into sections, and the most important feature of societies for a while has been the rise of experts and professionals. The reflection of this situation in the field of medicine was in the form of "specialization" [1]. Specialization in medicine is defined as an effort to understand more specific issues specific to a disease, an organ, an operation [2, 3]. This concept, which is in widespread demand among physicians, is seen as almost a necessity today [4, 5], and the choice of specialization turned to a crossroad for physicians who have graduated from medical school. The choice of specialty in medicine is an important decision of critical importance as it determines the professional future of a physician in her/his professional life [6]. This decision also affects the person's lifestyle, financial situation, work environment and circle of friends, and even the choice of spouse. For this reason, people in career professions such as the profession of medicine need to choose the field that suits their expectations, personality traits, abilities, and ideals [7]. However, most of the time, physicians have difficulties in finding an answer to the question of which specialty they will focus on, despite having an opinion about the "best" specialty while graduating from medical school. Sometimes they can get information about their specialty from family members, sometimes from other physicians, and sometimes from an outsider. It often seems more difficult to decide how or in which branch to become a physician than to decide to become a physician. This is because physicians almost take on a new identity with their chosen field of specialization [3].

However, although the choice of specialty in medicine seems to be a decision that only concerns the physician, it has both individual and social consequences. Although this choice is an individual decision for the physician, the characteristics of the chosen branch and the patient population of the branch cause social consequences. For example, it is reported that while the elderly population in the United States of America is estimated to increase almost twice between 2005 and 2030 the preference for internal medicine concerning this population has decreased by approximately 35% between 1985 and 2008, and this is likely to result in a doctor shortage in the future 8. Therefore, the selection of specialization is a complex, dynamic, and not fully understood process that includes many factors. Increasing socio-political and socio-economic factors predominantly shape the preferences of physicians in many countries today, and it is not yet known how this situation will affect the health sector and therefore society in the future [9–11].

It is reported in the literature that many factors affect physicians' choice of specialization. Studies have revealed that factors such as personality structure, workload, lifestyle, financial gain of the chosen branch, prestige, personal role model, familial reasons, experiences gained during medical education, talent, and gender are effective in the selection of specialization [12–15]. Among the studies designed with both qualitative and quantitative methods, no research was found using a mixed-method. The fact that the specialization selection decision is an individual decision increases the number of factors affecting the process and makes it difficult to understand the reasons behind the preferences. Understanding this complex process and the factors affecting it requires an in-depth perspective. From this point of view, it is aimed to determine the factors affecting the preferences of specialization in medicine in this study.

Material and Methods

Method. This study was designed as a mixed--method and "exploratory sequential design" from mixed-method research was used.

Research Group. In the qualitative phase of the research, the opinions of 14 physicians who are currently active in the health system were consulted. In the quantitative stage, it was carried out with 3rd, 4th, 5th, 6th-grade medical faculty students who are thought to have high awareness about the choice of specialty in medicine, and a total of 502 participants who were preparing for the specialty exam. Ethics Committee approval was obtained from Süleyman Demirel University for the research (29.05.2018/147130).

Sample. Although the number of samples is not mentioned in qualitative studies, sample selection methods are used when determining the sample group created for the research purpose. The maximum diversity sampling method was used to reflect the diversity of individuals at the maximum level by the research purpose of the qualitative data of the research [16].

While determining the sample at the quantitative stage, the number of scale items was taken as a basis. Although there is no consensus among researchers about the number of sample sizes made according to the number of items in the scale, it is often accepted that the criterion sample size should be at least 10 or at least 5 times the number of items in the scale [17]. In this study, the scale was applied to a total of 502 people and a sufficient sample size was achieved.

Working Group. The participants of the qualitative phase of the study consist of 14 specialist physicians who are currently working actively in the health system. Participants were coded as "H1, H2, H3" and their characteristics are given in **Table 1**.

The participants of the quantitative phase of the study consisted of 3rd, 4th, 5th, 6th-grade medical faculty students and physician candidates preparing for the specialty exam. The characteristics of the quantitative participants are shown in **Table 2**.

Data Collection. In the qualitative part of the study, the "Semi-Structured Interview" technique, one of the data collection methods, was used. To examine the subject in-depth, probing questions such as 'why can you give an example, can you explain a little more' were asked to the participants. The interviews were conducted in places where the physicians could be comfortable, at the places they preferred, on the day and time they determined, by voice recording. As a result of the interviews, a total of 604 minutes were interviewed. Qualitative research was carried out between January and May 2018.

The data of the quantitative phase of the research were collected with a measurement tool called "Physicians' Branch Preference Tendency Scale", which was developed to cover 11 themes obtained in the qualitative phase. This tool was created with the answers given by the qualitative participants of the research, to reveal the tendencies of the physicians on the choice of specialization and to determine whether the qualitative data are supported by the quantitative data. First of all, an item pool consisting of 100 statements was

Table 1. Characteristics of Qualitative Participants of the Study

Participant	Gender	Age	Branch	Years of experience in specialty
H1	Male	60	Radiology	35
H2	Female	48	Biochemistry (Public Health)	25
H3	Male	59	Pediatric Surgery	32
H4	Female	45	Biochemistry (Hospital)	22
H5	Male	46	Internal medicine	16
H6	Male	52	Clinical Microbiology and Infectious Diseases	10
H7	Male	46	Cardiovascular Surgery	18
H8	Female	39	Neurology	11
H9	Male	48	Psychiatry	23
H10	Male	43	Emergency Service	7
H11	Male	38	Orthopedics and Traumatology	11
H12	Male	49	Urology	24
H13	Female	42	Family Physician	9
H14	Female	41	Medical Pharmacology	10

Table 2. Characteristics of Quantitative Participants of the Study

Gender			Age				Grade					
Male	Female	N	20-23	24-27	28+	N	3	4	5	6	Graduate	N
219	283	502	213	215	75	502	53	61	82	175	131	502

written for the measurement tool, and the items were read by 3 faculty members and evaluated in terms of language, scope, and the number of items. As a result of the expert evaluation, it was reported that the items were appropriate for the way they were expressed and the purpose of the study, and a suggestion was made to reduce the number of items. It has been seen that the measurement tool is suitable for content validity. As a result of the expression reduction proposal, the measurement tool consisting of 55 statements, with at least 3 statements under each of the 11 themes in the measurement tool, was made ready for application. The measurement tool, which consists of 55 statements, includes 44 positive and 11 negative statements, and is structured as a 7-degrees Likert scale. After the measurement tool was applied to the research group, the answers were scored by considering positive and negative statements. Quantitative research was conducted between July and October 2018.

Analysis of Data: The raw data collected during the qualitative phase of the research were transcribed, transferred to Microsoft Word, converted into text, and the generated texts were read line by line. Then, the themes were determined, and studies were carried out on what the themes meant. Descriptive analysis and content analysis techniques were used to decompose the qualitative data of the research.

Quantitative data, on the other hand, were subjected to Exploratory Factor analysis using the SPSS 23.0 package program.

Validity-Reliability: To ensure the validity of the qualitative phase of the research, the data were sent to 2 faculty members who had worked on qualitative research, and the data were asked to be coded. As a result of coding, different coded themes were discussed between the researcher and the coders, and a consensus was reached between the themes. To confirm the research, the data obtained with the voice recorder during the research process, the themes, categories, and codings created are kept by the researcher for re-examination when necessary.

The reliability of the scale was determined by the Cronbach Alpha coefficient. This coefficient was found to be 0.924 out of 55 items. This result is evidence of reliable and consistent measurement.

Results

Qualitative Research

The opinions of the participants were conveyed within the ethical rules, by the principle of confidentiality, without revealing their identity information. Physicians are coded as "H1, H2, H3". The characteristics of the participants are shown in **Table 1**.

The qualitative findings obtained in the research were structured under three main themes "Individual Factors", "Occupational Factors" and "Systemic Factors". Sub-themes and codes were created under the main themes.

Main Theme 1: Individual Factors

Individual factors affecting the choice of medical specialization branches were divided into main themes and sub-themes in line with the answers of the participants. The main theme and sub-themes of individual factors and examples from the statements of the participants are given in **Table 3**.

Main Theme 2: Occupational Factors

Occupational factors that affect the choice of medical specialization branches were divided into main themes and sub-themes in line with the answers of the participants. The main theme and sub-themes of occupational factors and examples from the statements of the participants are given in **Table 4**.

Main Theme 3: Systemic Factors

The systemic factors that affect the choice of medical specialization branches were divided into main themes and sub-themes in line with the answers of the participants. The main theme and sub-themes of systemic factors and examples from the statements of the participants are given in **Table 5**.

Quantitative Research

The findings of the quantitative phase of the research include the descriptive statistics of the measurement tool and the results of the factor analysis applied to the data set.

Descriptive Statistics Related to Measurement Tool

Descriptive statistics of the measurement tool consisting of 55 expressions are shown in the table. When the mean scores of the participants

Table 3. Findings Related to Individual Factors Effective in Preferring Medical Specialization Branches

Themes and Sub-Themes / Statements of Participants
Main Theme 1: Individual Factors
Sub-Theme 1: Demographics
– *"The women already focused on dermatology and physical therapy so that I could think ahead and have a child anyway" (H10, male, 43 years old).*
– *"It is especially important for women …. Because there are housework, there are children, there are many things waiting for women" (H4, female, 45 years old).*
– *…of course, gender affects women, for example, they choose a branch by considering their future life. Because, due to the mission of women in society, women inevitably tend towards the comfortable branch (H8, female, 39 years old).*
Sub-Theme 2: Personal Features
– *"First of all, they prefer what they want to do according to their character. But the important thing is that he wants it personally. Depends on which part you want. They will also be happy if they can choose their ideal profession. For example, if you are an idealist, you prefer and do it even if you do two compulsory services. That is to say, in that case, I was not an idealist" (H14, female, 41 years old).*
– *"…..there are people who are such idealists. In other words, people who have determined their own branch a long time ago, who are different from others in order to reach their goal, who are more hardworking, who are different from the average group, who get high scores but who endure other conditions, think this directly when they make a choice" (H11, male, 38 years old).*
Sub-Theme 3: Reasons for Health
– *"Of course, this is also important here, if the person does not have any health problems, he should choose accordingly. For example, why can't someone with a hand injury choose orthopedics because they do power-based work? There may be health problems, for example, the person has a hand injury, is disabled, or has a crippled foot. For example, he cannot stand very long. Such people cannot opt for a surgery" (H5, male, 46 years).*
– *"….Or if there is a biological reaction reaction that he does not know about when he sees blood, this time he can quickly leave the surgical branches" (H1, male, 60 years old).*
– *"Physicians consider physical fatigue. In other words, they tend to where I get less physically tired (H11, male, 38 years old).*
– *"… There may be many reasons for this. For example, surgery, you know, is the job of surgery, the person who says I can't stand the sight of blood says that they will not choose a surgical branch, we can't do without seeing blood" (H12, male, 49 years old).*
Sub-Theme 4: Reasons for Ability and Experience
– *"For example, I practiced for 3 years. I went through such bad things when I was a practitioner that I hated the clinic and then turned to biochemistry" (H2, male, 48 years old).*
– *"…then talent is very important. It is very important for a physician to feel that he will be successful in that field. Because, you know, surgical branches are very dependent on manual skills. A physician cannot choose surgical branches if he does not have manual dexterity" (H13, female, 42 years old).*
– *"… medical school is more attractive to people now. You feel as if the end is very beautiful and bright, but of course, the practice period, compulsory service, emergency, the cases seen there, the environment, etc. guide your choice of branch" (H8, female, 39 years old).*
– *"…a doctor who chooses a specialty until he becomes a specialist can choose a branch either as much as he saw at the university or there are people he knows at the university somewhere around him, being influenced by them" (H12, male, 49 years old).*

in **Table 6** are examined, it is seen that they answered "strongly disagree", "somewhat disagree" and close to "disagree" to 9 statements in the scale, and "neither agree nor disagree" to 11 statements. When the average scores of the remaining 35 statements of the participants are examined, it is seen that the participants gave positive answers as "agree", "agree a little" and "strongly agree" to these statements. This shows that the qualitative data of the research is largely supported by the quantitative data.

Factor Analysis

In the study, (KMO) and Barlett's tests were used to determine whether the measurement tool was suitable for factor analysis (**Table 3**). The test result was 0.938, and it was seen that this value was quite sufficient for factor analysis. In addition, the significant result of Barlett's Sphericity test (Sig. Value = 0.000; $p < 0.05$) shows that the matrix formed by the relations between the variables is suitable for factor analysis.

When the total explained variance value was examined, it was seen that there were 6 factors with Eigenvalues greater than 1 in the measurement tool. The first factor (eigenvalue 13,622) explains 24.76% of the variance and the second factor (eigenvalue (3,878) explains 7.05% of the variance (**Table 7**) When the eigenvalues are examined, it is seen that the measurement tool consists of 6 dimensions. Appropriate expressions and factor load scores in the measurement tool will be revealed by subjecting them to exploratory factor analysis.

Table 4. Findings Related to the Professional Factors Effective in the Preference of Medical Specialization Branches

Themes and Sub-Themes / Statements of Participants
Main Theme 1: Professional Factors
Sub Theme 1: Working Conditions
– *" ….if he goes with this thought, young physicians will definitely prefer comfortable branches" (H4, female, 45 years old).*
– *"…in my opinion, it's also about the watch, and how many emergency services there are in the branch. Physicians determine their preferences accordingly. Of course, it can vary according to individuals" (H7, male, 46 years old).*
– *"… they choose not to communicate with the patient, so the radiology score is very good" (H8, female, 39 years old).*
Sub-Theme 2: Threatening behaviour that poses a risk
– *"…the attitudes of patients have also changed recently. As a result of the media being so active, people have realized something. Physician errors come up a lot" (H2, female, 48 years old).*
– *"…that is, patients complain and are investigated afterward. In addition, patients are constantly complaining, you cannot please them, who wants a branch that is constantly complained about" (H3, male, 59 years old).*
– *"I think in the history of the Republic, there has never been such a high intensity in preclinical branches. Lastly, I am following the intensity of this last semester with amazement. But I am not giving any rights to my fellow physicians. complications, malpractice, violence, such cases have now alienated physicians from this job" (H5 male, 46 years old).*
– *In other words, physicians are doing their best not to see patients today. Why, because there is violence, this is a separate issue in the first place, then he considers various alternatives and chooses to secure himself in a way, along with financial concerns (H11, male, 38 years old).*
Sub-Theme 3: Risk
– *"…they choose groups where seizures are low, where there is no malpractice, where there is no possibility of harming the patient, there are no complications, and they do not take such risks" (H5, male, 46 years old).*
– *"….and branches with less risk, more comfortable working conditions, a regular life, good salary, and high returns are generally preferred" (H14, female, 41 years old).*
– *"That's why they don't go to branches with high mortality rates and high complication rates. I think the risk and mortality rate of that branch is a factor that affects, even directly affects, the choice of branch today" (H11, male, 38 years old).*
Sub-Theme 4: Punishment
– *"I'm looking at what will make me happy. Everyone is looking at this now, so that when I make a choice, my peace should not be disturbed, I should not face a complaint or a court. In other words, the possibility of encountering punishment affects the choice" (H11, male, 38 years old).*
– *"….when you make a mistake, there is no one beside you, you are directly in court" (H9, male, 48 years old).*
– *"Of course they're running, wouldn't you? If the patient is well, he leaves without even thanking him, but if there is a mishap, his complaint, penalty, court is dragged out. Why doctor bother with this?" (H12, male, age 49).*
Sub-Theme 5: Specialization and Status
– *"Since the structure of the system, family type and capacity in Turkey is not built on capacity, it has to be an expert in a way. ….But the physician feels obliged to be an expert in terms of his apparent status, perspective and prestige of the society. Because otherwise he is classified as a second class doctor" (H7, male, 46 years).*
– *"Why specialist medicine is preferred? a little bit of status in my opinion. So this was the case in Roman times as well. So physicians were doing magical work. In other words, there were lawyers, clergy, and sociologists in the Senate at that time, but physicians were a profession preferred by the poor in order to advance faster in terms of status. In those days, medicine was a matter of status, today nothing has changed about expertise or status" (H11, male, 38 years old).*
– *"I tried to be an expert because I didn't want to stay a general practitioner. At that time, general medicine was not even considered as a physician. So is it now. Its status is very low, expertise is required. They feel compelled to be experts and they tend towards it like all of us…" (H9, male, 48 years old).*

Exploratory Factor Analysis

The dimensions and factor scores of the exploratory factor analysis applied to the measurement tool are shown in **Table 8**. According to **Table 8**, the factor loads of the items in the expressions that make up the scale are between 0.344 and 0.798. It is seen that 23 expressions in the scale are collected in the 1st dimension, 10 expressions in the 2nd dimension, 8 expressions in the 3rd dimension, 5 in the 4th dimension, 5 in the 5th dimension, and 4 in the 6th dimension.

Factorization

There are many techniques used for factorization while performing factor analysis. These techniques combine in two main points as principal component analysis and factor extraction techniques. A good factorization process should include variable reduction, ensuring unrelatedness between new variables, and making the obtained factors meaningful [18]. While factoring in the study, the statements in the dimensions were examined one by one with a faculty member, and the relationships and unrelatedness between the statements were evaluated. When the expressions in the dimensions were examined, it was seen that all of the expressions S24th, S25th, S11th, S18th, S50th, S3rd, S22th, S48th, S53th, and S9th in the 2nd dimension belonged to the opposite expressions and there was no corre-

Table 5. Findings Related to Systemic Factors Effective in the Preference of Medical Specialization Branches

Themes and Sub-Themes / Statements of Participants

Main Theme 1: Systemic Factors

Sub-Theme 1: Performance-Based Additional Payment Application

- *"...In recent years, preclinical branches, especially biochemistry, radiology, microbiology, laboratory departments are preferred" (H5, male, 45 years old).*
- *"Performance directly affects the system, namely income is the most important thing in a person's life. When you say income, you know that doctors are getting paid like a bird, if we can call it salary now, that is a separate issue. That's why people turn to performance. Why are laboratory branches preferred so much today? Because of the performance system, physicians think that I should not bother and get my doner" (H12, male, age 49).*
- *"Yes, I am talking about performance income. Today, physicians want to go to branches with high performance scores, just because of their income" (H13, female, 42 years old).*

Sub-Theme 2: Specialization Training

- *"...by the way, where you will do your specialization training is important. We said we should not leave Antalya, but the university here is good. When I think about it, for example, both the province and the clinic affected my choice of branch" (H13, female, 42 years old).*
- *"I want to specialize. First you think about what to do. For example, you choose a province, I choose one, I don't want to go to that province, let it be a place close to my hometown, Isparta Antalya, Denizli..."(H9, male, 48 years old).*
- *"But there is another point. It is the inadequacy and inequality of medical education. Today, he called me from the Emergency Department at 11:30 and said this is a general practitioner, the man has an heir, brother..." (H7, male, 46 years old).*

Sub-Theme 3: Health Policies

- *"Actually, we can say that here. Unfortunately, the system, today's system, forces physicians to do so. In other words, if a person who has always wanted to choose the same branch and continues in this direction changes his choice because of the system, there is a serious problem there" (H11, male, 38 years old).*
- *"If the health system in Turkey changes, the preferences will also change in that direction. Of course, health policies are effective... "(H12, male, 48 years old).*
- *"Health policies are also effective. The system is constantly changing. Healthcare has changed the most in the last 10 years. Doctors' preferences are also affected by these changes" (H6, male, 52).*

Table 6. Descriptive Statistics (n = 502)

Order	Minimum	Maximum	Average	Standard deviation	Order	Minimum	Maximum	Average	Standard deviation	Order	Minimum	Maximum	Average	Standard deviation
S1	1,0	7,0	4,76	1,79	**S19**	1,0	7,0	3,88	1,81	**S37**	1,0	7,0	4,40	1,81
S2	1,0	7,0	5,46	1,34	**S20**	1,0	7,0	4,93	1,80	**S38**	1,0	7,0	4,29	1,93
S3	1,0	7,0	3,20	1,75	**S21**	1,0	7,0	4,80	1,85	**S39**	1,0	7,0	5,96	1,29
S4	1,0	7,0	1,97	1,61	**S22**	1,0	7,0	1,91	1,51	**S40**	1,0	7,0	4,64	1,98
S5	1,0	7,0	4,37	1,99	**S23**	1,0	7,0	4,32	1,84	**S41**	1,0	7,0	5,00	2,01
S6	1,0	7,0	4,34	1,68	**S24**	1,0	7,0	2,38	1,66	**S42**	1,0	7,0	3,98	1,99
S7	1,0	7,0	4,51	1,71	**S25**	1,0	7,0	2,94	1,58	**S43**	1,0	7,0	3,19	2,05
S8	1,0	7,0	4,32	1,82	**S26**	1,0	7,0	5,02	1,80	**S44**	1,0	7,0	4,76	1,95
S9	1,0	7,0	5,18	1,58	**S27**	1,0	7,0	3,30	1,97	**S45**	1,0	7,0	4,71	1,76
S10	1,0	7,0	6,24	1,16	**S28**	1,0	7,0	4,06	1,97	**S46**	1,0	7,0	4,86	1,84
S11	1,0	7,0	2,72	1,47	**S29**	1,0	7,0	5,45	1,81	**S47**	1,0	7,0	4,45	1,79
S12	1,0	7,0	3,75	1,88	**S30**	1,0	7,0	5,13	1,67	**S48**	1,0	7,0	3,97	1,64
S13	1,0	7,0	5,25	2,01	**S31**	1,0	7,0	3,64	1,52	**S49**	1,0	7,0	3,18	2,02
S14	1,0	7,0	4,43	2,04	**S32**	1,0	7,0	5,14	1,81	**S50**	1,0	7,0	2,76	1,73
S15	1,0	4,0	2,34	1,13	**S33**	1,0	7,0	3,46	2,04	**S51**	1,0	7,0	4,92	1,65
S16	1,0	7,0	4,61	1,93	**S34**	1,0	7,0	5,00	1,87	**S52**	1,0	7,0	4,96	1,86
S17	1,0	7,0	5,07	1,86	**S35**	1,0	7,0	4,37	1,99	**S53**	1,0	7,0	2,96	1,72
S18	1,0	7,0	2,97	1,64	**S36**	1,0	7,0	4,41	1,94	**S54**	1,0	7,0	4,64	1,94
										S55	1,0	7,0	3,39	1,97

Table 7. View of Total Explained Variance

Dimensions	Total explained variance								
	Eigenvalues			Sums of Factor Loads			Rotated Sums of Factor Loads		
	Total	Variance %	Cumulative %	Total	Variance %	Cumulative %	Total	Variance %	Cumulative %
1	13,622	24,767	24,767	13,622	24,767	24,767	11,596	21,083	21,083
2	3,878	7,051	31,818	3,878	7,051	31,818	3,375	6,137	27,220
3	2,625	4,772	36,590	2,625	4,772	36,590	2,937	5,339	32,559
4	2,298	4,178	40,768	2,298	4,178	40,768	2,830	5,145	37,704
5	1,883	3,423	44,191	1,883	3,423	44,191	2,634	4,788	42,492
6	1,654	3,007	47,198	1,654	3,007	47,198	2,588	4,706	47,198

Table 8. Items and Factor Load Scores

Dimensions	Items and Factor Load Points
1 (23 expressions)	S34 (0,796), S32 (0,773), S46 (0,761), S44 (0,756), S52 (0,746), S54 (0,741), S40 (0,724), S21(0,715), S16 (0,713), S26 (0,706), S17(0,687), S38(0,682), S42(0,663), S29 (0,642), S5 (0,631), S28 (0,624), S13 (0,597), S14 (0,576), S36 (0,573), S15 (0,550), S8 (0,498), S31 (0,406), S55 (0,394)
2 (10 expressions)	S24 (0,640), S25 (0,543), S11 (0,541), S18 (0,502), S50 (0,488), S3(0,477), S22 (0,458), S48 (0,414), S53 (0,386), S9 (0,344)
3 (8 expressions)	S35 (0,705), S43 (0,688), S41 (0,614), S33 (0,575), S49 (0,514), S47 (0,798), S37 (0,736), S45 (0,725)
4 (5 expressions)	S39 (-0,693), S10 (-0,610), S30 (-0,541), S23 (-0,521), S27 (0,467)
5 (5 expressions)	S1 (0,736), S7 (0,695), S20 (0,625), S12 (0,546), S2 (0,432)
6 (4 expressions)	S6 (0,725), S51 (0,705), S19 (-0,619), S 4(0,555)

Table 9. Analysis of the Measurement Tool for the 1st Dimension of the Dimensional Instrument

Dimension	Items and Factor Load Points
1 (12 expressions)	S26 (0,752), S42 (0,740), S34 (0,728), S29 (0,723), S52(0,714), S44 (0,709), S28 (0,708), S46(0,666), S40(0,663), S17(0,609), S21 (0,578)
2 (7 expressions)	S13 (0,720), S8 (0,682), S55 (0,641), S16 (0,637), S5 (0,632), S27 (0,577), S31 (0,451)
3 (4 expressions)	S36 (0,782), S38 (0,630), S14 (0,602), S54(0,581)

lation in terms of the expressions in the dimension. Since 'S49', which is one of the expressions forming the 5th dimension, is meaningless in the dimension and S4, which is in the 6th dimension, is meaningless in the dimension, these expressions were excluded from the measurement tool. Then, the dimension structure of the whole measurement tool was re-examined, and although the expressions were grouped under dimensions, to create a more homogeneous scale, factor analysis was also conducted on 23 expressions that make up the first dimension, and it was determined that the expressions here were also divided into dimensions (**Table 9**).

After the dimensions and expressions in the measurement tool were determined, the dimensions were named. It was seen that the final version of the measurement tool consisted of 43 statements and 7 sub-dimensions. The names and expressions given to the dimensions are given in **Table 10**.

Discussion and conclusions

In the last century, there have been tremendous scientific and technological developments in the field of medicine. Medical knowledge that transcends borders has resulted in specialization in medicine. Since the beginning of the last century, the whole world has entered a very rapid transformation due to the beginning of specialization in medicine, discoveries emerging with technological advances in medicine, new diseases emerging with the increase in life expectancy, and political developments; The health sector has also been

Table 10. Names and Expressions Given to Dimensions

Dimension	
Risk	26 – I prefer branches with fewer seizures. 42 – I prefer branches with a low probability of encountering difficult patients. 34 – I prefer branches where the probability of encountering an administrative investigation due to the treatment or procedure applied to the patient is low. 32 – I prefer branches with low risk of malpractice in patients. 29 – I prefer branches where I will not be exposed to hostile attitudes from patients. 52 – I prefer branches where the possibility of paying compensation for the treatment or procedure applied to the patient is low. 44 – I prefer branches where the probability of being judged due to the treatment or procedure applied to the patient is low. 28 – I prefer branches that do not have emergency services. I prefer branches with low risk of complications in 46 – patients. 40 – I prefer branches with a low mortality rate in their patients. 17 – I prefer branches where I am less likely to be verbally insulted. 21 – I prefer branches where I am less likely to make mistakes.
Dimension	
Comfort	13 – If I get a high score in the TUS exam, I prefer comfortable branches. 8 – In order to increase the performance score, I prefer branches in which I will not exert much effort. 55 – Today, as a result of the TUS exam, I choose the branches most preferred by the physicians. 16 – I prefer branches with a light workload. 5 – I prefer branches with comfortable assistantship training. 27 – I prefer branches where I do not need to develop a dialogue with the patient. 31 – I prefer branches where the performance score is fixed every month.
Dimension	
Health problems	36 – I prefer branches that do not require much physical strength. 38 – I prefer branches that do not require me to run all the time. 14 – I prefer branches that do not require me to stand for a long time. 54 – I prefer branches where I will be less physically tired.
Dimension	
Status	43 – Since I think that specialist physicians look at general practitioners negatively, I will choose a branch. 35 – I will choose a branch because I think being a specialist is prestigious. 49 – I prefer a branch to go to the compulsory service later. 33 – I will choose a branch because of the social pressure on physicians. 41 – I will choose a branch because I do not want to stay as a general practitioner
Dimension	
Emotional Involvement	51 – I prefer branches that have the opportunity to do research. 19 – I prefer branches that require my lifelong reading and research. 39 – I prefer branches that suit my personal abilities. 30 – I always prefer branches that are in my ideal. 6 – I prefer branches that I think will work with high-level technology in the future. 23 – I prefer the branches of my professors that I was influenced by during my medical education. 10 - I prefer branches that suit my personality.
Dimension	
Earning	47 – I prefer branches with high performance gain. 37 – I prefer branches with high performance scores. 45 – I prefer branches where I will earn more.
Dimension	
Gender and Marital Status	1 – Gender is effective in choosing the branch of physicians. 7 – Male physicians tend to choose surgical branches. 20 – The number of shifts is important in the branch preference of female physicians. 12 – It is important that spouses are guided by the choice of branch of married physicians.

affected by these developments. Health reform movements have started in many countries, and health systems have been restructured by governments. As a result of this change and transformation, which affects many areas in health, the preference of physicians in their specialty has changed direction, branches that were popular in the past have lost their popularity, and the preferences of physicians have been reshaped. From this point of view, it is aimed to determine the factors affecting the preferences of specialization in medicine in this study. The choice of field of spe-

cialization and the factors affecting it have been the subject of many studies all over the world. In these studies, it was revealed that gender, marital status, desire for specialization, presence of a physician in the family, talent, personal interest, wage policies, workload, and working environment were effective in the preference of physicians [5, 19–24]. In this study, similar and consistent results were obtained with the studies carried out.

The qualitative findings of the research reveal detailed and rich data reflecting the views of physicians in the health system. The findings reveal that personality traits and idealism are important in choosing a specialty, although many factors affect the choice of specialty in medicine. Buddeberg Fischer and collaborators of her concluded that gender has a strong effect on the choice of branch, and that personality traits and ideals affect this choice [25].

Demographic characteristics related to individual factors were discussed by female participants in the context of gender and roles, and female physicians stated that they also care about the role of motherhood and the wishes of their spouses in their branch selections. Bedoya-vaca et.al. in their research on gender and specialty in medicine drew attention to the increasing employment of women in the field of medicine and stated that women, especially women who are surgeons, have difficulties in work life, and they consider the socio-cultural characteristics in the choice of specialization and observe the balance between family and professional life; Heiliger and Hingstman reported that especially female physicians consider the work and family balance in their study to reveal the branch choices of physicians, therefore they prefer the part-time working system. Similar results were obtained in our study. In addition, female physicians, who drew attention to the working conditions, reported that the branches with a low number of shifts were preferred [19, 26].

In this research, they stated that individual abilities are important and can affect their choices. Physicians drew attention to the importance of manual dexterity, tool use, and physical strength, especially in surgical branches. Han reported that the most important issue for physicians, especially those specializing in surgery, is talent and skill characteristics [27]. Similarly, Park et al. reported that teamwork is important for surgical branches, and talents and skills are very important for a successful professional life in a qualitative study conducted in Korea to reveal the perceptions of physicians regarding the specialty characteristics. In the study, one of the remarkable findings regarding the factors thought to affect the choice of the branch was the mortality rate of patients within the specialty [23].

The participants shared their experiences that they did not want to encounter difficult patients in their branch selection and that they empathized especially as a result of the death of the patient they treated, so they thought about branches that did not have a high mortality rate in their patients, and they chose this direction.

Threatening behavior that poses a risk emerged as another factor affecting the choice of a branch in the study, and almost all of the participants mentioned the recent increase in threatening behavior that poses a risk and stated that this situation caused a withdrawal in the physicians working in the system, and that important branches were not preferred for the physicians who were not yet in the system. In addition, physicians stated that they faced sanctions such as administrative investigations, courts, and penalties due to the risks of their profession, especially the risk of malpractice, which both affected their motivation and preferred comfortable branches to avoid such sanctions, and therefore some difficulties were experienced.

This study investigated the factors affecting the preferences of specialization in the field of medicine. The strengths of our study are the design of a mixed method study and the in-depth discussion of the views of specialist physicians.

The present study has some limitations. Personality traits are inherited and relatively constant throughout life, and important life events influence this personality development. Therefore, it is possible for a physician's personality would be affected because of medical education and/or career choice. Longer-term follow-ups will be necessary to gain a broader and more reliable table of how a physician's personality contributes to career choices and to shed light on questions of causation. We were not able to retain all specialties in the qualitative phase of the study, we retained representatives of the most preferred specialties and the least preferred fields. In terms

of data diversity, we could have consulted with more specialist physicians.

In the study, it was concluded that an important factor affecting the branch preferences is the performance-based additional payment application applied in Turkey, it was revealed that the application directly affects the physician preference and is the dominant factor due to economic reasons. In addition, the results of the research indicate that the risk of the branch, exposure to threatening behavior that poses a risk and performance-based additional payment application affect and guide this choice. This result was not surprising in our study, especially since threatening behavior that poses a risk is an important problem faced by healthcare professionals all over the world. Considering that factors other than personality and idealism that stand out in our qualitative findings are also deterrents, it is suggested that policymakers and researchers interested in the subject should work on these deterrent reasons in the future.

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The authors declare no conflict of interest.

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Authors' contributions
NÖ and MG designed the study; NÖ performed database searches for literature; two authors screened titles and extracted data; NÖ and MG performed initial analyses of data; two authors discussed results, finalized analyses, and potential implications of the results; NÖ and MG drafted the manuscript with tables and appendices; two authors read and approved the final manuscript.

Availability of data and materials
The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

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Ethics Committee approval was obtained from Süleyman Demirel University for the research.

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REVIEW PAPER

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A novel approach to alpha-lipoic acid therapy in the treatment of diabetic peripheral neuropathy

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ABSTRACT

Diabetic peripheral neuropathy (DPN) is a heterogenic disorder prevalent amongst patients suffering from diabetes mellitus (DM), with symptoms comprising neuropathic pain, paresthesia, and numbness in distal lower limbs. Alpha-lipoic acid (ALA) is proposed as a pathogenesis-oriented treatment option, targeting underlying causes of neural lesions such as hyperglycemia, metabolic and microvascular dysfunctions, and cellular oxidative stress. We performed a comprehensive review of controlled clinical trials demonstrating the clinical usefulness of ALA in the treatment of DPN, published in the last 5 years to determine the benefits of ALA monotherapy and combined treatments with other known antioxidants. We also investigated the differential efficacy of oral versus intravenous ALA administration. Clinical trials show the efficacy of ALA treatment, attributed to its anti-inflammatory, anti-hyperglycemic, and antioxidant properties, as well as its function in the endothelial activation and lipid metabolism parameters. ALA supplementation is associated with amelioration in nerve conduction velocity scores, clinically significant reduction of reported neuropathic pain, burning and paresthesia, as well as a decrease in serum triglycerides, improved insulin sensitivity, and quality of life.

Introduction

Diabetic peripheral neuropathy (DPN) is a symmetrical, length-dependent sensorimotor polyneuropathy related to metabolic and microvessel alterations as a result of chronic hyperglycemia exposure and cardiovascular risk covariates. Despite the advances in knowledge of the pathogenesis of neuropathy, there are still few causal therapeutic options. Alpha-lipoic acid (ALA) is suggested as a pathogenesis-oriented treatment option, targeting underlying causes of neural lesions. The efficacy of ALA in patients with diabetes mellitus (DM) is attributed to its anti-inflammatory, anti-hyperglycemic, and antioxidant properties, as well as its role in endothelial function, insulin sensitivity, and lipid metabolism parameters.

Pathophysiology of diabetic peripheral neuropathy

DPN is a neurodegenerative disorder, characterized by morphological changes and lesions mainly to the peripheral nerves. The major pathomechanisms include demyelination and thickening of the axon, contraction, and diminishment of Schwann cell as well as distortion of Ranvier nodes. An overall decrease in unmyelinated fibers which innervate organs of the abdominal cavity is usually present, however negative changes in peripheral nerves are observed more frequently. The pathogenesis of this disease has a complex mechanism. Nevertheless, two potential mechanisms of DPN are proposed: metabolic and ischemic. Hyperglycemia is considered a major factor causing disorders of the nervous system in DM [1].

The current state of knowledge indicates that hyperglycemia causes nerve damage in four mechanisms: activation of the polyol pathway and hexosamine pathway, kinase A activation, and oxidative stress. In the polyol pathway glucose is converted to sorbitol by aldose reductase and then to fructose by sorbitol dehydrogenase. Elevated levels of glucose, sorbitol, and fructose have an impact on the metabolism disorder of neurons. The abnormal activation of the polyol pathway increases the osmotic pressure which can result in nerve cell damage. In addition, activation of the polyol pathway decreases nicotinamide adenine dinucleotide phosphate (NADPH) levels, which is essential for the regeneration of glutathione (the main cellular antioxidant). The insufficiency of NADPH leads to disturbance in the redox potential may cause rapid intensification of oxidative stress. Chronic hyperglycemia induces oxidative stress which has a wide impact on nerves such as accelerated apoptosis of ganglionic cells and Schwann cells.

The hexosamine pathway is activated by high glucose concentration. Glucose is a substrate in glycolysis where it is transformed to fructose-6-phosphate, fructose-6-phosphate is then converted to glucosamine-6-phosphate and subsequently to N-acetylglucosamine. The connection of N-acetylglucosamine with serine and threonine leads to pathological gene expression of transforming growth factor-beta, plasminogen activator inhibitor-1. All those changes have a negative effect on blood vessels [2].

In the vascular theory of DPN, pathological changes in the vasa nervorum can be observed. Blood samples from the patients with DM show elevated levels of alfa-2-globulin, fibrinogen, and reduced level of albumin, all of which may lead to a lower concentration of nitric oxide, one of the diastolic factors in the cardiovascular system. All these factors reduce blood supply and lower the microcirculation around the nerve via vasoconstriction, increasing the osmotic pressure [3]. Additional risk factors in the development of DPN include immune system disorders, a disorder in axonal transport, hyperlipidemia and dyslipidemia [4, 5].

Current treatment options in DPN

Nowadays, the treatment of DPN is considered to be an extensive and demanding issue. The treatment is difficult to execute and often ineffective, especially in the advanced stages of DPN. No treatment has been invented yet, that could either fully cure, or at least entirely prevent neuropathic changes. Complete pain relief or reduction of its intensity by half is achieved only in about 50% of the cases [6]. Therefore, treatment goals are mainly focused on managing conditions that could lead to neuropathy, and secondly, on providing at least partial relief of symptoms.

The preventive treatment of DPN is based on education regarding risk factors and adapting

therapies and habits that alter pathological pathways. As evidenced by large observational and randomized control trials, such as DCCT (Diabetes Control and Complications Trial), strict control of glycemia is essential in the prevention and delay of symptoms onset of distal symmetrical polyneuropathy and cardiovascular autonomic neuropathy in type 1 DM [7]. It has been proven that intensive insulin therapy in the treatment of type 1 diabetes plays a crucial role in slowing down the progress of DPN. In addition, maintaining normalized blood pressure and body weight, supported by a non-sedentary lifestyle is also suggested in DPN prevention [8]. A balanced and healthy diet, smoking cessation, and alcohol restriction have been proven to have a positive impact on reducing the risk of DPN [9, 10].

Much evidence points to the role of oxidative stress in the pathogenesis of DPN [11]. Therefore, supplements containing α-lipoic acid (ALA), a potent antioxidative agent are used in the preventive treatment of DPN, along with other active agents such as angiotensin-converting-enzyme inhibitors (ACE-I), benfotiamine, an S-acyl derivative of thiamine (vitamin B1) and acetyl-l-carnitine [12].

It is important to note that DPN is symptomatic in only 50% of all diagnosed cases. Symptomatic treatment is focused on reducing pain that often accompanies DPN. It is a crucial aspect of the therapeutic approach, as pain management contributes to improving a patient's quality of life. In pharmacotherapy, anticonvulsant agents such as gabapentin or pregabalin, as well as antidepressants: duloxetine, and venlafaxine are recommended as first-choice medication for reducing pain and paresthesia. The standard therapeutic approach includes gradual dose increase until a therapeutic concentration of selected medication is set. In case of ineffectiveness, it is possible to attempt a combined agent therapy with two different types of medication or to change treatment to another medication entirely. A second-choice option in pharmacological treatment are tricyclic antidepressants (TCA), serotonin-noradrenaline reuptake inhibitors (SNRI), and α2-δ calcium channel ligands (the latter in case of autonomic nervous system neuropathy) [13, 14]. In difficult cases, with neuropathic pain resistant to standard treatment options, tramadol or other potent opioids can be administered, however, long-term use of opioids is not recommended due to multiple adverse effects and highly addictive properties. The use of over-the-counter analgesics such as metamizole(dipyrone) and mefenamic acid can be considered as a treatment option, but often proves to be insufficient in pain management amongst DPN patients. Adverse effects of certain drugs, such as anxiety, nausea, cardiovascular complications, or addiction are prevalent [15]. Other medications, while not listed in current treatment guidelines, can play a supplementary role in DPN pain management, preventing sorbitol accumulation and regulating polyol pathway (aldose reductase inhibitors), improving microvascular blood flow and functions of nerve fibers (protein kinase – C inhibitors), or by simply alleviating neuropathic pain (N-methyl-D-aspartate receptor antagonists, NMDAR) [5, 15].

ALA in current clinical recommendations

Due to the important clinical significance of ALA, it has been repeatedly mentioned as supplementary pathogenesis-oriented pharmacotherapy treatment of DPN over the past few years. This clinical recommendation remains consistent since 2014 and is underlined in 2022 guidelines issued by Diabetes Poland [14]. Currently, ALA is listed by Diabetes Poland as a first choice supplementary medication, alongside other agents such as benfotiamine and angiotensin-converting-enzyme inhibitors. The usefulness of ALA in DPN treatment was indicated in the 2022 guidelines of the International Diabetes Federation, listing ALA (600 mg/d, oral or i.v.) together with vitamin-B1 derivative Benfotiamine as pathogenically oriented treatment of symptomatic diabetic sensorimotor polyneuropathy. Currently, ALA is a drug licensed and approved for the treatment of DPN in several countries worldwide [16].

Material and Methods

We conducted detailed research of articles published in the English language, with full-text access available via the PubMed database. The database was searched using the following keywords: "a-lipoic acid" or "thioctic acid" and one

of the subsequent terms: "polyneuropathy", "diabetic neuropathy", "diabetes mellitus" and "hyperglycemia". The following inclusion criteria were applied: (1) studies published between 2017 and 2022, (2) controlled clinical trials conducted on humans and/or other mammals, (3) metanalyses meeting the criteria identified in (1) and (2). Using the aforementioned criteria, a total of 1,830 articles were identified, out of which 57 were found to be fully relevant and included in this review.

ALA monotherapy in DPN – evidence in clinical studies

Both hyperglycemia-triggered oxidative stress and defects in microvasculature are associated with the progression of nerve damage. By addressing each of these pathologies consecutively, ALA supplementation may bring successful outcomes in DPN treatment. Ziegler et al. metanalysis summarized the results of multiple double-blind clinical trials investigating ALA treatment [17]. Trials using alpha-lipoic acid infusions of 600 mg i.v. per day for 3 weeks, except for weekends, in diabetic patients with DPN were included. These were four trials: ALADIN I, ALADIN III, SYDNEY, and NATHAN II. Together 1258 patients (alpha-lipoic acid n = 716; placebo n = 542) were included in a meta-analysis. After 3 weeks the relative difference in favor of alpha-lipoic acid vs. placebo was 24.1% for Total Symptom Score (TSS) and 16.0% for Neuropathy Impairment Score of the lower limbs (NIS-LL). Among the individual components of the TSS, pain, burning, and numbness decreased in favor of alpha-lipoic acid compared with placebo, while among the NIS-LL components pin-prick and touch-pressure sensation, as well as ankle reflexes, were improved in favor of alpha-lipoic acid.

In detail, the ALADIN trial revealed clinically relevant improvement in Total Symptom Score, Neuropathy Disability Score (NDS), and Hamburg Pain-Adjective (HPA) results in patients who received daily (100/600/1200 mg/day, intravenous) ALA supplementation over the course of 3 weeks [18]. The objective of subsequent studies on ALADIN II and ALADIN III was to find an optimal therapeutic dose and regimen [19]. The ALADIN II study analyzed the response of patients with DM2 to ALA treatment (600/1200 mg/day oral) over the course of 2 years as measured by sensory nerve conduction velocity (SNCV) and sensory nerve action potential (SNAP) before and after treatment. After 2-year treatment with ALA, an amelioration of both clinical parameters was observed. ALADIN III broadened the scope of research, by introducing a combined treatment schedule, consisting of 3 weeks of intravenous, and subsequent 6 months of oral ALA supplementation (600 mg and 1800 mg/day). The clinical parameters applied in this study were TSS, NIS-LL, and general NIS. Treatment results showed improvement in TSS, NIS-LL, and NIS, as assessed by pin-prick, touch-pressure sensation, and ankle reflexes [20].

These findings correspond with outcomes of the SYDNEY clinical trial, where 3-week intravenous ALA supplementation (600 mg/day) proved effective in the amelioration of TSS, NIS, and Neuropathy Symptoms and Changes (NSC) versus placebo [21]. It should be noted that no adverse effects were observed in either of the clinical trials as presented in the Ziegler et. al. metanalysis except SYDNEY 2 and Mansoura studies. The former observed an incidence of dose-dependent (1200 mg/day and up) adverse effects, while the latter showed nausea as the most common adverse effect, with no correlation to the dose applied [22].

A long-term (4-year duration) Neurological Assessment of Thioctic Acid in Diabetic Neuropathy (NATHAN) reported improvement in pain, paresthesia, and numbness in participants suffering from DM2 and largely asymptomatic diabetic sensorimotor polyneuropathy. NATHAN I supplied evidence for the safety and efficacy of long-term ALA supplementation in the 600 mg/day dose in the treatment of neurological deficits related to DPN [23]. A recent clinical trial study by Agathos et al. researching the benefits of a 600 mg/day dose of ALA showed similar findings. In this trial, a significant improvement in the quality of life, as assessed by reduction of pain severity and pain interference, was observed amongst participants with painful DPN who were subjected to 40 days of oral ALA supplementation. Assessment with Neuropathy Symptom Score (NSS), Subjective Peripheral Neuropathy Screen Questionnaire (SPNSQ), and Disability Score showed a decrease in neuropathic signs, symptoms, and pain on day

40 versus baseline. A reduction in fasting triglyceride serum levels was also observed over the course of the trial. Similarly to earlier studies (ALADIN II, ALADIN III, NATHAN I) excellent safety profile of ALA was indicated as a key advantage of this therapeutic approach [24].

ALA has been also studied as one of the active agents directly addressing the underlying pathophysiology of DPN. ALA treatment over the course of 16 weeks in type 2 diabetic patients with symptomatic DPN showed a positive response to the treatment after just 4-weeks of high-dose supplementation [25]. Amelioration of nerve conduction velocity was also reported in a recent single-arm study conducted by S. Mrakic-Sposta et al., although the results of the study may be inaccurate due to the lack of a control group [26]. Importantly, this study indicates the low efficacy of short-term ALA supplementation on long-term patient outcomes, as anti-oxidative cell capacity returns to baseline level 60 days post-treatment termination, suggesting a need for optimal dose and treatment schedule to sustain therapeutic effect.

Effects of ALA supplementation as compared to other antioxidants. Benefits of ALA monotherapy versus combined agent treatment in patients with DPN

The isolated efficacy of vitamin B12, vitamin B9, vitamin E, vitamin D, and ALA supplementation in the treatment of DPN was investigated in several studies [27–29]. Vitamin B12 deficiency, associated with neurological disorders (peripheral, autonomic, and cardiovascular neuropathy) is a common occurrence in patients with type 2 diabetes (DM2) on metformin treatment, and those older than 60 years [30]. Combined ALA and B12 supplementation were shown to be successful in reducing burning sensation and pain in patients with DPN. A study by Han Y. et al. investigating the differential efficacy of methylcobalamin over ALA revealed that ALA treatment was superior in reducing burning and pain symptoms of DPN, while methylcobalamin reduced paresthesia and numbness to a greater extent [31]. ALA exhibited stronger antioxidant properties, while reduction of abnormal pressure sensation was caused by B12 supplementation only.

Vitamin D deficiency was found to be present in patients with DM2 and peripheral polyneuropathy, and current data shows that patients with DPN can benefit from high-dose cholecalciferol supplementation, leading to a decrease in neuropathy severity and amelioration of pain scores [28]. Similarly, the administration of folic acid is considered to enhance nerve conduction velocity in patients with DPN. Nano-curcumin supplementation was revealed to improve the total reflex score, and total score of neuropathy and reduce glycated hemoglobin in patients with diabetic sensory-motor polyneuropathy. Numerous research indicates that tocotrienol-rich vitamin E can improve nerve condition velocity in these patients due to its antioxidant, anti-inflammatory, and neuroprotective properties [32-35].

The efficacy of combined antioxidant therapy was confirmed in a randomized, double-blind trial conducted on a population of DM type 2 patients, all of whom experienced generalized neuropathy and underwent metformin treatment for at least four years [36]. The proposed treatment included a single tablet, four element combination of 10 mg superoxide dismutase, 570 mg α-lipoic acid, 300mg N-acetyl carnitine, and 250 mcg vitamin B12, administered daily for the period of 12 months. The study showed an improvement of the neurophysiological parameters in the study group, assessed by vibration perception threshold, conduction velocity, and amplitude of sural nerve. A notable improvement in the patient's condition was observed, with a pain reduction of 16% in the study group and improved quality of life score. Another study comparing the effectiveness of γ-linolenic acid with α-lipoic acid in pain management amongst painful diabetic peripheral neuropathy patients found no preference for either treatment [37]. Research conducted by Memeo et. al. showed the superiority of 600 mg/day ALA treatment over 1180 mg/day acetyl--L- carnitine treatment, with improved symptoms, electromyography findings, and reduced need for analgesics in patients receiving ALA supplementation [38].

The benefits of ALA monotherapy over combined treatment with ALA and other antioxidants require further research. A study by Huerta et al. suggests that α-lipoic acid in connection with

eicosapentaenoic acid helps to regulate adipose tissue metabolism, which may be beneficial in maintaining optimal blood sugar levels in DPN patients with dyslipidemia, preventing protein glycation and endothelial damage leading to neural ischemia and neuronal lesions [39]. The anti-inflammatory properties of ALA function are hypothesized to further enhance this result. The efficacy of commonly used vasodilatory drugs in DPN treatment can be strengthened by combining them with ALA. The effectiveness of combined alprostadil and ALA treatment is suggested by a recent study [40]. This result is attributed to the mechanisms of action of both substances, which simultaneously target microangiopathy and oxidative stress, which are considered major factors in DPN pathogenesis. When combined with alprostadil, ALA enhances the therapeutic effect by increasing the activity of Na+/K+-ATPase to protect the endothelium function of blood vessels, blocking protein glycation and increasing blood flow of neurotrophic blood vessels.

A meta-analysis of Jiang et al. showed that another vasodilator and anti-platelet drug, fasudil, when combined with either vitamin B12 or ALA, attenuates nerve conduction velocity more significantly than B12 or ALA monotherapy [41]. The superiority of combined epalrestat and ALA treatment compared to ALA monotherapy was recognized in the study of Zhao et al., with a greater reduction of high sensitivity C-reactive protein level and increase in nerve conduction velocity observed in the combined therapy group, later confirmed in a large meta-analysis study [42, 43]. However, a need for subsequent high-quality randomized controlled trials is expressed in the literature.

Finally, in patients with distal symmetric painful diabetic neuropathy, a greater reduction of pain intensity and diabetic neuropathy symptom score was obtained with concurrent administration of benfotiamine (B1) and ALA as compared to monotherapy with either agent. When compared with monotherapy, a combined approach of menhaden fish oil, enalapril, and ALA brought promising results in the reversal of corneal sensation and nerve loss in a type 2 rat model of chronic diabetes [44].

A study by Pieralice et al. investigating combined ALA ± palmitoyl-ethanolamide (PEA) treatment on patients with neuropathic symptoms (600 mg/day ALA ± 600 mg/ day PEA orally) reported a clinically significant reduction in neuropathy symptoms. Notably, the time needed for symptoms' relief was much shorter than indicated in studies focusing on isolated ALA supplementation, which was attributed to the anti-inflammatory and analgesic effects of PEA [45]. Further research is needed to investigate interactions between these two agents and their clinical application in DPN treatment.

In all cases, it is hypothesized that the superior therapeutic effect of combined therapies is derived from all agents working synergistically, each targeted at a different metabolic key point. There exists, however, a limited body of research on the combined effects of different antioxidants and ALA and the benefits of combined therapies as compared to monotherapy, underlying the importance of further studies.

Oral versus intravenous supplementation of ALA

In the ALADIN study, ALA was administered intravenously for three weeks in subjects with symptomatic DN at a dose of 600 or 1200 mg daily. This treatment reduced the symptoms of DPN without significant adverse effects [18]. SYDNEY study demonstrated that, in addition to DPN symptoms, nerve conduction was also improved by ALA, administered intravenously for five days in 14 perfusions [21]. In the SYDNEY 2 study, the oral 600 mg dose of ALA was proven the most effective dose (among 600, 1200, and 1800 mg) in reducing symptoms and with the fewest side effects during a follow-up of 5 weeks [22]. Similarly, in the 'Oral Pilot' (ORPIL) study, ALA, administered orally at a dose of 600 mg for three weeks, decreased DPN symptoms, including pain, burning sensation, paraesthesia, and numbness [46]. However, clinically relevant reductions in Total Symptom Score (i.e. >30%) were only observed with intravenously administered ALA at 600 mg/day for 3 weeks, but not with orally administered ALA at a dose of >600 mg/day for 3-5 weeks in the meta-analysis of Mijnhout et al. [47].

Based on the current research it can be concluded that both ways of administration may bring a clinically significant reduction of neuropathic symptoms such as burning, pain, numb-

ness, or paresthesia and amelioration of nerve conduction velocity, with probable best outcomes present while combining both IV and O. therapeutic approach [48].

Alfa-lipoic acid- biokinetics, clinically significant properties, and mechanisms of action as supported by the latest studies

ALA is characterized by several unique biochemical properties. ALA penetrates the blood-brain barrier, while its liposoluble and water-soluble character allows it to function both -intra and -extracellularly in the human organism. Evidence-based clinical applications of ALA include a variety of neuropathic pain disorders, such as carpal tunnel syndrome, peripheral neuropathic sciatic pain caused by herniated disc and chronic migraines (30% response rate in reduction of occurrence, duration and pain intensity). According to the neuropathic pain model of migraine pathophysiology, the onset of migraine pain is derived from the neurogenic inflammation affecting cranial vessels, which results in changes of blood perfusion. Therefore, a chemical agent such as ALA, targeting the underlying neurological cause of migraine attack, can prove to be an effective treatment strategy. Other clinical applications of ALA can be found in the treatment of neurodegenerative disorders, ischemia-reperfusion injuries, cataracts and chronic pain [19].

Current research on the biokinetics of drugs containing ALA shows rapid absorption of the active agent, with the highest serum ALA concentration levels after approximately 60 to 90 minutes post oral administration, respectively in tablet and capsule formula. The half-life of a 1200 mg ALA oral dose arrives at 81.2 ± 97.1 minutes in the human organism [49]. Alpha-lipoic acid is generally considered safe when taken as an oral supplement or used as a topical ointment. A maximum daily dose of up to 1800 mg is safe for adult patients. For maximum absorption, the supplements should be administered upon fasting [50].

Adverse effects are rare and may include insomnia, fatigue, diarrhea, skin rash, headache, muscle cramp, or a tingling "pins and needles" sensation, and will usually resolve once treatment is stopped. Current research on the long term safety of ALA indicates possible toxicity of doses 2400 mg /day or greater [51]. Due to potential adverse effects and no available clinical trials performed alpha-lipoic acid should not be used in the treatment of pediatric patients, pregnant women, or nursing mothers. ALA can lower blood sugar levels, thyroid hormone, or vitamin B1 levels. It can be dangerous in patients suffering from alcoholism where malnutrition and vitamin B1 insufficiency is already present.

ALA therapeutic efficacy is relatively low due to its pharmacokinetic profile. Due to fast hepatic degradation, reduced solubility, as well as instability in the digestive system and short half-life, bioavailability of ALA, oscillates around 30%. Liquid forms of ALA are more bioavailable than solid dosage forms. The former enhances the recovery of sensory and motor nerve conduction velocity altered by diabetic neuropathy in animal models [52,53]. New, improved forms of distribution, show better absorption rate and therapeutic outcomes. While the bioavailability of ALA depends on the patient's age, there is no correlation between the patient's gender and the absorption rate of the substance [52].

In its reduced form – dihydrolipoic acid (DHLA), ALA exhibits potent antioxidant properties, successfully eradicating reactive oxygen species (ROS), chelating metals, and regenerating other antioxidants [54]. These features of ALA have found clinical implementation primarily in the treatment of diseases associated with oxidative stress, for instance in diabetes, neurological and cardiovascular disorders. Some findings show improved arterial stiffness parameters and insulin resistance (IR) reduction in patients subjected to ALA treatment, suggesting that the antioxidant properties of ALA can be responsible for the improvement in arterial wall elasticity [55]. Moreover, intravenous infusion of ALA was associated with improved microcirculation in patients with DPN, as shown by accelerated time to peak capillary blood cell velocity (CBV). This effect was attributed to post-ALA-infusion improvement of nitric oxide (NO) mediated endothelium-dependent vasodilatation, as well as a reduction in malondialdehyde and increase in ubiquinol-10 plasma level.

Similarly, in Irbesartan and Lipoic Acid in Endothelial Dysfunction (ISLAND) study the

authors evaluated the ability of irbesartan, an angiotensin receptor blocker, and lipoic acid to affect endothelial function and inflammation in patients with the metabolic syndrome. Oral ALA supplementation improves endothelial function and reduces proinflammatory markers, improving endothelium-dependent flow-mediated vasodilatation [56]. ALA is also thought to attenuate renal and vascular endothelin I production and restore Ca++ levels, which allows it to successfully lower blood pressure in hypertensive animal models [57].

The presence of ALA in a human organism has been linked to several health benefits. ALA is known as a potent antioxidant and anti-diabetic substance, present in low quantities in almost all foods. Hyperglycemia is one of the main factors that initiate the production of free oxygen radicals, increasing the likelihood of oxidative stress present in the human body. Therefore, the anti-oxidative function of this featured acid might play a positive role in the treatment of diabetes and subsequent DPN. Due to bipolar properties, ALA may have an anti-oxidative effect on the majority of the body's tissues and cells, whereas other antioxidants can function only selectively.

ALA present in therapeutic doses mediates the effects of oxidative stress caused by Fenton's reaction. The sulfur group present in ALA causes the chelation of heavy metals such as iron ions, which are substrates in the Fenton reaction resulting in the generation of hydroxyl radicals. These compounds immediately react with lipids and cause lipid peroxidation in biological membranes. This process exhibits a negative effect on the cardiovascular system, contributing to the damage to endothelial lining. ALA and iron ions form a complex, resulting in a significant reduction of free oxygen radical production [58].

Recent studies have shown that ALA enhances glutathione synthesis, a crucial intracellular antioxidant [58, 59]. ALA is thought to act as a pro-oxidant, indirectly inducing antioxidant enzymes gene expression via binding nuclear factor E2-related factor 2 (Nrf2) [57]. Furthermore, ALA enhances the efficacy and increases the activity of other important antioxidants such as ubiquinone, L-ascorbic acid, or Vitamin E. Moreover, by interacting with Vitamin C and Vitamin E, ALA protects biological membranes and indirectly maintains cellular antioxidant status. Anti-oxidative functions of ALA reduce the risk of the blood vessel and nerve fiber degeneration. A recent study by Salehi et al. revealed that ALA improves blood flow in peripheral nerves affected by diabetic neuropathy by 50% [52]. There is also evidence that ALA shows an immunomodulatory effect, suggesting that ALA could be used in the treatment of autoimmune diseases including SLE (systemic lupus erythematosus), RA (rheumatoid arthritis), and primary vasculitis [60]. Furthermore, by activating the prostaglandin receptors (EP2 and EP4) ALA increases the synthesis of cAMP and in this way is responsible for the uprising of the immunomodulatory effect. Exploration of this hypothesis has shown different results respectively for animal and human models, with the latter group displaying increased numbers of Th cells associated with an increase in cAMP levels [61].

Dydoń-Pikor investigated the effects of ALA supplementation on the lipid-peroxidation process induced by a high-fat diet and showed promising outcomes [62]. The study was carried out on animal models, the diet of which was appropriately modified by introducing oxidized and non-oxidized lipoic acid and rapeseed oil in various combinations. Study results showed that animals receiving dietary ALA supplements exhibited reduced levels of high-fat diet-induced lipid peroxidation.

Beyond its powerful antioxidative properties, the biochemical role of ALA in the treatment of type 2 diabetes is furthermore associated with its participation in mitochondrial respiration as a cofactor. Yang et al. showed that ALA can prevent excessive fatty acid accumulation by increasing insulin sensitivity [63]. This results in the activation of the AMP-activated protein kinase (AMPK), an energy-sensing enzyme triggering insulin-sensitizing effect on muscle and adipose tissue. This further increases the glucose uptake in insulin–sensitive cells (primarily liver and skeletal muscles), connected with the translocation of GLUT4 glucose transporter and fatty acid (FA) oxidation. By increasing insulin sensitivity in human tissues and consequently reducing hyperglycemia, ALA directly eliminates the key factor contributing to DPN. Moreover, ALA lowers the number of noxious triglycerides in beta cells found in pancreatic islets. It was also observed that ALA significantly improved glucose metabolism (affecting glycolysis, glu-

coneogenesis, and glycogen pathways) in the livers of HFD-induced NAFLD mice via the modulation of key molecules such as ChREBP and GSK3β [63].

Conclusions

DPN is a heterogenous disorder prevalent amongst diabetic patients, with pathogenesis closely associated with hyperglycemia, inflammation and metabolic and microvascular disturbances. ALA supplementation addresses many of these underlying causes, therefore its efficacy in the treatment of DPN cannot be overstated. The powerful antioxidant, anti-hyperglycemic and anti-inflammatory properties of ALA, in connection with its role in the regulation of several gene transcription mechanisms, improving microcirculation, and normalizing serum triglyceride levels make ALA a promising agent in developing an effective therapeutic approach to diseases associated with neuropathic lesions and nerve damage, such as chemotherapy-induced neuropathy and trigeminal neuralgia. ALA, administered either intravenously or orally, is characterized by its good bioavailability and amphiphilicity as well as limited adverse effects. When combining these features of ALA with other agents exhibiting antioxidant properties, it is possible to achieve highly effective treatment inhibiting the progression and symptoms of DPN.

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

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REVIEW PAPER

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The role of the dietary patterns in the cardiovascular disease risk prevention

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ABSTRACT

Cardiovascular diseases (CVD) are a part of a wide group of diseases, which became main threat to the life and health of the population in highly developed countries. To prevent and treat CVD, in addition to implementation of pharmacological methods, there are a number of lifestyle components, including eating habits, that significantly influence the development of these diseases. The dietary patterns strongly correlate with the risk of cardiovascular disease. Modifications of the dietary habits allow to control many parameters such as: body weight, cholesterol/triglyceride levels or blood pressure. Alternative diets are frequently used to reduce the risk of developing a CVD. The main recommended dietary patterns includes Mediterranean diet (MD), the DASH diet (Dietary Approach to Stop Hypertension) and mild variants of vegetarianism. The more controversial nutritional styles includes the ketogenic or vegan diets. Due to various assumptions as well as the mechanisms of action of each diets, an attempt of its evaluation have been made. The aim of our study is to review and analyze the available data on the impact of various nutrition models regarding to cardiovascular diseases risk prevention.

Introduction

Cardiovascular diseases (CVD) are a part of a wide group of diseases, which became main threat to the life and health of the population in highly developed countries. According to World Health Organization (WHO), in 2019 almost 17.9 million people have died from cardiovascular diseases, accounting for 32% of all deaths in the world, with the number still growing annually [1]. To prevent and treat CVD, in addition to implementation of pharmacological methods, there are a number of lifestyle components that significantly influence the development of these diseases [2]. Lifestyle is a widely understood concept that describes the patterns of human behavior, which depends on factors such as the economic situation or socio-cultural norms [3]. Among the most impactful aspects, activities as smoking, alcohol consumption, physical activity and diet can be mentioned. The latter one is a specific model of nutrition depending on elements such as location, economic situation, socio-cultural influences or the level of education. The dietary patterns strongly correlate with the risk of cardiovascular diseases. Modifications of the dietary habits allow to control many parameters such as: body weight, cholesterol/triglyceride level or blood pressure (BP). The high content of saturated fatty acids, cholesterol or the total amount of calories may predispose to the development of cardiovascular diseases [4, 5]. Alternative diets are frequently used to reduce the risk of developing a CVD. The main recommended dietary patterns include Mediterranean diet (MD), the DASH diet (Dietary Approach to Stop Hypertension) and mild variants of vegetarian diets [6-8]. The more controversial nutritional styles include the ketogenic or vegan diets [9, 10]. Due to various assumptions as well as the mechanisms of action of each of diets, an attempt of its evaluation have been made

The aim of our study is to review and analyze the available data on the impact of various nutrition models regarding to cardiovascular diseases risk prevention.

The Mediterranean diet

The Mediterranean diet is currently the most proper diet recommended by WHO towards CVD risk improvement [11]. It is known from the 50s and 60s of the 20th century as a dietary pattern derived from Mediterranean Basin [12]. The region is bordered by 18 countries that are diverse in terms of economic and healthcare status, lifestyle, and dietary patterns. Duo to the fact that it is impossible to define a solitary version of MD, two scores methods that helps classify dietary patterns individually were invented. Mediterranean Adequacy Index (MAI) assesses amount of typical Mediterranean foods as fresh vegetables and fruits, legumes, wholegrains, seafood, olive oil and red wine and untypical foods as red meat, eggs, dairy products, and sweets which consumption should be limited. The second one is Mediterranean Diet Score (MDS) which determinates typical MD products intake as positive (1 point) and untypical foods intake as negative (0 points). The score totals from 0 to 9, and its higher result stand for better compliance to a traditional MD [13]. One of the biggest studies of health-promoting impact of MD is HALE project (Healthy Aging: a Longitudinal Study in Europe) that used data of individual long-term surveys as SENECA (Survey in Europe on Nutrition and Elderly: a Concerned Action) and FINE (Finland, Italy, the Netherlands, Elderly). It reports that MD (hazard ratio (HR): 0.77; 95% confidence interval (CI): 0.68-0.88) together with physical activity (HR: 0.63; 95% CI: 0.55-0.72), moderate alcohol usage (HR: 0.78; 95% CI: 0.67-0.91) and non-smoking (HR: 0.65; 95% CI: 0.57-0.75) were associated with a lower risk of all-cause mortality [14]. Over time the MD has been endeared by other European regions as a proper way of nutrition in many clinical conditions with particular references to CVD. The main preventive action of MD include high ratio of monounsaturated fatty acids to saturated fatty acids, high content of polyphenols and antioxidants, as well as reduced calorie intake [12, 15].

The study conducted by Estruch R et al. reports that MD with high amount of extra virgin olive oil or nuts followed for an average period of time by 4.8 years reduces the risk of CVD episodes as stroke, myocardial infraction or coronary heart disease [16]. The high consumption of monounsaturated fats (MUFA), mostly from extra virgin olive oil, combined with low intake of saturated fats (SFA) cause the decrease in plasma low-density lipoprotein (LDL) level. Keeping LDL

on possibly the lowest level may be as effective as statin's therapy which is used to treat progression of atherosclerosis that consequently leads to major CVD [17]. High MUFA/SFA ratio also has significant influence on decreasing CVD mortality and overall mortality at general as reported by a prospective investigation in Greece of Trichopoulou et al. [18]. Additionally, highly consume nuts and wholegrains are rich in omega-6 and omega-3 fatty acids and plant sterols which are involved in LDL lowering mechanism [14].

Another important function of the MD is its anti-oxidative and anti-inflammatory properties, mostly thanks to high content of polyphenols, vitamin C, vitamin E and β-carotene contained in vegetables and fresh fruits [14]. A cohort study of the MOLI-SANI reports that high-antioxidant enriched MD decreases glucose, lipids, CRP (C-reactive protein) and blood pressure levels what in turn reduces systemic inflammation damages and oxidative stress [19]. Those are highly corelated to endothelial dysfunction that initiate the atherosclerosis pathogenesis [20]. The main mechanism involved in the prevention of this disease include the inhibition of pro-inflammatory biomarkers production [7, 21]. The PREDIMED study observations indicate that one year of compliance with MD diet decrease VCAM-1 (vascular cell adhesion molecule-1), ICAM-1 (intercellular ahesionmolecule-1), IL-6 (interleukin-6), MCP-1 (monocyte chemotactic protein-1), TNF-α (tumor necrosis factor- α) compared with initial readings [22]. Simultaneously, transcription factor as NF-κB (nuclear factor kappa B) and signal transduction cascades are increasingly activated what leads to high production of inflammatory cytokines and to decrease the synthesis of NO (nitric oxide). Vasoactive features of NO have direct influence on cardiovascular system through LDL and triglycerides lowering effect, blood pressure improvement, decreasing of platelets aggregation (**Figure 1**) [23].

As mentioned above, the anti-oxidative and anti-inflammatory capacity of MD result from high content of phenolic compounds. They are present in the main key foods of this dietary pattern as extra-virgin olive oil, nuts, legumes, vegetables, fruits, and whole-grain cereals [24]. Additional sources of polyphenols in MD is light-to-moderate red wine consumption. The main vasoactive components of red wine are flavonoids. Its effect comprises LDL and triglyceride levels reduction, systolic and diastolic blood pressure improvement, endothelial vasodilatory stimulation, decrease of platelet aggregation and lowering the proinflammatory and oxidant mediators produc-

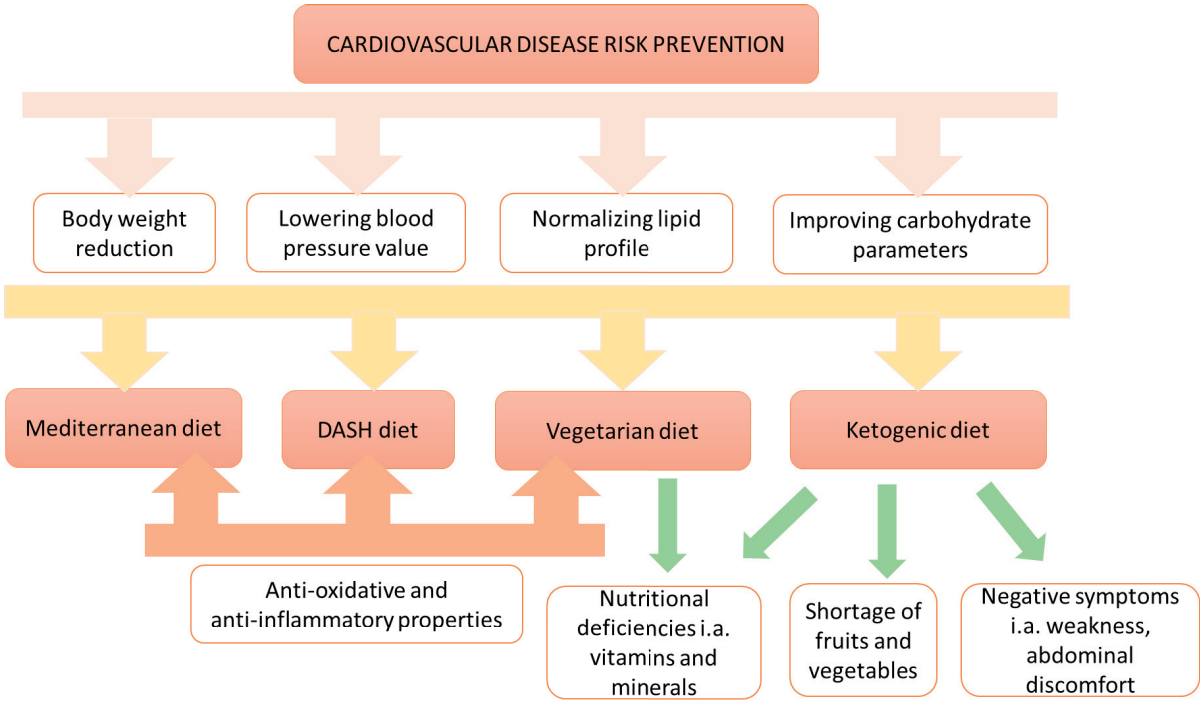

Figure 1. The effect of different dietary patterns on cardiovascular diseases risk prevention

tion [7]. Moreover, ethanol increases high-density lipoprotein (HDL) levels, promotes fibrinolysis and reduces systemic inflammation [21].

The important part of MD is high consumption of fishes and seafood. Those kind of food is rich in omega-3 fatty acids and provide high content of proteins, vitamin D, B vitamins, calcium, selenium as well as other nutrients (i.a. L-arginine) [25]. Data form clinical research have shown that fish consumption lead to reduce the potential risk of CVD including myocardial infarction [26], hypertension [27] or stroke [28]. The mechanism of protective fishes consumption involved their anti-inflammatory, anti-arrhythmia and anti-hypertension properties due to omega-3 fatty acids action. Moreover, unsaturated omega-3 fatty acids have triglyceride lowering as well as vasodilator effects [29]. A meta-analysis form 2020 indicated that high fish intake was correlated with a lower coronary heart disease incidence – CHD [relative risk – RR: 0.91, 95% CI: 0.84, 0.97]. What is more, the dose-response analysis demonstrated that the incidence and mortality of CHD were reduced by 4% together with a 20 g per day increment in fishes consumption [30]. In contrast not all studies show association between fish intake and CVD risk prevention [31], thus more analysis in this area are needed.

It is indicated that MD may be a useful dietary pattern in weight gain prevention and weight loss (**Figure 1**). Meta-analysis of randomized controlled trials showed that MD is an effective tool to reduce an excessive body weight, especially when it is energy-restricted, combined with physical activity, and lasts more than 6 months [32]. In other systemic review it has been observed that MD results in similar weight loss and CVD risk reduction as other comparator diets (low-fat diet, low-carbohydrate diet, the American Diabetes Association diet) in overweight or obese individuals [33]. Weight reduction is an important factor affecting the CVD risks. In one study Authors showed that 5-10% reduction of the initial body weight corelates with the decries in triglycerides, total cholesterol, LDL cholesterol and fasting glucose level. Losing more than > 10% of body weight produced improvements in cardiovascular risk factor in general [34].

The disadvantages of MD are difficult to define. There are singular reports on low or zero effect of MD on CVD risk improvement. Michalsen et al. [35] found no influence of MD on inflammation markers and metabolic risk in patients with artery disease. The MD is treated more like dietary pattern than strict regimen. Relevantly low-calorie intake and well-balanced structure make this diet easier to adherence. Nevertheless, the caloric value of the diet should be well-balanced according to the Body Mass Index (BMI). For example, the olive oil (regardless of its advantages) contains about 120 calories per spoon. Its uncontrolled, overload consumption may lead to body weight gain with adipose tissue accumulation in abdominal area. That condition may predispose to chronic low-grade inflammation, oxidative stress, and worsen of metabolic health at general. Thus, the benefits resulting from preventive properties of MD will be suppressed [36] and considerable as disadvantage. Worth mentioning is a fact that advantages of wine consumption are also still considered in terms of damaging influence of ethanol, which overconsumption in turn may contribute to the atherosclerosis [37].

In conclusion, dietary pattern has crucial influence on shape of cardiovascular condition and health as a whole. The MD bases on both quality and quintinite components. It has comprehensive impact on cardiovascular health mostly focused on anti-atherosclerosis prevention, which is departure point of major CVD as stroke, myocardial infraction or coronary heart disease. The individual form and flexibility of the diet makes it the most recommended due to particularly no contraindications and it approaches the gold standard for cardiovascular health.

DASH diet

DASH diet is considered as an effective nutritional change designed to reduce high blood pressure and the level of low density lipoprotein cholesterol [38]. Hemodynamic (hypertension) and metabolic (hyperlipidemia) stressors are considered as important risk factors in development of CVD [39]. The variety of studies confirms diet's positive effect on reduction of CVD risk factors (**Figure 1**) [38, 39].

DASH diet is similar to Mediterranean dietary pattern. It is based on high intake of fruits, vegetables as well as dairy products with low fats content. The DASH diet is rich in wholegrain

products, fishes, nuts and poultry. During following the diet the reduction of total and saturated fats, sweets, sugary drinks and meat is urged [40]. Thus, it supplies the food rich in calcium, potassium, magnesium, roughage and vegetable proteins [39]. Even though DASH diet may be considered as similar to Mediterranean one, according to various of studies, DASH diet has a more significant impact on BP. However, the analyzed studies confirms, that the adherence of both of these diets has a positive impact on the reduction of the cardiovascular disease risk factors [6].

While looking at DASH diet's mechanism, it exhibits protective effects for human various systems and its physiological functions. The mechanisms include the modification of antioxidant capacity, function of liver, inflammatory response, coagulation, activation of sympathetic system, control of insulin and glucagon, endothelial function and natriuresis [41].

According to research data, it may be considered, that several nutrients including sodium and potassium, are critical in high BP reduction during DASH diet undergo. Sodium intake affect negatively on the cardiovascular system–due to increase BP. Excess of sodium intake may be also correlated with CVD in mechanism not only related with hypertension [42]. Excess of sodium is corelated with intense albumin excretion, oxidative stress or increase of glomerular hydrostatic pressure [42].

The hypertension related to excessive sodium intake is connected with developed water retention that leads to increased blood flow in arteries. The increased BP in this case may lead to higher water and salt excretion [43]. This may lead to remodeling of arterial vessels, it may be corelated with changes in vascular resistances [44]. According to AHA (American Heart Association) it is highly recommended to reduce daily sodium intake to < 1500 mg/day in order to reduce all of the risk factors correlated with the development of CVD (both related and not-related to hypertension) [45]. The diseases have direct connection with excess of sodium intake include structural and functional impairment of the heart, kidneys and big vessels [42].

Independently of medicines intended to preventing hypertension, the diet should be an important lifestyle factor correlated with the reduction of the cardiovascular risk [46]. In the creating of the DASH diet sources of daily energy requirements are significant. The diet should consist of 15-20% from proteins, 25-30% from lipids and 55-60% from carbohydrates [47]. Clinical studies showed that the significant changes in systolic (SBP) and diastolic blood pressure (DBP) were observable in patients with hypertension following the DASH diet. The systematic review and meta-analysis conducted by Siervo M. et al., showed the significant decrease of SBP value (by 5.20 mmHg; $p < 0.001$) as a result of DASH diet undergo. Moreover, the reduction in the DBP value by 2.60 mmHg ($p < 0.001$) was also observed [39]. Lin P.H. et al. in their study also proved noticeable changes between control group and population following the DASH diet. The SBP values had been reduced approximately by 10.65 mmHg (±12.89 mmHg; $p = 0.023$) in the first week of the study. The effect of the SBP decrease were also noticeable on the second week of the experiment, where SBP values were lowered by 9.60 mmHg (±11.23 mmHg; $p = 0.039$) [48]. DBP also had been reduced in both cases by 5.95 mmHg (±8.01 mmHg; $p = 0.069$) in the first week of the study and by 8.60 mmHg (±9.13 mmHg; $p = 0.011$) after two weeks. The ions concentration also are crucial in reduction of BP in DASH diet. In simple linear univariate regression, the changed in urinary Na+, K+ and Na+/K+ proved the correlation with the reduction of SBP and DBP [48].

The diversity of researched people, in the study mentioned above conducted by Siervo M. et al. proved that BP reduction is most significant in African American population. More significant BP reductions were noticed in participants with the higher initial BP values and higher BMI [39].

The majority of the studies had proved that the aim of BP reduction is reached while following the dietary pattern. However, according to the study held by Appel LJ. et al. (PREMIER clinical trial), DASH diet's impact on blood pressure reduction was not as significant as it was assumed. According to the results – the decrease of SBP while following the DASH diet and established recommendations was only approximately 0.60 mmHg more significant than in participants with implemented established recommendations without diet [49].

It is worth mentioning that DASH diet's influence not only BP values but also modifies cho-

lesterol fractions concentrations in the blood (**Figure 1**). Hypercholesterolemia makes up the crucial risk of the development of variety of CVD including arteriosclerosis that may result in myocardial infarction [50]. The studies conducted by Mensink R.P. et al. [51] and Clarke R. et al. [52] showed that HDL level had been increased in case of DASH diet. Contrary, some researches submitted that DASH diet resulted in lowering the fraction HDL by 3.7 mg/dl, what may be considered an issue in connection with cardiovascular risk [53]. Such an effect may result from low dietary fat intake, since the reduced-fat dietary patterns lead to decrease of HDL level [53].

DASH diet makes also an impact on the other aspects directly correlated with reduction of cardiovascular risk. One research showed a considerable improvement of insulin sensitivity as well as the reduction of oxidative stress on the DASH diet (**Figure 1**) [54]. The factors which's values significantly change while following DASH diet included fasting glucose concentration and HOMA-IR level (Homeostatic Model Assessment – Insulin Resistance). Those are major cardiovascular risk factors that occur in abnormal glucose tolerance or type 2 diabetes which often lead to micro- and macroangiopathy. Both of them may be reduced by following the DASH diet's rules [54]. The recent studies proved also diet's impact on minimalizing cardiovascular incidence as stroke [55]. These results were confirmed in a systematic review and meta-analysis on observational prospective studies performed by Salehi-Abargouei A. et al. [56].-

Another aspect worth mentioning is the influence of DASH diet on body weight and composition. Soltani et al. showed that this dietary pattern is effective in weight management (especially weight reduction) in overweight and obese subjects. The analysis of 13 articles pointed out that adults on DASH diet lost more weight (weighted mean difference – WMD: -1.42 kg, 95CI: -2.03, -0.82), BMI (WMD: – -0.42 kg/m^2, 95%CI: -0.64, -0.20) and waist circumference (WMD: – -1.05 cm, 95%CI: -1.61, -0.49) when compared with other low-energy diets [57]. Also other randomized controlled trial demonstrated that following the DASH diet for 8 weeks among patients with non-alcoholic fatty liver disease (NAFLD) had beneficial effects on not only to body weight and BMI, but other metabolic parameters (triglycerides, markers of insulin metabolism and inflammatory response) correlated with CVD as well [58].

To conclude it may be observable that DASH diet had caused an decrease of SBP and DBP, however some researches performed its insignificant impact in this specific case. According to analyzed studies, DASH diet is correlated with the decrease of total cholesterol and LDL cholesterol fraction, it may be surely interpreted as a valuable aspect in decrease of cardiovascular risk. Many implications including an impact on glucose, HDL and triacyclglycerols level are still missing, because of the ambiguous research results [59]. The DASH diet had been created in order to prevent the hypertension and persistent metabolic diseases. The reduction of those risk factors may be connected with lowering possible complications of CVD. Overall, the DASH diet then is highly recommended for adults mainly with pre-hypertension or stage 1 hypertension, but also for the population in general.

Ketogenic diet

Ketogenic diet (KD) is a dietary pattern characterized by low intake of carbohydrates (< 50 g/day) [60], increased consumption of fat and an adequate amount of protein. Although this diet is mainly used for treating pediatric patients suffering from epilepsy [61], it has been gaining popularity among people trying to reduce an excessive body weight. Due to poor consumption of carbohydrates organism has to adapt and find a new energy source for the peripheral tissues and the brain, which leads to break down the fatty acids by the liver and produce ketone bodies (KB – acetoacetate, beta-hydroxybutyrate and well known acetone) [62] as an alternative sources of energy [61]. These situation cannot be compared to pathophysiological ketosis which occurs in type 1 diabetes. The latter cause significantly increase in concentration of ketone bodies results in blood pH decreasing and a life-threatening situation. On the other hand ketonemia from the diet does not increase ketone bodies to dangerous levels because of usage them by central nervous system (**Table 1**) [61].

Ketogenesis occurs in liver's cells mitochondria matrix [63]. Ketone are transported via blood to tissues such as central nervous system and

Table 1. Blood levels during a normal diet, ketogenic diet and diabetic ketoacidosis

Blood Levels	Normal Diet	Ketogenic Diet	Diabetic Ketoacidosis
Glucose (mg/dL)	80–120	65–80	>300
Insulin (μU/L)	6–23	6.6–9.4	≈0
[1]KB (mmol/L)	0.1	7/8	>25
pH	7.4	7.4	<7.3

[1]KB – ketone bodies.
Based on: [61].

skeletal muscles and metabolized by them. Then KB are converted into acetoacetyl-CoA, which afterwards is transformed into 2 acetyl-CoA, which are used in Krebs cycle for energetical purposes of the cells [61].

Hypertension represents one of the main components of the metabolic syndrome. It has been observed that ketogenic diet helps to reduce an excessive body weight which ensure the control of blood pressure rate (**Figure 1**) [64]. A study conducted on 377 patients put on VLCKD (very low calories ketogenic diet) diet, showed an improvement in SBP (−10.5 ± 6.4 mmHg, $p < 0.001$), as well as in DBP (−2.2 ± 3.1 mmHg, $p < 0.001$). Although, the effect has only been observed only up to 3 months of the studies with no further changes after 1 year of observation [65]. On the other hand, one meta-analysis found a significant difference in SBP between diet groups in favor of low-CHO (low -carbohydrate) diets (−2.74 mm Hg; 95% CI: −5.27, −0.20, p =.03), but no difference in DBP [66]. Moreover, a metanalysis (13 studies) conducted by Beuno NB. et al. showed difference in DBP (-1.43 mmHg; 95% CI: -2.49, -0.37, 1298 patients) but not in SBP in patients put on VLCKD [67].

Ketogenic diet may also influence the carbohydrate metabolism (**Figure 1**). The study performed on 12 overweight patients (BMI ≥ 25 kg/m^2) with type 2 diabetes, that were put on very low calories ketogenic diet for 32 weeks, has proven a significant reduction in hemoglobin A1c (HbA1c) serum levels (−0.8%, 95% CI: −1.1%, −0.6%) compared to participants from the control group. Moreover, participants from intervention group lost more weight and lowered their triglyceride levels [68]. Decreased blood glucose levels as a result of KD were confirmed in another studies [69, 70]. What is more, VLCKD has been proven to increase tissues' insulin sensitivity [70].

Dyslipidemia is another risk factor for the development of CVD. A results from clinical research have shown significant positive changes in lipids profiles among individuals following ketogenic diet (**Figure 1**). It included the reduction of: concentration of triglycerides and total cholesterol levels as well as an increase in HDL concentration [50]. In a meta-analysis of randomized controlled trials, occurring type 2 diabetes patients, improved lipid profiles in terms of lower triglyceride (standardized mean difference – SMD: -0.45; $p = 0.01$) and greater high-density lipoprotein (SMD: 0.31; $p = 0.005$) have been evaluated [71]. On the other hand, not all studies conformed this results. In other meta-analysis no difference was found in total cholesterol, HDL, and LDL levels after 3, 6, and 12 months of treatment ($p > 0.05$) in type 2 diabetes patients put on VLCKD [72].

It has been proven, that ketone bodies have a suppressant effect on appetite, which predispose to body weight reduction [73]. It seems that the ketogenic diet can also induce the reduction of adipose tissue without loss of lean body mass [74]. Studies on obese individuals showed that during exercise an increase efficiency of adipose tissue oxidation is present. This effect may be caused by higher free fatty acids level, which enhance transport across the mitochondrial membrane and therefore more substrate for fat oxidation [75]. Thus, the ketogenic diet is also an effective way to reduce body weight. Goss et al. demonstrated that a very low carbohydrate diet may be beneficial for older obese adults by depleting the amount of adipose tissue and improving the metabolic health (insulin sensitivity and the lipid profile) [76]. Also in a meta-analysis mentioned above, besides improvements the metabolic dysfunction and minimalizing the risk of CVD in obese subject, ketogenic diets led also to substantial weight reduction (SMD, -0.46; $p = 0.04$) [71].

On the other hand some publication shows the negative effects of a ketogenic diet [77]. This diet is poor in most of fruits and vegetables, which are an important source of vitamins and minerals. For this reason some patients on the keto-

genic diet may suffer from hypovitaminosis (thiamin – B1, folate, pyridoxine – vitamin B6, vitamin A, vitamin K), minerals deficiency (calcium, magnesium, iron, and potassium), dyselectrolytemia or dehydration [78, 79]. What is more patients on low calories ketogenic diet are predispose to suffer from bone mass and structure impairment [80]. Shortage of fruits and vegetables, which are main sources of protective substances such as antioxidants and polyphenols can induce increased exposure to free radicals. This imbalance might be harmful especially for patients with type 2 diabetes, where free radicals play an important role in the development of its metabolic complications [81]. The already mentioned dehydration causes decrease in thirst due to presence of ketones as well as low supply of fiber, due to low fruit and vegetables contents in the diet, what may induce constipations in some patients [82]. Moreover, low water supply combined with excessive production and reduced excretion of uric acid can cause higher risk of hyperuricemia and formation of urate stone in individuals following ketogenic diet. Thus, it is important to control level of uric acid in these patients [83]. One of the most common negative symptoms associated with ketogenic, are abdominal discomfort, nausea or vomiting. These symptoms may intensify after consuming larger amounts of the fatty acids [84]. Other adverse effects associated with the ketogenic diet are constipation and headache (**Figure 1**) [78].

To sum up, ketogenic diet has increased in popularity among many people. It has been proved to have positive influence over the human body in particular cardiovascular risks, such as: blood pressure, glucose serum level, obesity and dyslipidemia. Although, unbalanced KD may cause some side effects. Moreover, there are no clearly results on only negative or positive influence of KD on human organism, thus more randomized controlled trials in this area are needed.

Vegetarian diet

Due to significant raise in popularity, vegetarian diet is considered as an important alternative dietary pattern. There are several reasons, including environmental, ethical and medical factors, for whom the Western civilizations are deciding to withdraw animal products from their daily nutrition [85]. This type of diet is also highly promoted as healthy and therefore it should be examined how it correlates with cardiovascular risk and its factors [86].

There are several variations of so-called plant-based diets (PBDs). Each type is characterized by the range of excluded animal products. These variations are summarized in **Table 2**,

Table 2. The types of vegetarian diet

Type of diet	Summary of definitions
Flexitarian (semi-vegetarian)/meat reductionism/reducetarian	Occasional inclusion (less than once per week) of flesh foodstuff (meat, poultry, and fish) and permits eating all other animal products (e.g., eggs, milk, honey)
General vegetarian diets	Whenever not specified, a vegetarian diet is often an ovo-lacto-vegetarian diet
Pescetarian (pesco-vegetarian)	Includes seafood/fish, but not flesh of other animals (meat, poultry), and permits eating all other animal products (e.g., eggs, milk, h oney). This diet is sometimes included in the semi-vegetarian group
Pollo-vegetarian	Poultry is the only animal flesh consumed, as well as dairy and egg products. This diet is sometimes included in the semi-vegetarian group
Ovo-lacto-vegetarian	Excludes all types of flesh foodstuffs (meat, poultry, fish), but permits eating all other animal products (e.g., eggs, milk, honey)
Lacto-vegetarian	Excludes flesh foodstuffs and eggs but allows dairy products, honey
Ovo-vegetarian	Excludes consumption of all animal products with the exception of eggs
Vegan	Diet which excludes all animal products (both as ingredients and processing aids, the latter being an important aspect); an exception is human mother's breast milk, given voluntarily; veganism can also imply excluding all items of animal origin (e.g., made from wool, silk, leather materials) Other subcategories of a vegan diet are: – Vitarian (raw vegan): permits consumption of organic, raw, and fresh foods only; excludes coffee and tea – Fruitarian: excludes flesh foodstuffs, animal products, and vegetables, cereals permitted are only fruit, nuts, seeds, which can be gathered without damaging the plant – Sproutarian: eating foods in the form of sprouted plant seedlings, such as grains, vegetables, fruits

Based on: [87].

based on division that is most often used in research [87].

In 1999 Key et al. analyzed 5 prospective studies with 76 172 participants on vegetarian diet and established a 24% lower mortality from ischemic heart disease (IHD) [88]. However there was no association between this particular dietary pattern and any other significant cause of death. In 2012 Huang et al. confirmed these conclusions in their meta-analysis. Seven studies were examined with 124 706 participants overall and it was found that vegetarians had 29% lower chance of death from IHD [89]. Another evidence comes from EPIC-Oxford cohort study where risk or fatal cases of IHD were taken into consideration [90]. The study showed a 32% lower chance of suffering from IHD when following a vegetarian diet. What is important, taking into account other risk factors as sex, age, BMI and smoking, the diet had an important influence on CVD occurrence [90]. Potential mechanisms responsible for these results will be discussed below.

Crowe et al. stated that one of the key elements lowering the risk of IHD in the vegetarian group is its effect on non-HDL cholesterol [90]. Dyslipidemia is a well-studied risk factor for cardiovascular diseases [91]. Zhang et al. studied the effects of vegetarian diet on BMI and lipid profile in Chinese vegetarians and concluded that this dietary pattern corelates with favorable lipid profile and lower BMI [92]. The occurrence of BMI lowering during vegetarian diet is emphasized in several other studies [93-95]. A meta-analysis of randomized controlled trials evaluated a significant benefits on weight reduction compared to non-vegetarian diets. Subjects from vegetarian groups reduced more weight comparing to the control i.a. non-vegetarian diet groups (WMD: -2.02 kg; 95 % CI: -2.80, -1.23). Participants consuming a vegan diet were characterized by significant weight reduction (WMD: -2.52 kg; 95 % CI: -3.02, -1.98) and individuals consuming lacto-ovo-vegetarian diets showed lower effect due to weight loss (WMD: -1.48 kg; 95 % CI: -3.43, 0.47). What is more, vegetarian diets with energy restriction revealed even a greater weight reduction (WMD: -2.21 kg; 95 % CI: -3.31, -1.12) [96]. Therefore, due to the fact that weight reduction has an positive effect on blood lipids and lipoproteins [97], vegetarian diet may help to reduce both risk factors.

There is an evidence that plant-based dietary pattern has blood pressure lowering properties (**Figure 1**). Lee et al. in their meta-analysis noted that vegetarians have lower the SBP (WMD: −2.66 mmHg; 95% CI: −3.76, −1.55, $p < 0.001$) and DBP (WMD: −1.69 mmHg; 95% CI: −2.97, −0.41, $p < 0.001$) compared to omnivores [98]. Similar outcomes were found by Yokoyama et al. in 2002 [99]. It is believed that those results emerge from the diet's effectiveness in weight reduction as well as high potassium, magnesium and fiber content. Ernst et al. also proved that vegetarians tend to have lower blood viscosity, which possibly have impact on their BP [100].

As was already mentioned, PBDs are characterized by large levels of dietary fiber [101], especially this classified as water-soluble. It is believed that water-soluble fiber enhance satiety, therefore may be crucial in maintenance of body weight [102]. Kromhout et al. in the Seven Countries Study pointed out that dietary fiber is crucial in obesity and cardiovascular diseases prevention [103].

There are few mechanisms suggested the preventive properties of vegetarian diet in the context of CVD. Besides a clear impact on lipid profile, high intake of antioxidants is also signalized as a meaningful factor that prevents from atherosclerosis (**Figure 1**) [104]. Studies focuses mainly on ascorbate and alpha-tocopherol. Szeto et al. made a small cross-sectional study on 30 long-term vegetarians which supported their hypothesis that plasma vitamin C may be used as a general health marker due to its inverse relation with high sensitive CRP [105]. This thesis is consistent with other studies presenting the relation between ascorbate levels and cardiovascular health [106]. In addition, not only PBDs assure vital nutrients such as dietary fiber or antioxidants due to high vegetable intake, but also remove meat consumption (particularly red meat) with high content of nucleic acids, which may result in higher uric acid levels among omnivores. Urate is considered as an independent mortality predictor of mortality in patients with coronary artery disease (CAD) [107]. In 2013 Koeth et al. examined the metabolism of L-carnitine which leads to production of trimethylamine-N-oxide (TMAO) [108]. TMAO is proatherogenic and therefore its accumulation elevates cardiovascular risk. Indeed, the researchers found that dietary levels

of L-carnitine was associated with cardiovascular event risk (p = 0.04). The same study claims that vegetarians and vegans have significantly lower L-carnitine levels as well as plasma TMAO concentration. Adding up to the benefits of meat removal, Micha et al. established that processed meats are associated with coronary heart disease occurrence [109], however it is stated that red meat has no such influence. To our knowledge, it seems that further research is needed.

In 1985 Snowdon et al. brought up a hypothesis that vegetarian diet reduces risk of suffering from type 2 diabetes [110]. It is proven that the pattern helps to improve insulin sensitivity [111] and also lowers HbA1c levels (p = 0.046) which is corelated to high vegetable intake [112]. In addition, Villegas et al. concluded that vegetarians are significantly more protected against T2D incidence [113]. To add up, research provided data suggesting that low-fat vegan diet may have better results in glycemic control of patients already suffering from diabetes [114]. The pattern was compared to a diet suggested by American Diabetes Association [115] with results over 22 week clinical trial [114], which have been shown in the **Table 3**.

Data from clinical studies often evaluates the effect of plant-based diets on risk of CVD as general without showing the exact dietary pattern (vegan or vegetarian). There is no doubt, that the elimination of animal products is beneficial in the context of metabolic health. A meta-analysis and systematic review of prospect cohort studies indicated that an overall plant-based diet was significantly associated with a lower risk of CVD mortality (pooled HR: 0.92, 95% CI: 0.86, 0.99, p = 0.0193) and risk of CVD incidence (pooled HR: 0.90, 95% CI: 0.82, 0.98, p = 0.0173) [116]. Other prospective study from UK Biobank compared vegetarians, fish, poultry, and meat-eaters in the context of CVD incidence. It has been showed that fish-eaters rather than meat or poultry had lower risk of adverse cardiovascular outcomes. Vegetarians had only lower risk of CVD incidence [117]. It seems that plant-based diet minimalize the risk of CVD mainly due to consumption of low calorie and fat food as well as modification of lifestyle in general. Nevertheless, vegetarianism is characterized by a lower risk of nutritional deficiencies (i.a. proteins, vitamins and minerals), thus often is recommended as alternative dietary pattern in different population groups [8].

The main concern about PBDs, especially vegan diet, are possible deficiencies (**Figure 1**). According to Craig, it is important to be aware of an effective incorporation of calcium, zinc, iron, long chain omega-3 acids, vitamin D and vitamin B12 [118], the latter being most often discussed. A review performed by Pawlak et al. in 2013 strongly stated that vegetarians are at significant risk of developing B12 deficiency [119]. Two years later Pawlak suggested that such abnormal levels of the vitamin may lead to hyperhomocyteinemia, increasing CVD risk [120]. In addition, small cross-sectional study performed by Weikert et al. in 2020, presented an additional recommendations that vegetarians should care more about iodine intake [121]. What seems crucial in case of previous discussion, this trial's results did not signalize differences in B12 levels between vegans, vegetarians and omnivores which probably was an effect of supplementation, therefore it underlines the importance of additional intake of this vitamin.

In conclusion, plant-based dietary patterns may reduce cardiovascular risk in several different ways. While regular supplementation is needed, it seems that vegetarian or vegan diet should be considered in clinical approach, particularly when patients may find them attractive not only

Table 3. Elected results from randomized controlled trial (n = 99, 22 weeks) showing the benefits from low-fat vegan diet vs a diet following the American Diabetes Association (ADA) guidelines

Elected results/diet	Low-fat vegan diet ([2]n = 49)	Diet followed by [1]ADA recommendation (n = 50)
% of participants that reduced diabetes medications	43%	26%
[3]HbA1C decrease (P = 0,089)	0,96 percentage points	0,56 percentage points
Body weight decrease(P < 0,001)	6,5 kg	3,1 kg

[1]ADA – American Diabetes Association; [2]n – number of participants; [3]HbA1C – hemoglobin A1c.
Based on: [114].

because of health benefits. Additionally, vegan diet tends to have great potential as a dietary pattern for people suffering from type 2 diabetes and further research in this topic should be made.

Conclusion

Lifestyle changes correlate with the prevalence of CVD. Studies indicate that well balanced nutrition modify the risk of chronic diseases occurrence. Moreover, diet is one of the most important factors which may have the influence on the cardiovascular risk and it has been proven that diet components play an important role in CVD prevention. A number of the most commonly used dietary patterns including Mediterranean, DASH, vegan and ketogenic were analyzed in the context of the development of cardiovascular risk. As illustrated in this review, these eating habits, when used correctly, reduce the risk of occurrence CVDs. As a consequence of following diets there have been reported effects such as lower blood pressure, reduction in glucose, total cholesterol, LDL and triglycerides levels. However, the most recommended dietary patterns in prevention and treatment of CVD are MD and DASH diet. These are the best studied and there are the most evidence that its components may reduce the risk. Patients with these eating habits presented lower level of specific cholesterol fraction, what contributed to the reduction of the CVDs incidence rate. In the case of the DASH diet, antihypertensive properties were also observed. Simultaneously both provide all the necessary nutrients and there are no negative effects of the use. Ketogenic is an alternative diet which is also confirmed to have a positive effect on the cardiovascular system, but the supply of fruit and vegetables is insufficient, what may cause hypovitaminosis, dehydration, bone impairment and increased exposure to free radicals. Other side effects include constipation, hyperuricemia, nausea, and vomiting. A vegetarian diet is increasingly being recommended in CVD prevention, however an improperly balanced vegetarian diet can cause numerous nutritional deficiencies.

Despite the large amount of evidence supporting the effectiveness of these diets, long-term studies of the effect of various diets on the incidence of CVD are still needed.

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REVIEW PAPER

JMS Journal of Medical Science

Biodegradable and biocompatible synthetic polymers for applications in bone and muscle tissue engineering

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ABSTRACT

In medicine, tissue engineering has made significant advances. Using tissue engineering techniques, transplant treatments result in less donor site morbidity and need fewer surgeries overall. It is now possible to create cell-supporting scaffolds that degrade as new tissue grows on them, replacing them until complete body function is restored. Synthetic polymers have been a significant area of study for biodegradable scaffolds due to their ability to provide customizable biodegradable and mechanical features and a low immunogenic effect due to biocompatibility. The food and drug administration has given the biodegradable polymers widespread approval after they showed their reliability. In the context of tissue engineering, this paper aims to deliver an overview of the area of biodegradable and biocompatible synthetic polymers. We also discussed the frequently used synthetic biodegradable polymers in tissue scaffolding, scaffold specifications, polymer synthesis, degradation factors, and fabrication methods. Particular examples of synthetic polymer scaffolds are investigated to emphasize the many desired properties and corresponding needs for skeletal muscle and bone. Increased biocompatibility, functionality, and clinical applications will be made possible by further studies into a novel polymer and scaffold fabrication approaches.

Introduction

Since its development, synthetic polymer chemistry has advanced tremendously, thanks to decades of invention and advancement, leading to the variety of plastics people use daily. These polymers are suitable for various applications because of their highly functional characteristics, including toughness, stability, and durability. Degradation is one of the fascinating features of several polymers that has great significance in biomedicine and global waste management. Biodegradable polymers are crucial to developing

polymer chemistry because they are intrinsically susceptible to harsh conditions and environmental deterioration. Since biodegradability is highly valued in many sectors, medicine and tissue engineering have shown a particular interest in these polymers.

The dearth of donor sites in autologous grafts and the absence of the necessity for subsequent or repeated operations to eliminate non-degraded material are two advantages of tissue engineering over conventional grafting techniques [1]. The scaffold seeks to replicate the extracellular matrix (ECM), a structure surrounding the cells [2, 3]. A scaffold usually has various functions like monitor water and ion absorption, transport glucose and waste products, and protect cells from external strain pressures. As a therapy method, tissue engineering platforms can be transplanted into tissue defect locations or employed in vitro to create more accurate disease models [4]. The biodegradable scaffold will keep cells in place and then decay at a regulated pace so that the cells proliferate and produce their own ECM to substitute the scaffold, resulting in fully functioning regenerated tissue in the end [5]. Because fewer operations are needed to remove non-biodegradable scaffolds, and fewer long-term immunosuppressant medications are required, biodegradable polymers exhibit considerable benefits over the other substrates utilized as tissue scaffolds [6]. To achieve the ideal balance between functional qualities and biodegradation, biodegradable scaffolds must be adjusted.

Therefore, ideal tissue scaffolds should have high biocompatibility in both their scaffold and degraded forms. They should also have the necessary mechanical qualities to tolerate stress forces and supporting cells in vivo [7]. Additionally, scaffolds should have proper surface chemistry and be highly porous and permeable to allow cell adhesion and movement inside the scaffold while tolerating the required nutrition exchange [7]. These characteristics guarantee that tissue scaffolds perform at their peak levels, offering cells an environment to develop functional tissue-like structures [1]. In order to maintain acceptable structural characteristics during deterioration and finally be substituted by the regenerated tissues, the pace of degradation of scaffolds must also be configurable to their specific uses. Additionally, the precise mechanical and compositional characteristics and needs of a scaffold fluctuate greatly depending on the tissue type in a question and patient variations like age and gender [8]. Therefore, when evaluating certain biodegradable materials for the implant, a highly adaptable and flexible scaffold design is crucial.

Although natural polymers, like collagen, may be the most biocompatible and closely mimic the in vivo environment, they still have limits due to their poor mechanical characteristics and immunogenicity [7, 9]. Natural polymers, like fibrin and collagen, have the advantage of incorporating cell recognition and adhesion sites, like the arginine-glycine-aspartate (RGD) motif, which was first identified in natural polymers [10]. Therefore, the focus of this review will be on biodegradable synthetic polymer-based scaffolds, with an application-focused discussion of the advantages of composite materials with natural polymers. This review will first look into the processes of biodegradation and the unique physicochemical properties of biodegradable polymers, which enable and regulate this process. The usefulness of such materials in medical operations will next be highlighted through a study of production methods and examples of the implementation of specific biodegradable and biocompatible polymers in skeletal and bone tissue engineering.

Frequently used polymers

Numerous synthetic polymers, such as polyurethanes, polyacetals, and polyanhydrides, have the characteristics necessary for biodegradable scaffolds, as described above [6]. However, synthetic aliphatic polyesters, particularly poly (-caprolactone) (PCL), polylactic acid (PLA), which comes in two optically isomeric forms (D and L) and a racemic form (DL), and polyglycolic acid (PGA), as well as their copolymers, are the most frequently and widely utilized polymers for tissue engineering [6, 8, 11]. These polymeric materials are vulnerable to hydrolytic degradation through de-esterification, and the derived monomers are readily excreted from the body, making them extremely attractive as tissue scaffolds [12]. They also have good biocompatibility and sustainable production methods [6]. They have effectively been employed in clinical goods

because of their well-researched biodegradable and bioabsorbable qualities [5]. Pure versions of these polymers do, however, have some inherent drawbacks that must be considered. PLA's relatively poor cytocompatibility and biological inertness are two of the material's most significant flaws when used to create biodegradable tissue scaffolds [13]. Pure polymers frequently support decreased cellular contact and tissue regeneration. These polymers are commonly mixed in blocks with other polymers to tailor their degradability and mechanical characteristics to create better biomimetic and biocompatible scaffolds [5]. Numerous different chemical alterations, including the addition of hyaluronan [13], metallic nanoparticles [14], ceramics [15], or hydroxyapatite [16], have been demonstrated to increase the bioactivity of many polymers, enabling more efficient use in tissue engineering.

Blending synthetic polymers with substances like the aliphatic polyester group polyhydroxyalkanoates (PHA), which includes poly-3-hydroxybutyrate (PHB) and poly-3-hydroxyoctanoate (PHO), is another alteration [17]. Although these polymers may be synthesized, microbes often create them in purposefully imbalanced environments [18]. PHB may be synthesized from various monomers, such as BBL [19], propylene oxide, and carbon monoxide, to create syndiotactic PHB with lower crystallinity and a more significant transition melting temperature than its isotactic bacterial version [20]. Polyorthoesters and polyanhydrides are surface-eroding biomaterials, in contrast to aliphatic polyesters, which are bulk-eroding. This enables them to deliver pharmacological payloads for an extended period at a controlled gradual rate while maintaining structural integrity. The only surface-eroding biomaterial that has received FDA approval is polyanhydrides. Yet, its complex manufacture and weak mechanical properties have prevented them from finding broader usage. Another more popular polymer is polyethylene glycol (PEG), a cross-linked hydrogel with soft gel-like properties that has potential in drug administration and wound healing. Based on its position in the body, PEG degrades in a different manner [21].

Many biodegradable synthetic polymers are used in tissue engineering as scaffold materials presenting unique properties, as shown in **Figure 1**. It is crucial to remember that compounds are frequently copolymerized or changed for a specific usage by changing various parameters. It would be too huge to be practical to create a complete library in tabular form that lists all the available polymers, together with copolymers, composites, and other types of modified polymers, along with their many production pro-

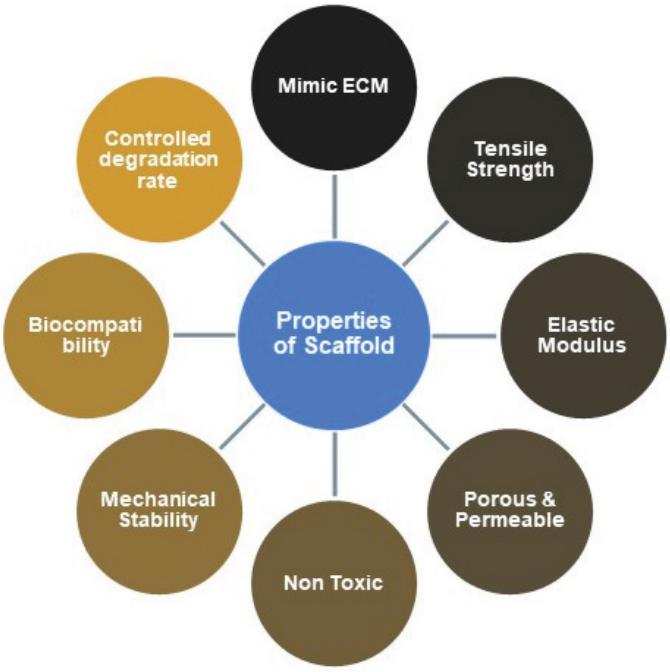

Figure 1. Properties of scaffolds used for application in tissue engineering

cesses, characteristics, and uses. However, compiling this data into a clear and concise database would help researchers and industrial players to comprehend the present state of the art for specific polymers and enable future research and industry to make decisions more quickly. This database may be connected to primary research publications and reviews like this one, allowing people to look deeper into the intricacies after choosing particular polymers or materials.

Mechanism of degradation

Prior to describing how biodegradable polymers are utilized in tissue engineering, it's critical to talk about the degrading traits that make them desirable as biodegradable scaffolds. Oxidative biodegradation and hydrolytic biodegradation are the principal in vivo polymer degradation processes [11]. The former depends on reactive radical molecules created in vivo by phagocytic assault. Contrarily, hydrolytic degradation is a passive process that breaks down chemical bonds susceptible to interaction with water [11]. Passive hydrolysis stands out as the primary breakdown method in biological settings because of synthetic polymers' relatively reduced sensitivity to enzyme activity [22]. Due to hydrolytic breakdown, polymers can erode on their surface or in bulk. The macroscopic scale polymeric scaffold shrinks while keeping its structure at a uniform degradation rate because surface erosion only affects the polymer surface, as the name implies. Contrarily, bulk erosion occurs throughout the polymer, maintaining the polymer's size even when the degradation rate is not linear [22]. For tissue scaffolds and their intended uses, it is crucial to know whether the form of erosion is prevalent [15, 22]. The hydrophobic nature of scaffolds affects the diffusion of water into and across the polymeric scaffold, which in turn affects the pace of hydrolytic action. This is where polymeric scaffolds' pore size comes into play since bigger pore sizes allow for easier osmosis into the scaffold, which favors mass erosion.

Additionally, amorphous parts of polymers are destroyed first in biological settings because they are packed less densely and more favorable to diffusion. As a result, the crystalline areas remain intact for a more extended period [23]. It follows that a higher polymer crystallinity correlates with greater stiffness and strength as well as a slower rate of degradation. Another crucial factor is the polymer's glass transition temperature (Tg), particularly when considering the mechanical needs of scaffolds [23]. For instance, bone scaffolds often need long-lasting mechanical qualities; therefore, their Tg has to be higher than body temperature to guarantee an acceptable level of stiffness while still delaying early degradation [23] (**Figure 2**).

The molecular weight of the polymer has a substantial influence on the rate of degradation [24]. A rise in molecular weight causes more secondary bonds and entanglements to form between chains, which results in stronger bonding between polymer chains and a slower rate of disintegration [24]. A property of polymers that can store this data is dispersity, calculated as the ratio of weight average molecular weight and number average molecular weight (Mw/Mn).

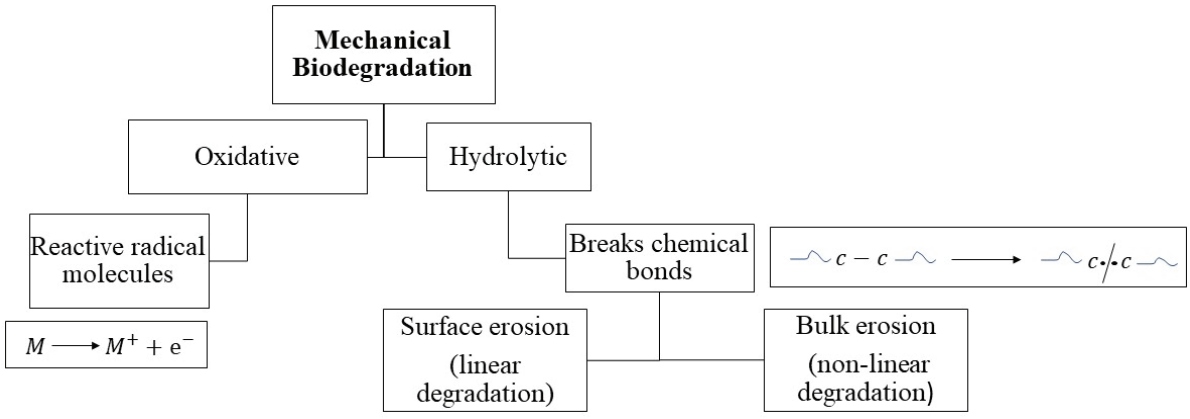

Figure 2. Schematic Diagram of mechanical degradation

Higher dispersity values highlight a smaller Mn because smaller molecules are easier to break down and may be broken down more quickly. Hence, biodegradable polymers need less dispersity, implying less variation in the chain length of the polymer, enabling better forecasts of the breakdown rate within shorter durations that consequently avoid problems like infection and inflammation [24, 25].

The versatility of synthetic polymers for tissue engineering applications makes it appealing to adjust the breakdown rate to fit specific needs. Copolymerization, in which the result consists of blocks of several degradable polymers, illustrates how to do this. Poly-L-lactide (PLLA), poly (-caprolactone) (PCL), and polyurethane, to mention a few, have all been fine-tuned using this approach [26–28]. Other strategies to control the degradation rate include blending, surface modification, and the inclusion of plasticizers [29]. By utilizing these methods on the wide variety of readily accessible polymers, polymer breakdown may be tailored to its role in tissue engineering.

Synthesis of polymers

The polymers themselves must be produced before specialized 3D scaffolds can be created. There are two ways to make synthetic polymers: (i) step-growth polymerization of hydroxy-acid or combinations of diacid/diol monomers; or (ii) chain growth by ring-opening of cyclic monomer units [30]. The former is often quicker and generates more monomers with a greater molecular weight [31]. The second method is frequently preferred since, for aliphatic polyesters, that's not an issue [32]. The removal of severe reaction conditions, the elimination of undesirable byproducts, and more command of stereochemistry and molecular weights, which results in higher-quality polymers, are further advantages of chain-growth polymerization [33, 34]. In order to produce PCL, PGA, and PLA, it is therefore commonly employed [33]. Tin (II) bis (2-ethylhexanoate), often known as Sn (Oct) 2, is used in the manufacture of PLA in the vicinity of alcohol (ROH), although ring-opening needs catalyst-initiators [30, 33]. There is no study to find metal-free catalysts that accomplish the same reaction rate [32, 35]. Adding heavy metal catalysts like Sn (Oct) 2 risks contamination during manufacturing, raising costs and potential toxicity of the finished product. Particularly, organocatalysts have increased their potential for ring-opening polymerization of racemic PLLA [19].

Other polymers, such as polyurethane (PU) and polyurethane urea (PEUU), are better suited for manufacture by step-growth polymerization because it is less expensive and more efficacious [31]. They are created mainly by processing hexamethylene-diisocyanate (HMDI) with a diol, then reacting with other polymers like PCL to generate a block polymer that can be broken down by de-esterification [36, 37].

Enzymatic polymerization is the third technique of polymer synthesis that is being increasingly researched as a more ecologically friendly substitute for both step and chain-growth processes [38]. Here, the immobilized enzymes, like ionic-liquid-coated lipases extracted from bacterial culture and deposited in a solvent solution, are combined with synthetic polymers like PCL [38]. Although reaction optimization and economic feasibility are still being investigated, this approach may create large molecular weights of polymers such as polyesters [39].

The production method frequently uses many monomers. Based on their combination ratio, block polymerization functions to integrate the qualities of its component homopolymer sections [40]. Contrarily, copolymerization of numerous monomers is frequently utilized to produce materials with unique features, perhaps with reduced stiffness, enhanced crystalline nature, or higher deterioration than any homopolymer. These manufacturing processes frequently result in straightforward pre-polymer forms, and their ultimate structure is usually achieved by the inclusion of extra blocks of polymer or side chains. These procedures enable atomic-level manipulation and control of polymer characteristics [40].

Fabrication of polymeric scaffolds

Once these polymers are created, they may be transformed into scaffolding structures to perform the desired tissue engineering functions. The method utilized to create the scaffold can significantly impact how it functions in vivo.

There are several techniques; the most popular ones include solvent casting, gas foaming, electrospinning, particle leaching, and additive manufacturing. Each produces a distinct structure and usefulness [9, 41]. These techniques' economic aspect is also crucial [42]. Particulate leaching refers to the addition of particles that are soluble in the polymer while it is still being formed. Later, the particles disintegrate in deionized water, leaving a web of porous holes behind [43]. Laminating layers of separately leached sheets can create a 3D scaffold [41]. Particulate leaching is a straightforward and inexpensive method, but it lacks precise structural control since pore interconnectivity depends only on the size and number of particles supplied [43, 44]. Since this ensures strong pore interconnectivity, it is suitable for scaffolding constructions with exceptionally high porosity leading to low load-bearing capacities, such as endothelial tissue [43].

One of the most extensively studied methods of scaffold production is electrospinning [43]. Mixing a biodegradable polymer, such as PCL, with a conductive polymer and injecting the mixture from needles under high voltage produces electrically active fibers. With the introduction of an external magnetic field, nanofibrous electrospun materials feature tuneable porosities, a high surface area-volume ratio, and adjustable porosities [45]. This method is quite effective, but it necessitates the optimization of several variables, including the applied voltage, solution concentration, and system humidity [43]. The fundamental issue is that the scaffold's need for a considerable amount of conductive polymer might change its mechanical characteristics [45] (**Figure 3**).

Innovative methods for producing scaffolds have also been made possible by advancements in additive manufacturing technology. Size, porosity, and shape may be finely controlled throughout the scaffold using fused-deposition modelling, selective laser sintering, and stereolithography [46]. Using a computer-aided design model, thermoplastic polymers like PCL and PLA are extruded in fused-deposition modelling to create layer-by-layer depositions. It makes it possible to create intricate porous scaffolds with precise dimensions [4, 48]. Optimization of additive manufacturing processes is highly desirable because of its accuracy and patient-specific potential, and this research topic is quite active [46, 49, 50]. 3D printing is among the promising technologies in tissue engineering and regenerative medicine to develop advanced scaffolds. This technique has been shown to successfully seed cells that lead to effective bone growth, and it may be utilized to generate tailored scaffolds

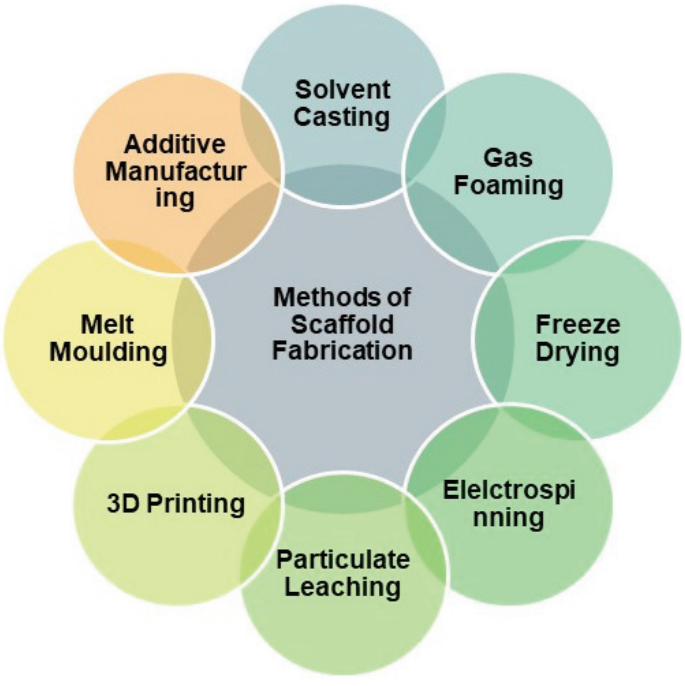

Figure 3. Various fabrication methods for scaffolds

using patient CT images [47]. Various materials are used for the 3D printing of scaffolds. Photosensitive resins, cross-linkable hydrogels, temperature sensitive polymers, thermoplastics, and ceramic paste are commonly used in scaffold 3D printing. It enables the creation of more precisely crafted biomimetic scaffolds with added bioactive ingredients to improve their functionality. Unprecedented potential for producing tissue structures from bone to skin has been demonstrated by 3D bioprinting stem cells. It allows for the delivery or mounting of cells and physicochemical factors essential for tissue regeneration, thus making 3D bioprinting a promising technology for future regenerative medicine.

Applications

The body has several different tissue types, each with unique structural characteristics and ECM compositions. When contemplating the sort of tissue that has to be replaced, the ability to customize the scaffold's qualities is quite helpful. The body is supported and protected by bones, which give more robust physical stability and a higher ECM: cell ratio primarily comprised of collagen [2]. In contrast, skin and muscles need to stretch rather than exert such mechanical strength, which causes the fraction of elastin in the ECM to grow [51]. Tendons and ligaments flexibly transmit stresses between muscles and bones, extending and recoiling to increase movement effectiveness. These require excellent tensile and mechanical strength, attained using plenty of aligned collagen structures [52, 53]. This summary clarifies that different tissue types have different scaffolding needs, which must be considered when choosing polymers and production techniques [5].

This section examines the particular needs and uses of synthetic biodegradable polymers in a couple of tissue types—skeletal muscle and bone-selected for their contrast. Studies on bone biomaterials have had a lot of success; there are currently clinical studies and various products on the market as a result [9, 54]. To the authors' knowledge, there isn't a clinically tested scaffold for skeletal muscle. These variations in patient accessibility have tissue complexity as their underlying cause. Still, they are also influenced by financial accessibility and the compatibility of existing authorized approved polymers to their needs [55, 56]. Here, we'll concentrate on the synthetic scaffolding methods utilized or investigated in these two subfields.

Scaffolding of Bone tissue

In order to enable regenerated bone to substitute the supports lost from the scaffold, the complicated interaction between mechanical support and time for degradation must be regulated due to the specific mechanical needs of bone-tissue scaffolds. For bone regeneration or osteoinductivity, a porosity of between 80 and 90 percent [57] and a pore size greater than 300 m are desirable [8, 58]. This might be improved by including osteoinductive, or growth, substances that can be released during disintegration [59]. Bone is a composite substance mostly made of the polymer collagen and the inorganic ceramic apatite [60]. Therefore, simulating this natural environment using composite scaffolds made of inorganic and polymeric phases may help in regeneration. Numerous polymers and polymer composites have been used to create clinical-grade scaffolds that successfully regenerate bone and have led to the development of commercial goods by including the optimum qualities for bone-tissue engineering scaffolds. Aliphatic polyesters like PGA, PCL, and PLA have been used quite often because they were given US FDA permission. The following will include specific scientific publications that accelerated commercial development, followed by illustrations of particular goods that are presently on the market.

In vitro osteogenic differentiation was demonstrated by [60] using a PLGA electrospun scaffolding with integrated silica nanoparticles, which led to increased bone nodule development and collagen secretion. A rat model used a different PLGA composite functionalized with a peptide similar to the osteoinductive bone morphogenetic protein 2 (BMP-2) to heal a critical-sized cranial lesion [61] successfully. The PLGA composite employed in this work is an appealing scaffold for bone tissue engineering applications due to its mechanical similarities and displaying that it may induce osteogenic differentiation as well as bone formation in vivo. For hip replacement surgery, PLA has been employed as a biodegradable bone graft with a metal core, demonstrating that it is

biocompatible and mechanically stable for effective bone regeneration [62].

Inorganic material has been added to scaffolds in several research areas to promote biomimicry and bone tissue regeneration. As early as 1986, a PLA/hydroxyapatite composite presented efficacy as a bone-filling scaffold in vivo [63]. Lately, a composite of PCL/hydroxyapatite helped bone marrow mesenchymal stem cells proliferate and differentiate [64]. Beyond biomimicry, adding hydroxyapatite to these investigations removes the drawbacks of pure hydroxyapatite's fragility and weak mechanical strength [65]. Recent research has demonstrated that it is possible to employ 3D printing to enhance hydroxyapatite content in a PLA composite without drastically changing the scaffold's mechanical characteristics. Investigation into hydroxyapatite-containing polymer composite is still underway [65]. The prior study found that this scaffold was beneficial both in vitro and in vivo; problems persisted with increased acidity levels during PLA breakdown that might cause inflammation. Even if the amount of hydroxyapatite has grown, further study is still needed before this scaffold is widely used in clinical settings.

Natural polymers could be added with synthetic polymeric scaffolds or utilized as coatings with the aim of promoting cell adhesion because of the advantage of possessing cell-binding RGD sites. Collagen coating was used to achieve this effect, which improved cellular adhesion and differentiation in PLGA [66] and PLLA [67]. In a different research, the persistent release of the BMP2-related peptide P28 was combined with a scaffold made of small intestine submucosa (SIS) and PLA to improve bone regeneration [68]. Collagen I and glycosaminoglycan-containing SIS were combined with PLA to create a highly biomimetic scaffold with tuneable bone-tissue development and breakdown. The specifics of several further experiments, including various polymer kinds, production processes, applications, and degradation timeframes, may be found in review papers [69, 70].

The studies cited above support the potential utility of specific polymers in bone regeneration. This has sparked numerous clinical investigations and the development of commercial goods. Zimmer Biomet produces a range of implants and screws called LactoSorb® that are made of PLA and are used in craniofacial procedures [71]. Narayanan et al. [69] provide a summary of several other commercially available PLA-based solutions, including RapidsorbTM and Biocryl®. Although several clinical investigations, such as using 3D-printed PCL scaffolding in dental surgery, are now underway, the economic success of other polymer kinds is less obvious.

Scaffolding of skeletal muscle tissue

To efficiently transfer force along the tissue, muscle has precise alignments and lengths of fibrils [72]. The skeletal muscle stem cells, or satellite cells [73], multiply, develop into multinucleated myoblasts, and subsequently merge into myotubes when forming new tissue [74]. Promoting satellite cellular migration into the scaffold is crucial because a skeletal muscle scaffold should be able to efficiently control cell migration and development to form these parallel, highly ordered fibers [72]. The architecture of skeletal muscle, mainly regulated mechanically, adds to the complexity of tissue scaffolding. A static scaffold cannot provide these physical development signals; therefore, the myotubes develop randomly [45].

Due to this, cultured skeletal muscle performs poorly when subjected to force in vivo [75] [76]. The scaffold must be exposed to a rhythmic mechanical or electrical component to simulate actual muscle usage in order to prevent this random direction of myoblast development [74]. Incorporating conductive polymers into the scaffold of skeletal muscle has been one of the main study areas [77]. Electrical stimulation has been found to cause muscle contractions and orient myoblasts parallel to the vectors of the electric field [78], suggesting that it may be a straightforward and affordable way to guarantee alignment and contractile capabilities. Electrospinning, which needs a highly conductive component to work, is well suited for the job since it can generate regulated alignment of polymer fibers. To create such an aligned polymer fiber scaffold, Chen et al. employed an electrospinning approach with a mixture of PCL and polyaniline (PANi) [45].

In vitro mouse myoblasts showed higher myotube fusion and cell proliferation than a non-aligned PCL/PANi combination. Similar outcomes were obtained by Jun et al. [79] using a mixture of poly(L-lactide-co-epsilon-capro-

lactone) (PLCL) and PANi, which combined the stiffness and brittleness of PANi with the highly elastic PLCL to produce a scaffold that was more suited than either pure polymer alone. A PANi-PLCL ratio of 3:7 was able to produce 170 percent strain, which is more than skeletal muscle can. However, PANi does not disintegrate despite being biocompatible [40, 45]; therefore, additional research into its polymer structure is necessary before it can provide the qualities that a biodegradable scaffold needs.

The usage of hydrogels is another method for scaffolding skeletal muscle. The FDA has cleared PEG, a highly biocompatible hydrogel for internal ingestion. Its cross-linking density, Mn, and water: polymer ratio may all be easily changed to change its qualities. After cells are suspended inside, its derivative, PEGDA, which is made by replacing its terminating hydroxyl groups with acrylate, may gelate from a liquid to a solid form when exposed to UV radiation. So, rather than relying on a premade scaffold form to specify the shape of the muscle tissue, the scaffold may be constructed after the myoblasts have matured into it. PEG, when mixed with a biological cell-adhesive foundation like fibrinogen, produces a scaffold with both tunable physical features and cell-signalling capabilities, facilitating blood vessels' formation and skeletal muscle regeneration in vitro [74]. Han et al. employed PEG in vivo as an injectable scaffolding cell-delivery method because it can be functionalized with maleimide groups, which makes it possible to store stem cells and adhere to patient tissue [18]. Dong et al. could combine the advantages of hydrogels with those of conductive polymers [40]. They mixed PEG with polyglycerol sebacate (PGS), a very hydrophilic polymer but also elastic [40]. Aniline pentamer (AP) side chains were added by esterification to the resultant polymer PEGS to increase conductivity. PEGS films possessed mechanical qualities that prevented mechanical fatigue and encouraged myoblast growth. Further investigation is warranted because this conductive, stretchy hydrogel can provide mechanical and electrical stimuli to control tissue development.

The combination of aliphatic polyesters and polyurethanes represents a relatively recent development in biodegradable skeletal muscle. Hydrophobic PCL and hydrophilic PEG copolymer soft segments and PU hard segments are combined to create thermoplastic PU and PEUU copolymers (TPUs) [37]. The facile customization of the synthetic process to adjust the soft and hard segment ratios to fit TPU for skeletal muscle scaffolding is made possible by the tunability of synthetic polymer manufacture. A 3D-printed scaffold made of oriented TPU filaments was described as "soft yet robust, sturdy, elastic, and hydrophilic" by Goyker et al. in 2021 [37]. They detected myoblast regeneration and capillary development to some extent at the implant site when assessed in vivo four weeks after implantation, with a recovery in the function of 86% [37].

Cartilage tissue engineering is a promising method for regenerating cartilage tissue damaged by disease or trauma. Articular cartilage has a limited capacity for healing and regeneration, making its restoration one of the biggest problems in musculoskeletal medicine. Cartilage tissue can be repaired when the polymer scaffold's mechanical strength and structural toughness are met with the requirements. The migration of metabolically active cells ECM components that are synthesized and turned over in large quantities is restricted. And hence, artificial cartilage to develop materials that can mimic natural cartilage is preferred. Self-assembly and biomineralization are crucial steps for cartilage repair because they mimic the natural ECM process. For biomineralization, a composite material resistant to high compressive loads can be formed by nucleation and alignment of hydroxyapatite crystals onto bundles of polymeric fibres. One potential process by which highly selective nucleation could occur is illustrated by a creating model that uses a co-polymer hydrogel system. It is believed that controlled nucleation may develop in bone by a similar approach [80].

Another approach to forming hybrid scaffolds for artificial biofunctionalization is combining synthetic polymers with short peptide sequences; scaffolds can be rationally designed with specified biofunctionality. Bioactive precursors with chemically reactive functional groups, such as amines, thiols, and carboxyls, or end-functionalized hydrophilic polymers, like PEG, which function as physical or chemical crosslinkers, can be used to create hybrid multifunctional networks. Of these multifunctional systems, the most adaptable and distinctive ones are based on PEG-peptide hydrogels. The peptides in this

system are made to be substrates vulnerable to proteases produced on the surfaces of migratory cells, such as plasmin and matrix metalloproteinase (MMPs). The field of cartilage reconstruction is expanding at a fast pace with many recent developments [81].

Conclusion

This field of study has particular difficulties because of the special biocompatible and biodegradable needs of tissue scaffolds and the intricacy of their interconnections within the human body. A scaffold must not only perform and decay correctly, but it should also do so for the proper tissue type, as each has specific mechanical and morphological needs. Even though a low level of dispersion is ideal, other parameters like crystallinity, Tg, and strain values are all influenced by the kind of tissue. Polymer breakdown kinetics must be managed to prevent gaps and inflammation throughout healing. In order to combine their properties into a scaffold that is much more appropriate for its function than either polymer in its pure state, well-researched polymers like PLA and PCL are frequently used in conjunction with other less biodegradable but more tissue-compatible polymers, either in block form or as a modification. The variety of fabrication processes that a polymer may be made to construct the 3D scaffolding the body demands, adjusting permeability and fiber arrangement to govern cell migration and proliferation, further enhances this control over the scaffold characteristics and mechanics. Bone, which has high porosity and stress requirements, has given rise to PLA-based scaffolds of bone that have been effectively used in clinical studies. Until now, PCL and PEG-based polymer scaffolds have been the sole in vitro success for muscle, which requires a conducting and elastic scaffold capable of experiencing mechanical loading. Clinical research has been restricted to a small number of biodegradable scaffolding polymers since only a few of them have received FDA and MHRA approval. Despite extensive study on their characteristics, these polymers aren't ideal for scaffolding in their pure forms. Even a perfect scaffold is still ten years away from being used in the general population since research into novel polymer architectures is now restricted to in vitro experiments, and rigorous testing is needed before clinical trials can be conducted. Synthetic polymers can be tuned in terms of both mechanical and biodegradable properties [56]. This review has just scratched the surface of the field's possible polymer and scaffold architectures because of the tunability, co- and block polymerization, and differences in the scaffolding approach. Establishing a list of known scaffold polymers accessible to the public and containing information on their characteristics, the impacts of various manufacturing methods, and the effects of copolymer additions would be the perfect next step for the discipline. Such a thorough evaluation of these polymers could guide and improve further research. Tissue engineering with biodegradable scaffolds is a relatively new area. Until this technology is used often in therapeutic settings, there are still numerous obstacles to be solved. But more research into the function of ECM in cell development, together with the evaluation of copolymers and cutting-edge production methods, will only expand the potential of this exciting area of study.

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Histamine intolerance (HIT)

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ABSTRACT

Histamine intolerance (HIT) is food intolerance of non-immunological origin, and it results from an imbalance between the consumption of histamine with food and the organism ability to metabolize it. The patients with HIT were found to have a significantly reduced concentration of histamine-degrading enzymes, diamine oxidase (DAO) and histamine N-methyltransferase (HNMT). Factors which have been identified that are conducive to histamine intolerance are: genetic factors, dysbacteriosis, chronic diseases – especially allergic and intestinal diseases, chronic infections, mastocytosis and some drug use. Clinical symptoms of histamine excess may affect various organs and systems - the most common are skin symptoms (pruritis, erythema) nausea, vomiting, abdominal pain, diarrhea, headaches and sometimes severe reactions with shortness of breath, arrhythmias, blood pressure drop, and even cardiac arrest.

Diagnostic methods in HIT utilize determination of serum DAO, determination of histamine metabolites in urine, measurement of the histamine wheal in the 50th minute of the skin prick test (SPT), gastroscopy with intestinal biopsy, diagnostic and therapeutic test, oral histamine-challenge test and genetic tests.

The mainstay of histamine intolerance treatment if a low-histamine diet. Patients should avoid products belonging to the three groups of food: containing large amounts of histamine, histamine liberators, and products inhibiting the activity of DAO. Additionally, supplements containing DAO and antihistamines can be used.

Introduction

Histamine intolerance is food intolerance of non-immunological origin, and it results from an imbalance between the consumption of histamine with food and the organism ability to metabolize it [1]. In recent years, there has been a significant increase in the reported frequency of adverse food reactions. More and more patients come to the doctor for diagnosis or perform tests on their own. The most common tests are for food allergy or lactose intolerance. Meanwhile, in many patients it is not possible to make a proper diagnosis on their basis. Therefore, this article discusses a very important issue regarding the histamine intolerance reaction. More attention by clinicians should be paid how challenging objective diagnosis of histamine intolerance is due to lacking reliable laboratory test or biomarkers and clear diagnostic criteria as well. According to the latest data, the disorder may affect up to 3–6% of the population and is more common in children than in adults (the percentage given is for HIT only, not for food intolerance in general) [2, 3]. The patients with HIT were found to have a significantly reduced concentration of histamine-degrading enzymes, diamine oxidase (DAO) and histamine N-methyltransferase (HNMT). As the symptoms

of histamine intolerance are very similar to those of an allergic reaction, the term pseudoallergy is often used for HIT. Contrary to allergic reactions, in which even the smallest amount of the allergen can cause severe systemic symptoms, in patients with histamine intolerance, the severity of symptoms depends on the amount of histamine consumed with food. Current diagnostic methods utilize for example determination of serum DAO, determination of histamine metabolites in urine, measurement of the histamine wheal in the 50th minute of the skin prick test (SPT), and genetic tests. The mainstay of treatment is a low histamine diet, the use of antihistamines, and supplements containing DAO.

Factors predisposing to HIT

So far, many factors have been identified that are conducive to histamine intolerance. These include:

- genetic factors – histamine intolerance is most often associated with a single-nucleotide polymorphism (SNP) in the DAO gene that limits the production of the resulting protein [4];
- dysbacteriosis – disturbances of the intestinal bacterial flora may lead to increased concentrations of histamine [5]. Some bacteria can produce and secrete histamine, and significant disturbances of the intestinal microflora have been found in patients with HIT [6];
- other chronic diseases – especially allergic (atopic dermatitis, allergic rhinitis, asthma) [7, 8] and intestinal diseases (large intestine polyps, food allergies, celiac disease) [9]. Moreover, HIT is more common in patients with chronic infections and mastocytosis;
- drug use – DAO activity is lowered by: mucoactive agents (ambroxol, acetylcysteine), antiemetics (metoclopramide), antidepressants (amitriptyline), anti-arrhythmic drugs, antihypertensive drugs (dihydralazine), and clavulanic acid [10].

Symptoms of histamine intolerance

Histamine is a biogenic amine normally present in the human body. It is synthesized and secreted by many cells, most importantly by mast cells and basophils. Accumulation of histamine, both exogenous and endogenous, can cause a variety of symptoms. The amine affects the cells via several known subtypes of histamine receptors [11].

H1 receptors are found primarily in the endothelium, airway smooth muscle, skin, and subcutaneous tissue. H2 receptors are located in mucosa and epithelium cells, immune cells and smooth muscle, and are involved in, inter alia, secretion of gastric juice. H3 receptors are found in the nervous system, and H4 ones in various cells of the immune system. Therefore, clinical symptoms of histamine excess may affect various organs and systems [12, 13]. The most common symptoms include:

- skin symptoms – typically pruritis, erythema (often sudden facial erythema), hives, and edema;
- gastrointestinal symptoms – nausea, vomiting, abdominal pain, diarrhea;
- respiratory symptoms – runny nose, nasal mucosa swelling, shortness of breath;
- nervous system symptoms – very frequent headaches, including migraine;
- circulatory symptoms – arrhythmias, blood pressure drop, and even cardiac arrest [12].

The severity of HIT symptoms depends directly on the concentration of accumulated histamine. The concentration of 1–2 ng/ml increases the secretion of gastric juice and heart rate, and induces skin erythema. The level of 3–5 ng/ml causes headache, pruritis, hives, and tachycardia. At 7–12 ng/ml bronchospasm and dyspnea can occur, and histamine concentrations above 100 ng/ml cause cardiac arrest [10].

Histamine-rich foods and histamine-releasing foods

Symptoms of histamine intolerance result from an imbalance between the excess of exogenous and/or endogenous histamine and the body ability to degrade it.

The foods that cause HIT are divided into three groups: the first group includes foods that contain large amounts of histamine, the second are foods that cause increased release of histamine from cells (so-called histamine liberators), and the third group comprises foods that are natural DAO inhibitors [14, 15].

Foods rich in histamine include: fish and seafood, ripened cheeses (e.g. parmesan), ripened meats (salami, skilandis), pickles (sauerkraut), fresh yeast and their extracts, some fruit and vegetables (tomato, spinach, eggplant, avocado).

Histamine liberators include: some fruit and vegetables (strawberries, raspberries, papaya, citrus fruit, spinach, eggplant, tomato, asparagus), alcohol (wine, champagne, beer), coffee, tea, cocoa, chocolate, nuts, and egg white.

The DAO inhibitors include: fish, cured meats, and sauerkraut.

HIT diagnostics

HIT diagnosis is based on a detailed medical history of the patient ailments and their possible relationship with the consumed food. It may be helpful if the patient keeps a diary and notes down their symptoms and menu. A detailed history is also necessary regarding chronic diseases that may accompany HIT. Physical examination should assess clinical symptoms of histamine intolerance and accompanying conditions.

Additional tests helpful in HIT diagnosis:

- determination of serum concentration and activity of DAO (ELISA) Currently, DAO enzyme activity in serum is the most studied but still controversial laboratory diagnostic approach and cannot be considered conclusive, mainly because level in serum doesn't entirely correspond to level in intestinal tissue. However there are some studies, that confirm the concentration and activity of DAO are statistically significantly lower in patients with histamine intolerance [16];
- histamine 50 – skin-prick test – this is the assessment of the histamine wheal after a 50-minute skin prick test (SPT). A wheal with a diameter ≥ 3 mm is considered as denoting HIT [17]. Sensitivity of this test is 79% and its specificity is 81%. SPT should always be performed to confirm or rule out possible IgE-mediated allergy, which manifests itself similarly to histamine intolerance, and these two conditions may overlap in many patients;
- gastroscopy with intestinal biopsy – determination of intestinal concentration of DAO is a highly sensitive and specific method. Its disadvantages include invasiveness and high costs [18];
- determination of histamine and its metabolites in urine – a convenient and non-invasive method involving UHPLC-FL chromatography. In the future it may become a routine test used in everyday practice [19];
- diagnostic and therapeutic test – in patients suspected of HIT, a physician may recommend a low-histamine diet or DAO supplementation for four to eight weeks. If clinical symptoms significantly cease or disappear, the test can be considered one of the criteria confirming histamine intolerance [20];
- oral histamine-challenge test – consists in administering a solution containing 75 mg of histamine and observing clinical symptoms. The test may trigger severe systemic symptoms and must be performed in hospital conditions. Due to its low specificity (possible positive results in healthy people), this test is not recommended in the diagnosis of HIT [21];
- determination of serum histamine concentration – currently not used in routine workup due to high cost and low availability [22];
- genetic testing of DAO and HNMT SNPs in blood or oral mucosa samples. These tests are not an element of routine diagnosis.

Treatment

The mainstay of histamine intolerance treatment if a low-histamine diet. Patients should avoid products belonging to the three groups mentioned above, that is those containing large amounts of histamine, histamine liberators, and products inhibiting the activity of DAO [23, 24].

Additionally, supplements containing DAO can be used, preferably as an addition to a low-histamine diet, not instead of it. The European Food Safety Agency (EFSA) has approved a pig-kidney extract with 0.3 mg of DAO. The available supplements should be taken 2–3 times a day, 10–15 minutes before a meal (the maximum dose of DAO is 0.9 mg per day) [25, 26].

Antihistamines that block both H1 and H2 receptors are recommended to relieve symptoms associated with the consumption of too much histamine. They are not to be taken on daily basis to combat histamine intolerance, and no clinical

trials have so far confirmed that implementation of antihistamines yields a beneficial therapeutic effect.

If life-threatening systemic symptoms occur as a result of histamine intolerance, the therapeutic procedure is analogous to anaphylaxis and involves administration of adrenaline, corticosteroids, and sufficient hydration.

If comorbidities are found, treatment of the underlying disease is of key importance, as it may also lead to HIT symptoms disappearance.

Summary

Histamine intolerance has recently become an increasingly common problem in everyday medical practice. Its symptoms can be mild but also life-threatening. Due to the multifactorial etiology of the disease, the search for its possible causes is often difficult. As the HIT symptoms closely resemble those of an allergy, they usually result in a comprehensive diagnostic workup focused on allergies. In many patients, problems related to allergic diseases are additionally observed, which hampers the diagnosis and proper therapeutic management. For this reason, each patient in whom HIT is suspected on the basis of clinical symptoms should be carefully and individually diagnosed.

However, as HIT reactions may be triggered by a vast array of products, dietary management poses a huge challenge for the patient. The problem of histamine intolerance certainly requires further research to improve the diagnosis and therapy of this food intolerance.

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

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The first page of the manuscript should contain the title of the article, authors' full names without degrees or titles, authors' institutional affiliations including city and country and a running title, not exceeding 40 letters and spaces. The first page should also include the full postal address, e-mail address, and telephone and fax numbers of the corresponding author.

Abstract
The abstract should not exceed 250 words and should be structured into separate sections: Background, Methods, Results and Conclusions. It should concisely state the significant findings without reference to the rest of the paper. The abstract should be followed by a list of 3 to 6 Key words. They should reflect the central topic of the article (avoid words already used in the title).

The following categories of articles can be proposed to the Journal of Medical Science:

ORIGINAL RESEARCH

Original articles: Manuscripts in this category describe the results of original research conducted in the broad area of life science and medicine. The manuscript should be presented in the format of Abstract (250-word limit), Keywords, Introduction, Material and Methods, Results, Discussion, Perspectives, Acknowledgments and References. In the Discussion section, statements regarding the importance and *novelty of the study* should be presented. In addition, the limitations of the study should be articulated. The abstract must be structured and include: Objectives, Material and Methods, Results and Conclusions. Manuscripts cannot exceed 3500 words in length (excluding title page, abstract and references) and contain no more than a combination of 8 tables and/or figures. The number of references should not exceed 45.

Brief Reports: Manuscripts in this category may present results of studies involving small sample sizes, introduce new methodologies, describe preliminary findings or replication studies. The manuscript must follow the same format requirements as full length manuscripts. Brief reports should be up to 2000 words (excluding title page, abstract and references) and can include up to 3 tables and/or figures. The number of references should not exceed 25.

REVIEW ARTICLES

Review articles: These articles should describe recent advances in areas within the Journal's scope. Review articles cannot exceed 5000 words length (excluding title page, abstract and references) and contain no more than a combination of 10 tables and/or figures. Authors are encouraged to restrict figures and tables to essential data that cannot be described in the text. The number of references should not exceed 80.

A THOUSAND WORDS ABOUT... is a form of Mini-Reviews. Manuscripts in this category should focus on *latest achievements of life science and medicine.* Manuscripts should be up to 1000 words in length (excluding title page, abstract and references) and contain up to 5 tables and/or figures and up to 25 most relevant references. The number of authors is limited to no more than 3.

OTHER SUBMISSIONS

Invited Editorials: Editorials are authoritative commentaries on topics of current interest or that relate to articles published in the same issue. Manuscripts should be up to 1500 words in length. The number of references should not exceed 10. The number of authors is limited to no more than 2.

Images in Clinical Medicine: Manuscripts in this category should contain one distinct image from life science or medicine. Only original and high-quality images are considered for publication. The description of the image (up to 250 words) should present relevant information like short description of the patient's history, clinical findings and course, imaging techniques or molecular biology techniques (e.g. blotting techniques or immunostaining). All labeled structures in the image should be described and explained in the legend. The number of references should not exceed 5. The number of authors is limited to no more than 5.

The Rationale, Design and Methods of New Studies: Manuscripts in this category should provide information regarding the grants awarded by different founding agencies, e.g. National Health Institute, European Union, National Science Center or National Center for Research and Development. The manuscript should be presented in the format of Research Project Objectives, Research Plan and Basic Concept, Research Methodology, Measurable Effects and Expected Results. The article should also contain general information about the grant: grant title, keywords (up to five), name of the principal investigator and co-investigators, founding source with the grant number, *Ethical Committee permission number,* code in clinical trials (if applicable). Only grant projects in the amount over 100,000 Euro can be presented. Manuscripts should be up to 2000 words in length (excluding references) and can include up to 5 tables and/or figures. The abstract should not exceed 150 words. The number of authors is limited to the Principal Investigator and Co-investigators.

Acknowledgements

Under acknowledgements please specify contributors to the article other than the authors accredited. List here those individuals who provided help during the research (e.g., providing language help, writing assistance or proof reading the article, etc.). Also acknowledge all sources of support (grants from government agencies, private foundations, etc.). The names of funding organizations should be written in full.

References

All manuscripts should use the 'Vancouver' style for references. References should be numbered consecutively in the order in which they appear in the text **and listed at the end of the paper.** References cited only in Figures/Tables should be listed in the end. Reference citations in the text should be identified by Arabic numbers in square brackets. Some examples:

This result was later contradicted by Smith and Murray [3].
Smith [8] has argued that...
Multiple clinical trials [4–6, 9] show...

Journal names should be abbreviated according to Index Medicus. If available alwaysprovide Digital Object Identifier (DOI) or PubMed Identifier (PMID) for every reference.

Some examples

Standard journal articles

1. Petrova NV, Kashirskaya NY, Vasilyeva TA, Kondratyeva EI, Marakhonov AV, Macek Jr M, Ginter EK, Kutsev SI, Zinchenko RA. Characteristics of the L138ins (p.Leu138dup) mutation in Russian cystic fibrosis patients. JMS [Internet]. 2020 Mar 31;89(1):e383. doi: 10.20883/medical.383.

Books

Personal author(s)

1. Rang HP, Dale MM, Ritter JM, Moore PK. Pharmacology. 5th ed. Edinburgh: Churchill Livingstone; 2003.

Editor(s) or compiler(s) as authors

2. Beers MH, Porter RS, Jones TV, Kaplan JL, Berkwits M (editors). The Merck manual of diagnosis and therapy. 18th ed. Whitehouse Station (NJ): Merck Research Laboratories; 2006.

Chapter in the book

1. Phillips SJ, Whisnant JP. Hypertension and stroke. In: Laragh JH, Brenner BM, editors. Hypertension: pathophysiology, diagnosis, and management. 2nd ed. New York: Raven Press; 1995. p. 465–478.

TABLES: Tables should be typed on sheets separate from the text (each table on a separate sheet). They should be numbered consecutively with Arabic numerals. Tables should always be cited in text (e.g. table 2) in consecutive numerical order. Each table should include a compulsory, concise explanatory title and an explanatory legend. Footnotes to tables should be typed below the table body and referred to by superscript lowercase letters. No vertical rules should be used. Tables should not duplicate results presented elsewhere in the manuscript (e.g. in figures).

FIGURES: All illustrations, graphs, drawings, or photographs are referred to as figures and must be uploaded as separate files when submitting a manuscript. Figures should be numbered in sequence with Arabic numerals. They should always be cited in text (e.g. figure 3) in consecutive numerical order. Figures for publication must only be submitted in high-resolution TIFF or EPS format (*minimum 300 dpi resolution).* Each figure should be self-explanatory without reference to the text and have a concise but descriptive legend. All symbols and abbreviations used in the figure must be defined, unless they are common abbreviations or have already been defined in the text. Figure Legends must be included after the reference section of the Main Text.

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SUPPLEMENTARY ONLINE MATERIAL: Authors may submit supplementary material for their articles to be posted in the electronic version of the journal. To be accepted for posting, supplementary materials must be essential to the scientific integrity and excellence of the paper. The supplementary material is subject to the same editorial standards and peer-review procedures as the print publication.

Review Process

All manuscripts are reviewed by the Editor-in-Chief or one of the members of the Editorial Board, who may decide to reject the paper or send it for external peer review. Manuscripts accepted for peer review will be blind reviewed by at least two experts in the field. After peer review, the Editor-in-Chief will study the paper together with reviewer comments to make one of the following decisions: accept, accept pending minor revision, accept pending major revision, or reject. Authors will receive comments on the manuscript regardless of the decision. In the event that a manuscript is accepted pending revision, the author will be responsible for completing the revision within 60 days.

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