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Analysis of the level of non-specific and specific immunity parameters in saliva of children with *osteogenesis imperfecta* and study of relationships between selected proteins, disease symptoms and sociodemographic factors

Analiza stężeń parametrów odporności nieswoistej i swoistej w ślinie dzieci z *osteogenesis imperfecta* oraz badanie zależności pomiędzy wybranymi białkami a objawami choroby i czynnikami socjodemograficznymi

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Keywords

osteogenesis imperfecta, cathelicidin LL-37, defensins, lysozyme, secretory IgA

Summary

Introduction. *Osteogenesis imperfecta* is characterized by dental symptoms, including *dentinogenesis imperfecta* (DGI). Carious process can be modified by factors such as patient's hygiene habits, diet, saliva and its components. Saliva's elements of non-specific immunity with antibacterial and immunomodulatory action are hBD1, hBD2 defensins, cathelicidin LL-37, lysozyme and specific immunity – secretory immunoglobulin A.

Aim. Analysis of levels of hBD1 and hBD2 defensins, cathelicidin LL-37, lysozyme and sIgA in saliva of children with *osteogenesis imperfecta* and in a comparative group of healthy children.

Material and methods. In the years 2015-2018, 62 individuals with brittle bone disease were examined, samples of non-stimulated saliva were collected from 30 patients and from 30 subjects of the comparative group. Levels of the examined parameters were measured using ELISA immunoassays tests. Statistical analysis of laboratory test results was carried out. Statistically significant differences/relationships were established at the significance level of $p < 0.05$.

Results. The concentration of the studied proteins in saliva did not differ significantly between the groups. There were statistically significant positive correlations between: age and sIgA concentration in saliva of the examined patients; age and concentration of hBD1 and hBD2 in saliva of children from the comparative group; age and sIgA concentration in saliva of patients from the study and comparative groups. For the comparative group, statistically significant differences in sIgA concentrations in saliva between girls and boys were observed.

Conclusions. Laboratory test results of hBD1 and hBD2 defensins, cathelicidin LL-37, lysozyme and sIgA levels in saliva indicate that there is a need for further laboratory tests, which could explain the low levels of caries index in children with *osteogenesis imperfecta*. The increased levels of sIgA in saliva with growing age of the patients (with OI and healthy children) may indicate that specific immunity system matures with age or may reflect the impact of external environment on this parameter.

Słowa kluczowe

osteogenesis imperfecta,
katelicydyna LL-37, defensyny, lizozym,
sekrecyjna IgA

Streszczenie

Wstęp. *Osteogenesis imperfecta* towarzyszą charakterystyczne objawy ze strony układu stomatognatycznego, m.in. *dentinogenesis imperfecta* (DGI). Do czynników modyfikujących proces próchnicowy zaliczamy nawyki higieniczne pacjenta, dietę oraz działanie śliny i jej składowych. Obecne w ślinie o działaniu antybakteryjnym oraz immunomodulującym są elementy odporności nieswoistej – defensyny hBD-1, hBD-2, katelicydyna LL-37, lizozym oraz odporności swoistej – wydzielnicza immunoglobulina A.
Cel. Analiza stężeń defensyn hBD1 i hBD2, katelicydyny LL-37, lizozymu oraz sIgA w ślinie dzieci z *osteogenesis imperfecta* oraz w grupie porównawczej.
Materiał i metody. W latach 2015-1018 zbadano 62 pacjentów z wrodzoną łamliwością kości, od 30 z nich oraz 30 pacjentów z grupy porównawczej pobrano próbkę śliny niestymulowanej. Pomiar poziomu badanych czynników przeprowadzono, posługując się testami immunoenzymatycznymi Elisa. Przeprowadzono analizę statystyczną wyników badań laboratoryjnych. Różnice/zależności istotne statystycznie ustalono przy poziomie istotności $p<0,05$.
Wyniki. Zawartość badanych białek w ślinie nie różniła się istotnie statystycznie w obu badanych grupach. Stwierdzono istotne statystycznie dodatnie korelacje pomiędzy: wiekiem a stężeniem sIgA w ślinie badanych pacjentów; wiekiem a stężeniem hBD-1 i hBD-2 w ślinie dzieci z grupy porównawczej; wiekiem a stężeniem sIgA w ślinie pacjentów z grupy badanej i porównawczej. Dla grupy porównawczej dostrzeżono istotne statystycznie różnice w stężeniu sIgA w ślinie pomiędzy dziewczynkami i chłopcami.
Wnioski. Wyniki badań laboratoryjnych śliny pod kątem stężeń badanych białek wskazują na konieczność dalszych badań laboratoryjnych, które mogłyby wyjaśnić niskie poziomy próchnicy u dzieci z OI. Wzrost stężenia sIgA w ślinie wraz z wiekiem pacjentów (z OI i z grupy porównawczej) może świadczyć o dojrzewaniu układu odporności swoistej w miarę upływu lat lub może odzwierciedlać wpływ na ten parametr środowiska zewnętrznego.

Introduction

Osteogenesis imperfecta (OI) is a hereditary disorder of the connective tissue caused by mutations in genes encoding collagen type I – COL1A1 and COL1A2, or in a small percentage in genes encoding proteins involved in collagen biosynthesis. Most often it is inherited in an autosomal dominant pattern. The phenotype of the disease ranges from mild to severe and even lethal. It is characterized by reduced mass and density of the bones, increased brittleness, which leads to repeated fractures even due to minor injuries and deformations of the long bones (1-3). Patients with *osteogenesis imperfecta* are also characterized by short stature, laxity of the joints, they may experience hearing impairment, blue sclerae and dental abnormalities. Characteristic features of their stomatognathic system include *dentinogenesis imperfecta* type I (DGI), malocclusion, lack of permanent tooth germs, ectopic eruption of permanent teeth (4, 5).

Tooth decay is a local, post-eruption, pathological process of external origin that causes decalcification of enamel, decay of tooth's hard tissues and leads to tissue loss (WHO). It develops when components such as bacterial biofilm, carbohydrates supplied with food and the host's susceptibility coexist in the appropriate period of time (6). The caries process can be modified by factors such as hygienic habits of the patient, diet and action of saliva and its components.

Elements with antibacterial and immunomodulatory properties present in saliva include: hBD1 defensins, hBD2, cathelicidin LL-37, lysozyme (non-specific immunity), and secretory immunoglobulin A (specific immunity). Defensins and cathelicidin LL-37 belong to antimicrobial peptides, they have bactericidal effect on Gram-negative and Gram-positive bacteria, they neutralize toxins, furthermore they have antiviral, antiparasitic and antifungal properties. Moreover, they exhibit a number of immunomodulatory functions, such as induction and inhibition of inflammatory process, influence on cell differentiation and chemotaxis. Cathelicidin LL-37, acting on the FPRL1 receptor, activates fDCs (follicular dendritic cells) to produce CXCL13 chemokine, which stimulates B lymphocytes through the CXCR5 receptor, further increasing production of BAFF activating factor. In this way, it increases proliferation of B lymphocytes and secretion of immunoglobulins, including immunoglobulin A. Secreted IgA prevents adhesion of microbes to mucosal epithelial cells, and by agglutinating removes them from the oral cavity, neutralizes microbial toxins, enzymes and viruses. Lysozyme is an enzyme that has bactericidal effect on Gram-positive and Gram-negative bacteria (7-10).

Type I of DGI, present in OI, is characterized by changes in tooth color, ranging from brown to opalescent blue or gray. There is often enamel loss as a result of abnormal dentin structure, exposure of dentin and its rapid abrasion to the level of gingiva, which could enhance the carious process (11).

Aim

Analysis of hBD1 and hBD2 defensins, cathelicidin LL-37, lysozyme and sIgA concentrations in saliva of children with *osteogenesis imperfecta* and in the comparative group.

Material and methods

In 2015-2018, 62 patients with OI, from Department of Paediatrics, Newborn Pathology and Metabolic Diseases of Bones of the Central Clinical Hospital – University Center of Paediatrics in Łódź were examined and subjected to interview and the physical examination, dmft/DMFT index was also calculated. The p/P component included changes with code 3 according to ICDAS II, when a localized lesion in the form of opaque or discolored enamel is visible, with no visible signs of dentin involvement, located in the place of stagnation of plaque and with codes 4, 5 and 6 as carious lesions with different advancement affecting the dentin. In 30 of them and in 30 patients of the Department of Paediatric Dentistry of Medical University of Łódź (the comparative group), laboratory tests of saliva were performed. The study included children aged from 3 to 16 years, whose legal guardian/parent consented to the planned procedures, moreover the study group included individuals with *osteogenesis imperfecta*, and the comparative group included children not affected by this disorder.

The laboratory part was performed at the Department of Microbiology and Laboratory Medical Immunology, Medical University of Łódź. In order to perform statistical analysis of the laboratory tests results, the patients were divided four times: by gender (girls vs boys); by presence or absence of *dentinogenesis imperfecta*; by severity of *osteogenesis imperfecta* (mild vs severe) and by presence or absence of OI (the comparative group) (tab. 1).

Thirty patients with *osteogenesis imperfecta*, in the morning hours, after breakfast, had a sample of unstimulated saliva in the amount of at least 2 cm^3 collected into round-bottomed polypropylene tubes. The children spat out saliva directly into the test tube or, in case of difficulties, it was collected from the bottom of the oral cavity with a sterile syringe with simultaneous massage of the sublingual and submandibular areas. The procedure lasted about 5 minutes and depended on the degree of patient cooperation. The same procedure was performed, also in the morning, in the comparative group of 30 children who were patients of the Department of Paediatric Dentistry of Medical University of Łódź.

Within an hour after collection, the saliva samples were transported in a portable refrigerator at 5°C to the Department of Microbiology and Laboratory Medical Immunology, Medical University of Łódź. After proper preparation in accordance with instructions attached to diagnostic tests, the samples were stored in freezers at -70°C until tests were performed. The samples were defrosted before testing. The saliva samples required additional preparation by centrifugation – 3000 rpm for 10 minutes at 19°C, then the supernatant was poured into Eppendorf tubes. In order to measure levels of the studied agents, Elisa enzyme immunoassay tests were used. For analysis the following tests were used: 1) defensins (hBD1 and hBD2) – ELISA Kit for Beta-defensin 1 test and ELISA Kit for Beta-defensin 4A test from ElAab Science, Wuhan, China; 2) Cathelicidin LL-37 (CAMP) – ELISA Kit for Cathelicidin Antimicrobial Peptide (CAMP) test from Cloud-Clone Corp, San Jose, USA; 3) lysozyme – Lysozyme ELISA test from Immunodiagnostic, Hameenlinna, Finland; 4) secretory immunoglobulin A (sIgA) – Demeditec Diagnostics Secretory IgA test, Demeditec, Kiel, Germany. Altogether 300 determinations were made for 60 saliva samples. Spectrophotometric measurement of absorbance (OD) was performed with a Multiskan GO device (Thermo Scientific). Concentrations of agents was calculated on the basis of calibration curves. The results for hBD1 were given in pg/ml, for sIgA in µg/ml, for hBD2, CAMP and lysozyme in ng/ml.

For each tested parameter in saliva of the study group and the comparative group, the following measures of distribution were determined: arithmetic mean, median, range (min-max) and standard deviation. The Shapiro-Wilk and U Mann-Whitney tests were used for statistical analysis, and the Spearman's rank correlation factor was used to assess a connection between two measurable features. Statistically significant differences/correlations were established at the significance level of $p < 0.05$. The results of laboratory tests were statistically analyzed by GRETL and Excel programs.

Tab. 1. Distribution of patients for statistical analysis

Division of patients		Average age	Gender		*Dentinogenesis imperfecta*		Severity of the disease		Presence of OI	
			Girls	Boys	DGI	Without DGI	Mild	Severe	OI	Without OI
Number of people in the group	Study group	8 years and 6 months	15	15	13	17	19	11	30	–
	Comparative group	8 years and 7 months	16	14	–	–	–	–	–	30

Results

The values of the dmft/DMFT index for patients with OI are presented in table 2. In patients with *dentinogenesis imperfecta*, no signs of active caries were found (tab. 2). The dmft/DMFT rates were low in primary dentition, and they equaled for 3-year-old children: 0.14; for 5-year-old children: 0; for 7-year-old children: 0.2; in adolescents with permanent teeth: for 12-year-olds: 0; for children aged 15: 4.67.

The mean value of sIgA in saliva was slightly higher in the comparative group (188.24 μg/mL) than in the study group (185.43 μg/mL). Mean values of hBD1 and hBD2 defensins were at a higher level in the study group than in the comparative group and equaled respectively: for hBD1 – 184.91 and 149.69 pg/mL and for hBD2 1.0962 and 0.98891 ng/mL. For cathelicidin LL-37, the mean value was higher in the study group – 3381.8 ng/mL, similarly, the mean value of lysozyme was higher in the study group – 2782.4 ng/mL. The obtained differences were not statistically significant (tab. 3).

The Shapiro-Wilk test was used to check the normal distribution of a random variable. It was shown that the studied feature (proteins content in saliva) did not have normal distribution. Hence, the Mann-Whitney U test was used to determine whether the protein content in saliva in these two groups (the OI group and the comparative group) did not differ. The content (median) of the studied proteins in saliva – sIgA, hBD1, hBD2, CAMP and lysozyme did not differ statistically in both groups.

Relationship between age and the studied parameters

A positive correlation was found between age and concentration of sIgA in saliva of the studied patients (R Spearman = 0.544, p = 0.002), i.e. the concentration of sIgA in saliva increased with age. A similarly statistically significant

Tab. 2. Value of dmft/DMFT index in children with OI compared to epidemiological data

Age	dmft/DMFT in patients with OI		Epidemiological data (19)
	Total (patients with DGI and without DGI)	Patients with DGI	
		Primary dentition	
3 years old	0.14	0	2.4 (2015)
5 years old	0	0	4.7 (2016)
7 years old	0.25	0	5.61 (2016)
		Permanent dentition	
12 years old	0	–	3.75 (2016)
15 years old	4.67	–	5.75 (2015)

Tab. 3. Measurements of distribution of the studied parameters in saliva in the study and comparative groups

	Study/ comparative group	Arithmetic mean	Median	Minimum	Maximum	Standard deviation
sIgA [μg/mL]	S	185.43	142.49	35.977	398.54	121.47
	C	188.24	161.51	22.059	838.60	157.76
hBD1 [pg/mL]	S	184.91	86.186	0.00000	1455.7	317.85
	C	149.69	95.739	0.00000	1468.7	265.66
hBD2 [ng/mL]	S	1.0962	0.53189	0.00000	9.3696	1.9097
	C	0.98891	0.62138	0.00000	7.2044	1.4175
CAMP [ng/mL]	S	3381.8	1884.9	203.01	33289.	6067.4
	C	2892.6	2074.3	224.38	13483.	2835.0
Lysozyme [ng/mL]	S	2782.4	1076.5	464.48	28645.	6681.7
	C	1989.3	894.07	98.054	19514.	4244.8

positive correlation was observed between the age of children in the comparative group and the concentration of hBD1 defensin (R Spearman = 0.502, p = 0.005) and hBD2 defensin (R Spearman = 0.421, p = 0.02), i.e. the concentration in saliva increases with age for both hBD1 and hBD2. The results considered simultaneously for the study group and the comparative group showed a statistically significant positive correlation only between age and the concentration of sIgA in saliva (R Spearman = 0.365, p = 0.004) (tab. 4).

Relationship between the number of broken bones and the studied parameters

There was no statistically significant correlation between the studied parameters and the number of broken bones in the study group (tab. 5).

In order to compare the average values of the analyzed parameters in two groups, the Mann-Whitney test was used (because the distribution of the examined features differed from normal distribution, which was determined using the Shapiro-Wilk test).

Tab. 4. Analysis of relationships between the studied parameters in saliva and age of the subjects

Correlation between age and:	n	Rank correlation coefficient by Spearman	Statistical significance p
Study group			
sIgA	30	0.544187	0.001879*
hBD1	30	-0.251212	0.180548
hBD2	30	-0.193814	0.304784
CAMP	30	0.160992	0.395391
Lysozyme	30	0.091707	0.629825
Comparative group			
sIgA	30	0.164674	0.384524
hBD1	30	0.501910	0.004713*
hBD2	30	0.420678	0.020622*
CAMP	30	0.218596	0.245834
Lysozyme	30	0.202039	0.284312
Study and comparative groups			
sIgA	60	0.364681	0.004173*
hBD1	60	0.142909	0.276027
hBD2	60	0.134170	0.306759
CAMP	60	0.200545	0.124442
Lysozyme	60	0.161453	0.217796

*Statistically significant

Comparison of average values of the analyzed parameters for gender (girls vs boys)

For the study group, no statistically significant differences were found in saliva concentrations of the tested parameters between girls and boys. Higher mean values of sIgA, cathelicidin LL-37, hBD1 and hBD2 defensins, and lysozyme were observed in girls (tab. 6). For the comparative group, there were statistically significant differences in the concentration of sIgA in saliva between girls and boys (the value of the Z statistic in the Mann-Whitney test = 2.27, p = 0.02). Higher mean values of defensins and lysozyme were observed in girls, whereas sIgA and cathelicidin LL-37 in boys (tab. 7). In total, for the study group and the comparative group, no statistically significant differences were noticed in the concentration of the tested parameters between girls and boys. Higher values of defensins, cathelicidin LL-37, and lysozyme were observed in girls, and of sIgA in boys (tab. 8).

Comparison of average values of the analyzed parameters depending on the presence of *dentinogenesis imperfecta*

No statistically significant differences were found in saliva concentrations of the tested parameters between patients with and without *dentinogenesis imperfecta* (tab. 9).

Comparison of average values of the analyzed parameters depending on the severity of *osteogenesis imperfecta* (mild vs severe)

There were no statistically significant differences in saliva concentrations of the tested parameters between patients with mild and severe *osteogenesis imperfecta* (tab. 10).

Discussion

Laboratory studies compared concentrations of hBD1 and hBD2 defensins, cathelicidin LL-37, lysozyme, secretory immunoglobulin A in saliva of children with *osteogenesis imperfecta* and children from the comparative group. According to results of clinical trials, the dmft/DMFT index ratios

Tab. 5. Analysis of relationships between the studied parameters in saliva and the number of broken bones in the study group

Correlation between the number of broken bones and:	n	Rank correlation coefficient by Spearman	Statistical significance p
sIgA	29	0.109299	0.572485
hBD1	29	0.092140	0.634520
hBD2	29	0.131124	0.497771
CAMP	29	-0.008408	0.965474
Lysozyme	29	0.012117	0.950258

Tab. 6. Comparison of values of the analyzed parameters depending on the sex of subjects in the study group

Parameter	n	Value of the Z statistic in the Mann-Whitney test	Statistical significance p	Average values of the studied parameters for	
				girls	boys
sIgA	30	-0.12443	0.900972	196.037	174.832
hBD1	30	-0.22902	0.818851	187.900	181.913
hBD2	30	-0.78843	0.430443	1.127	1.065
CAMP	30	-1.11991	0.262754	4788.155	1975.383
Lysozyme	30	0.00000	1.000000	2943.380	2621.329

Tab. 7. Comparison of values of the analyzed parameters depending on sex of subjects in the comparative group

Parameter	n	Value of the Z statistic in the Mann-Whitney test	Statistical significance p	Average values of the studied parameters for	
				girls	boys
sIgA	30	2.272887	0.023034*	131.580	248.668
hBD1	30	0.178509	0.858323	175.197	122.488
hBD2	30	-0.376280	0.706709	1.212	0.751
CAMP	30	0.375520	0.707274	2463.725	3350.018
Lysozyme	30	0.652220	0.514260	2006.612	1970.936

*Statistically significant

Tab. 8. Comparison of values of the analyzed parameters depending on sex of the subjects in the study and comparative groups

Parameter	n	Value of the Z statistic in the Mann-Whitney test	Statistical significance p	Average values of the studied parameters for	
				girls	boys
sIgA	60	1.507543	0.131673	162.769	211.750
hBD1	60	0.043443	0.965348	181.344	152.201
hBD2	60	-0.808768	0.418649	1.171	0.908
CAMP	60	-0.598689	0.549381	3588.449	2662.700
Lysozyme	60	0.627542	0.530305	2459.887	2296.132

Tab. 9. Values of the analyzed parameters depending on presence of DGI

Parameter	Value of the Z statistic in the Mann-Whitney test	Statistical significance p
sIgA	1.38111	0.167247
hBD1	-1.70163	0.088825
hBD2	-1.71669	0.086038
CAMP	1.13000	0.258478
Lysozyme	-0.25111	0.801729

Tab. 10. Values of the analyzed parameters depending on the severity of OI

Parameter	Value of the Z statistic in the Mann-Whitney test	Statistical significance p
sIgA	-0.86073	0.389388
hBD1	-0.62647	0.531005
hBD2	-1.46389	0.143225
CAMP	-0.55947	0.575839
Lysozyme	-0.21518	0.829625

were low in relation to respective age groups, compared to the epidemiological data. The level of caries in children with OI from the current study and in children from epidemiological studies conducted under the program "Monitoring of the oral health of the Polish population for 2016-2020" in primary dentition was – for 3-year-old children, respectively: 0.14 and 2.4; for 5-year-old children: 0 and 4.7; for 7-year-old children: 0.2 and 5.61. Also, in adolescents with permanent dentition, the differences between these groups were significant for 12-year-olds – 0 and 3.75, respectively, but the differences between them decreased for the age of 15 and amounted: 4.67 and 5.75, respectively. Moreover, there were no active signs of caries in patients with *dentinogenesis imperfecta*. Literature shows that teeth with DGI lack normal enamel, which in 1/3 of the cases shows hypoplastic or hypomineralization lesions and quickly crumbles. Exposed dentin is characterized by abnormal histological structure (12).

Agents that can be tested in saliva were selected: antimicrobial proteins – hBD1 defensins, hBD2, cathelicidin LL-37, lysozyme (non-specific immunity), and secretory immunoglobulin A (specific immunity), which could have influence on the lack of active carious lesions in individuals with brittle bone disease. This choice was related to the function (including antimicrobial, immunomodulating) of these agents.

Caries is the most common oral disease of children in developed countries. *Streptococcus mutans* is considered to be the causative factor of caries. The carious process can be modified by action of saliva and its proteins with antimicrobial properties (13, 14). In this study, no statistically significant differences were found in saliva concentrations of the tested proteins of individuals with inborn *osteogenesis imperfecta* and the comparative group.

Antimicrobial proteins – cathelicidin LL-37, hBD1 and hBD2 defensins showed slightly higher values in the study group than in the comparative group. Davidopoulou et al. (15) demonstrated a lower concentration of cathelicidin LL-37 in saliva in patients with caries compared to subjects without caries. On the other hand, Ribeiro et al. (16) found no differences in the level of LL-37 in saliva of children with caries and without active caries lesions. Similar results for cathelicidin LL-37 and for hBD2 were obtained by Phattarataratip et al. (17). In a study by Colombo et al. (18), the values of antimicrobial protein levels, including LL-37, hBD2, also did not differ in the studied groups – children without caries, with early childhood caries (ECC) or its acute form (S-ECC). However, Colombo et al. (18) showed a positive correlation between concentration of hBD2 and the number of *Streptococcus mutans* bacteria. Moreover, they revealed a positive relationship between dmfs and LL-37 and dmfs and hBD2. Malcolm et al. (19) found a positive correlation between concentration of LL-37 and the number of *S. mutans*. This group of studies may confirm that higher ratios of the caries index and higher numbers of *S. mutans* in children with early childhood caries are associated with higher concentrations of such antimicrobial peptides as LL-37 and hBD2 in saliva.

The obtained mean concentrations of lysozyme in saliva were slightly higher in the study group than in the comparative group. Moslemi et al. (20) showed a higher concentration of lysozyme in unstimulated saliva of patients without signs of caries, compared to subjects with ECC. However, in other studies, Hao and Lin (21) did not find significant correlations between the concentration of lysozyme and the condition of patients' dentition. Lertsirivorakul et al. (22), comparing the activity and level of lysozyme in saliva of children without signs of caries and children with early childhood caries (ECC), showed opposite results – increased values and greater activity of the tested enzyme in the S-ECC group. Similarly, Jentsch et al. (23) in four-year research proved lower levels of lysozyme in saliva of young adults with low caries index compared to individuals with high caries index. If studies on larger population confirmed that higher rates of caries are accompanied by higher levels of lysozyme in saliva, this could indicate that the enzyme is more active when the number of cariogenic microorganisms increases.

In the present study, sIgA concentration was slightly lower in the study group. The level of secretory sIgA depends on many factors, including age, emotional state, physical activity, diet, hormonal factors, smoking, moreover, genetic conditions and fluctuations should be taken into account (24). Hence, there are large differences in results of studies available in literature describing the relationship between concentration of sIgA in saliva and intensity of caries. Higher levels of sIgA in children susceptible to caries were found in studies by Thaweboon et al. (25), Bruno et al. (26), Bagherian et al. (27), de Farias and Bezerra (28), Sikorska et al. (29). A different result – higher concentration of sIgA in a group of caries-free children, was obtained in studies by Omar et al. (30), Chawda et al. (31). Similarly, Gregory et al. (32) and Tenovuo et al. (33) demonstrated decreased levels of sIgA directed against *Streptococcus mutans* in saliva of patients with high caries incidence. No relationship between concentration of sIgA and intensity of caries was demonstrated by Hocini et al. (34) and Naspitz et al. (35).

Babies are born with programmed non-specific immunity, and specific immunity develops with age. As mentioned above, concentration of sIgA in saliva depends on patient's age. A study by Jafarzadeh (36) showed increased IgA concentration with age, reaching its maximum values in 51-60-year-olds, and a decrease in 61-70-year-olds. sIgA values obtained by Tappuni and Challacombe (37) were higher in older people. Similarly, in a study by Wan et al. (38), concentration of IgA increased from birth to 18 months of age.

In this study, a statistically significant positive correlation was found between age and concentration of IgA in saliva. Moreover, it was shown that sIgA levels increase with age of all patients – the study and comparative groups. In the

present study, a statistically significant positive correlation was also noted between age of children in the comparative group and concentration of hBD1 and hBD2 defensins, no similar results were obtained for the study group. The average age of both groups was similar – 8 and a half years old. Defensins are factors of non-specific immunity. An increase in defensin concentration can be explained by maturation of the innate immune system. This study also showed statistically significant differences in concentration of sIgA in saliva between girls and boys from the comparative group, this relationship was not found in the study group. Higher values were found in boys.

In the present study, a relationship between severity of OI disease and concentration of the parameters studied in saliva was sought. The criterion of severity was the number of bone fractures in patients with *osteogenesis imperfecta* and the type of *osteogenesis imperfecta*, types I and IV were mild, whereas II and III were severe. No statistically significant differences were found in both analyses. Mean values of the analyzed parameters were also compared depending on presence of *dentinogenesis imperfecta* (DGI). There were no statistically significant differences between the results of patients with DGI and without DGI, while concentrations of cathelicidin LL-37, lysozyme, sIgA were higher in OI patients without DGI, and the levels of hBD1 and hBD2 defensins in patients with OI with DGI. However, it should be emphasized that occurrence of DGI was assessed only on the basis of a clinical trial. As it is known, a lack of clinical and radiological symptoms does not necessarily mean that there are no lesions in histological structure of dental tissues. There are no similar studies available in literature to which the obtained results could be compared, therefore their interpretation at this stage is extremely difficult.

Conclusions

The results of saliva analyses for concentrations of hBD1 and hBD2 defensins, LL-37 cathelicidin, lysozyme, sIgA indicate that there is a need for further laboratory studies that could better explain low level of caries in children with *osteogenesis imperfecta* and thus translate these observations into limiting high levels of caries index in children not affected by this pathology. The growth of salivary sIgA concentration that increases with the age of patients (with *osteogenesis imperfecta* and in the comparative group) may indicate that the specific immune system matures with age or may reflect the influence of external environment – like contact with pathogens such as, cariogenic microorganisms – on the value of this parameter.

Conflict of interest
Konflikt interesów

There was no conflict of interest at the time of writing the article.
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