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Salivary antioxidant status and oral health in children and adolescents

Status antyoksydacyjny a stan zdrowia jamy ustnej u dzieci i młodzieży

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Keywords

antioxidants, oxidative stress, saliva, dental caries

Summary

Introduction. The pathogenesis of oral diseases may be associated with oxidative stress. Salivary antioxidant system constitutes one of the key salivary defence mechanisms against pathogens and a protective factor for oral cavity.
Aim. To investigate the relationship between oral health (hygiene level, gingival and dental health), age and gender and antioxidant capacity parameters in children and adolescents with permanent dentition.
Material and methods. A total of 87 patients were examined. DMFT/DMFS and white spot lesions (WSL), oral hygiene level and gingival health were assessed. Salivary samples were collected from all participants. Unstimulated salivary flow was calculated and salivary samples were assayed for total antioxidant capacity (TAC) and ferric reducing antioxidant power (FRAP).
Results. Antioxidant capacity parameters were lower in patients with caries, active caries, white spot lesions, poor oral hygiene and gingivitis, but the differences were not statistically significant. Oxidative stress parameters were significantly higher in low unstimulated salivary flow. Spearman's rank correlation analysis revealed no relationship between TAC or FRAP values and patients' gender, but there was a positive correlation between TAC/FRAP and patients' age.
Conclusions. Salivary antioxidant capacity parameters differ in certain oral conditions. There is a correlation between salivary antioxidant capacity parameters and patients' age.

Słowa kluczowe

przeciwutleniacze, stres oksydacyjny, ślina, próchnica

Streszczenie

Wstęp. Patogeneza chorób jamy ustnej może być związana ze stresem oksydacyjnym. Układ antyoksydacyjny śliny jest jednym z kluczowych mechanizmów obronnych śliny skierowanych przeciwko patogenom, a także czynnikiem ochronnym jamy ustnej.
Cel pracy. Analiza związku między stanem zdrowia jamy ustnej (poziomem higieny, stanem dziąseł i zębów), wiekiem i płcią a parametrami potencjału antyoksydacyjnego u dzieci i młodzieży z uzębieniem stałym.
Materiał i metody. Badaniem objęto 87 pacjentów. Dokonano oceny wskaźników PUWZ/PUWP i białych plam próchnicowych (ang. *white spot lesions* – WSL), poziomu higieny jamy ustnej oraz stanu dziąseł. Od wszystkich pacjentów pobrano próbki śliny. Oznaczono wydzielanie śliny spoczynkowej (niestymulowanej) oraz dokonano badania próbek śliny pod kątem całkowitego potencjału antyoksydacyjnego (ang. *total antioxidant capacity* – TAC) oraz aktywności antyoksydacyjnej FRAP.

Wyniki. U pacjentów z próchnicą, aktywnym procesem próchniczym, białymi plamami próchniczymi, niskim poziomem higieny jamy ustnej i stanem zapalnym dziąseł odnotowano niższe parametry potencjału antyoksydacyjnego, jednak różnice te nie były statystycznie istotne. U pacjentów z niskimi wartościami wydzielania śliny spoczynkowej stwierdzono istotnie wyższe parametry stresu oksydacyjnego. Analiza korelacji rang Spearmana nie wykazała związku między wartościami TAC i FRAP a płcią pacjenta, ale wykazano dodatnią korelację między wartościami TAC/FRAP a wiekiem pacjentów.
Wnioski. Parametry potencjału antyoksydacyjnego śliny różnią się w zależności od stanu zdrowia jamy ustnej. Potencjał antyoksydacyjny śliny jest skorelowany z wiekiem pacjenta.

Introduction

Oxidative stress is defined as an imbalance between excessively reactive oxygen species (ROS) and antioxidant mechanisms (enzymatic and non-enzymatic). ROS, such as the superoxide radical, hydrogen peroxide and hydroxyl radical, are products of cellular metabolism (1). This imbalance may be a result of infection, inflammation or disease (2). Several studies indicate that the pathogenesis of various systemic inflammatory diseases, including oral ones, can be associated with oxidative stress. Many of these include periodontitis and mucosal lesions, but also dental caries (3-6). Salivary antioxidant system is one of the key salivary defence mechanisms against pathogens and a protective factor for oral mucosa (4). There are multiple different antioxidant compounds in human saliva. The salivary antioxidant system is made of enzymes (for example: peroxidase, super oxide dismutase, glutathione peroxidase, catalase), as well as small molecules such as uric acid or ascorbic acid (7, 8). According to Battino el al., these elements have additive effects rather than act alone. For this reason, measuring individual antioxidants separately can be confusing and lead to misinterpretation. Studies should focus on the whole antioxidant status, which can be expressed, for example, as total antioxidant capacity (TAC) or ferric reducing antioxidant power (FRAP) (9-11).

Aim

The aim of the study was to investigate the relationship between oral health (hygiene level, gingival and dental health), age and gender and antioxidant capacity parameters (TAC, FRAP) in children and adolescents with permanent dentition.

Material and methods

The study conformed to the STROBE guidelines (STrengthening the Reporting of OBservational studies in Epidemiology).

Study population

The study was conducted in 87 patients aged 11-16 years with permanent dentition. The participants were recruited from the Department of Paediatric Dentistry, Medical University of Warsaw. The research was conducted in full accordance with the World Medical Association Declaration of Helsinki. The Bioethical Commission of Warsaw Medical University (No. KB/194/2015) approved the study and informed written consent was obtained from all parents. The inclusion criteria were as follows: no systemic diseases, no chronic pharmacotherapy, no developmental defects of tooth hard tissues, no previous orthodontic treatment. Patients who did not meet the above mentioned criteria were excluded from the study.

Clinical examination

Clinical examinations were conducted in a dental office by one examiner using standardised WHO (World Health Organization) criteria. Caries index, expressed as mean DMFT/DMFS and white spot lesion (WSL) score, was evaluated (12). Oral hygiene level was assessed using ODI-S (the Debris Index), part of Oral Hygiene Index by Greene and Vermillion (13) and Approximal Plaque Index (API%) index by Lange et al. (14). Gingival health was assessed based on the Gingival Index (GI) by SILNESS and Löe (15), on four surfaces of permanent teeth 16, 12, 24, 36, 32, 44.

Sample collection for salivary analysis

Saliva samples were collected from all participants in the morning (8.00-10.00 am). The participants were fasted. Salivette collection tube (Sarstedt, Nümbrecht, Germany) was used. The swab was placed in the mouth and allowed to absorb resting saliva for 5 min. Immediately after collection, the tube was stored at +4°C and centrifuged within 2 hours. The centrifugation to recover saliva from the swab was performed at 1000 x g at +4°C for 15 min. Unstimulated salivary flow rate was calculated by dividing the patient's salivary volume by collection time. The saliva samples were frozen and stored at -80°C until the analysis.

Total antioxidant capacity of saliva

TAC was assessed using a modified method developed by Erel (10). The assay was based on the principle of reduction of 2,2'-azino-bis (3-ethylbenz-thiazoline-6-sulfonic acid)

cation radical (ABTS •+) (whose solution is blue-green) to ABTS (colorless solution) by antioxidants present in the sample. The reaction changed the colour of the reaction mixture. The green-blue colour disappeared. The reaction was measured spectrophotometrically at 660 nm. The change in absorbance was linked with the level of antioxidants in the sample. Calibration of the results was performed using Trolox. The results of TAC were expressed in mmol Trolox Equiv./L.

Ferric reducing antioxidant power

FRAP was determined using a method developed by Benzie and Strain (11). The method is based on the ability of antioxidants to reduce the complex of ferric tripyridyltriazine (Fe3+-TPTZ) to Fe2+-TPTZ at low pH (3.6). As a result of reaction, the yellow colour of Fe3+-TPTZ changed during the reduction to Fe2+-TPTZ, developing an intense blue colour with a maximum absorbance at 593 nm. The reaction was monitored spectrophotometrically. The change in absorbance was directly proportional to the level of antioxidants with a reduction potential in the sample. FRAP value of a sample was determined using calibration curve method. Calibration was performed using aqueous solutions of known Fe2+ (iron (II) sulphate heptahydrate ($FeSO_4 \times 7H_2O$)) concentration. The results are expressed in mmol/L.

Statistical analysis

The analyses were performed using Statistica 12.0 software and R package. For quantitative variables, descriptive statistics characterising their variability were calculated. For binomial variables, the proportions (%) with division into groups were calculated. Comparison of quantitative variables between two groups was performed using the Mann-Whitney U test and the chi-square test, while the comparison of three or more groups was performed using the ANOVA test. In the case of categorical variables (including binomial variables), chi-square test was used to compare the groups. Spearman's rank correlation coefficient was calculated. In all analyses, the statistical significance level was set at 0.05. Missing data were excluded from the analysis.

Results

A total of 87 patients, including 46 (52.9%) boys and 41 (47.1%) girls, were examined. The mean age ± SD was 13 ± 2 years. Caries (DMFT > 0) was present in 81 patients (93.1%), active caries (DT > 0) in 47 patients (54.3%), and white spot lesions (WSL > 0) in 41 patients (47.1%). The results for the whole group and those depending on sex are presented in table 1.

The mean ODI-S index value was 1.01 ± 0.56 overall (1.06 ± 0.49 and 0.95 ± 0.63 respectively in boys and girls).

Tab. 1. Caries prevalence in the study group

Parameters	Total	Boys	Girls	p
		Incidence [%]		
DMFT[a] > 0	93.2	93.6	92.7	0.868
DT[b] > 0	54.3	57.4	51.2	0.562
WSL[c] > 0	47.1	41.3	53.6	0.251
		Mean ± SD		
DMFT[a]	6.30 ± 4.98	6.30 ± 5.09	6.37 ± 4.92	0.948
DT[b]	2.09 ± 2.81	2.30 ± 3.02	1.85 ± 2.56	0.458
MT[d]	0.06 ± 0.28	0.02 ± 0.15	0.10 ± 0.37	0.181
FT[e]	4.18 ± 3.78	3.98 ± 3.78	4.41 ± 3.81	0.599
DMFS[f]	8.93 ± 8.82	9.02 ± 9.73	8.83 ± 7.78	0.921
WSL[c]	3.47 ± 5.60	3.96 ± 6.30	2.93 ± 4.71	0.395

[a]Decayed, Missing, Filled Teeth
[b]Decayed Teeth
[c]White Spot Lesions
[d]Missing Teeth
[e]Filled Teeth
[f]Decayed, Missing, Filled Surfaces

The mean API% index was 67.5 ± 29.1 overall (72.4 ± 26.8 and 61.9±30.9 respectively in boys and girls). Gingivitis (GI > 0) was present in 60 patients (69%). The characteristics of oral hygiene and gingival state is presented in table 2. Unstimulated salivary flow rate ranged from 0.4 to 2.1 mL/5 minutes (mean 1.31 ± 0.45 mL/5 min). It was considered low in 44 (50.6%), average in 34 (39.1%), and high – 9 (10.3%) patients.

The mean TAC values were 0.478 ± 0.257 in mmol Trolox Equiv./L (0.490 ± 0.261 mmol Trolox Equiv./L for boys and 0.465 ± 0.250 mmol Trolox Equiv./L for girls), and the mean FRAP values were 0.465 ± 0.171 mmol/L (0.465 ± 0.182 mmol/L for boys and 0.465 ± 0.159 mmol/L for girls). The intersex differences were not statistically significant. The results showed that the antioxidant capacity parameters were lower in patients presenting with caries, active caries, white spot lesions, bad oral hygiene and gingivitis, but the differences were not statistically significant (tab. 3). Oxidative stress parameters were significantly higher in low unstimulated salivary flow (for TAC p = 0.032; for FRAP p = 0.005). Spearman's rank correlation analysis revealed no relationship between TAC or FRAP values and sex (p = 0.592; p = 0.873, respectively). There was a positive correlation between TAC or FRAP and age (r = 0.253, p = 0.018 for TAC and r = 0.204, p = 0.05 for FRAP).

Discussion

The salivary antioxidant system is a part of salivary defence against pathogens. It also constitutes a protective factor for oral mucosa. Salivary antioxidants are the first defence mechanism against oxidative stress caused by free radicals (16, 17). Several research described that the initiation of many oral diseases may be connected with decreased antioxidant capacity of saliva and, consequently, oxidative stress (4, 5, 18). According to Krawczyk et al. (19), the presence of cariogenic bacteria forces an increased response of leukocytes (neutrophils and monocytes) which generate ROSs in a respiratory burst process, producing a bactericidal effect. This may be the possible cause of TAC/FRAP decrease in the saliva of patients with dental caries. Increased levels of salivary ROS intensify the oxidative stress as a result of reduction of the total antioxidant capacity of saliva (19). The results of the present study were in accordance with some previous reports. Reduced levels of TAC in patients with high caries experience were reported by Krawczyk et al. (19) and Rahmani et al. (16). However, their results were statistically significant, as opposed to the present study. On the other hand, many studies show that the total antioxidant capacity of saliva may be also increased in dental caries (4, 20-22). Ahmadi et al. (4) showed that the level of TAC was significantly higher in the caries active group than in caries free subjects. According to Silva et al. (21), oxidative damage was significantly lower in S-ECC (Severe-Early Childhood Caries) children than in caries-free children, while salivary total antioxidant capacity was significantly higher in the S-ECC group. The explanation for this can be that TAC level in individuals with high caries intensity is a form of compensation against oxidative stress. In the present study, we also investigated the gingival condition and oral hygiene, and calculated unstimulated salivary flow rate. These parameters were rarely assessed in previous research concerning the relationship between antioxidant capacity and caries prevalence (19, 23), and taking the unstimulated salivary flow rate into consideration is novel. In the present study, we failed to confirm significant relationships between oral

Tab. 2. The characteristics of oral hygiene and gingival health in the study group

Parameters	Incidence [%]			p
	Total	Boys	Girls	
ODI-S[a] good oral hygiene	24.1	15.2	34.1	0.039*
ODI-S[a] fair oral hygiene	50.6	58.7	41.5	0.109
ODI-S[a] poor oral hygiene	25.3	26.1	24.4	0.856
API%[b] optimal hygiene	5.7	4.3	7.3	0.553
API%[b] quite good hygiene	18.4	10.9	26.8	0.055
API%[b] average hygiene	24.1	26.1	22.0	0.653
API%[b] bad hygiene	51.7	58.7	43.9	0.168
GI[c] > 0	69.0	69.6	68.3	0.898

[a]Debris Index
[b]Approximal Plaque Index
[c]Gingival Index
*statistically significant p < 0.05

Tab. 3. TAC and FRAP values (mean ± SD) in different oral conditions

TAC [mmol Trolox Equiv./L]			
Oral conditions	**Group**		**p**
Caries	DT[d] + WSL[e] = 0	DT[d] + WSL[e] > 0	
	0.479 ± 0.273	0.477 ± 0.251	0.983
	DT = 0	DT > 0	
	0.496 ± 0.273	0.463 ± 0.244	0.558
ODI-S[a] hygiene	good	bad or fair	
	0.483 ± 0.271	0.461 ± 0.254	0.736
API%[b] hygiene	optimal or good	average or bad	
	0.483 ± 0.271	0.461 ± 0.245	0.393
Gingivitis	GI[c] = 0	GI[c] > 0	
	0.493 ± 0.265	0.471 ± 0.255	0.720
Unstimulated salivary flow rate	low	average or high	
	0.515 ± 0.255	0.418 ± 0.247	0.032*
FRAP [mmol/L]			
Oral conditions	**Group**		**p**
Caries	DT + WSL = 0	DT + WSL > 0	
	0.456 ± 0.187	0.485 ± 0.163	0.458
	DT = 0	DT > 0	
	0.485 ± 0.211	0.448 ± 0.126	0.319
ODI-S[a] hygiene	good	bad or fair	
	0.493 ± 0.161	0.456 ± 0.173	0.393
API %[b] hygiene	optimal or good	average or bad	
	0.493 ± 0.161	0.456 ± 0.173	0.736
Gingivitis	GI[c] = 0	GI[c] > 0	
	0.491 ± 0.162	0.453 ± 0.174	0.351
Unstimulated salivary flow rate	low	average or high	
	0.536 ± 0.170	0.414 ± 0.157	0.005*

[a]Debris Index
[b]Approximal Plaque Index
[c]Gingival Index
[d]Decayed Teeth
[e]White Spot Lesions
*$p < 0.05$

hygiene and TAC/FRAP levels, which corresponded with the results presented by Krawczyk et al. (23). However, they performed another investigation in 2014 (19), which revealed a significant negative correlation between OHI-S and TAC (r = -0.4368, p = 0.000001). The significant relationship between TAC/FRAP and unstimulated salivary flow rate is an original finding and has not been reported before. It may be caused by greater dilution of ROS in a higher salivary volume, which may reduce TAC/FRAP levels, which regulate ROS concentration.

Compared with the previous studies, the obtained results indicate that the relationship between dental caries, gingiva and oral hygiene and salivary antioxidant systems is not fully explained. The differences may result from different laboratory methods used for TAC/FRAP evaluation, or differences in patients' age and, consequently, dentition type (24). In the present study, we observed that the total antioxidant capacity of saliva increases with age, which corresponds with the results obtained earlier by Hegde et al. (2). Ahmadi et al. (4) suggested a relationship with sex (lower TAC values in females), however, neither we nor other researchers confirmed this finding (16).

Some authors suggest that salivary oxidative stress parameters may potentially be used for screening and monitoring oral diseases, including dental caries (3, 21). However, the authors of the present study remain sceptical as the results of the studies are ambiguous. Therefore, further investigations should be conducted, with a participation of larger patient groups of different age, dentition and caries intensity or gingival health.

Conclusions

Practitioners should bear in mind that particular oral conditions (caries, gingivitis, poor oral hygiene) can modify salivary antioxidant capacity parameters as a result of oxidative stress. There is a correlation between salivary antioxidant capacity parameters and patients' age, but there is no such correlation with patients' gender.

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Conflict of interest
Konflikt interesów

None
Brak konfliktu interesów

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