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ORIGINAL ARTICLE

Life style and risk of development of dental caries in a population of adolescents

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Abstract

Introduction. Oxygen is an essential element for sustaining the life of aerobes; however, in certain conditions it may be toxic for these organisms. This is due to so-called reactive oxygen species – ROS. Factors which cause the production of free radicals include ionizing radiation, UV radiation, high temperature, and hazardous substances, such as phenols, carbon monoxide, e.g. in smokers, also air pollution and drugs.

Objective. The objective of the study was analysis of the total antioxidant status (TAS) in stimulated and unstimulated saliva, according to the number of active carious lesions in generally healthy non-smokers aged 15–17.

Materials and method. The study covered 113 adolescents aged 15–17 in whom the state of dentition was evaluated using the DMFT (Decayed-Missing-Filled) index, and oral hygiene assessed based on the OHI-S (Oral Hygiene Index-Simplified) index. TAS in saliva was determined by means of a Randox Labolatories Ltd. test kit, by the spectrophotometric method.

Results. Based on the results of the study, it was found that in the population examined an increase in the number of carious lesions was accompanied by a significant decrease in the TAS, both in stimulated and unstimulated saliva.

Conclusions. A health-promoting life style, maintenance of basic principles of oral hygiene and care of general health through the elimination of harmful habits decrease the risk of dental caries.

Key words

dental caries, reactive oxygen species, oxidative stress, TAS, environment of the oral cavity

INTRODUCTION

It is common knowledge that oxygen, which is essential for sustaining the life of aerobes, under certain circumstances may become toxic for these organisms. This may happen due to so-called reactive oxygen species (ROS), which include: superoxide anion radical ($O_2^{\bullet-}$), hydroxyl radical ($^{\bullet}OH$), hydrogen peroxide (H_2O_2), singlet oxygen ($^1O_2$), peroxide radical ($ROO^{\bullet}$), nitric oxide radical ($NO^{\bullet}$), and peroxynitrite radical ($ONOO^-$) [1, 2, 3, 4].

The production of ROS is also affected by such factors as: ultrasounds, radiation (ionizing, ultraviolet, gamma, X-ray), environmental pollution (containing, e.g. ozone) and tobacco smoke. Cigarette smoking generates high temperature in the oral cavity, and hazardous substances, such as phenols and carbon monoxide, penetrate into the organism. These factors are conducive to the production of ROS. The increase of ROS production is also affected by bacteria and then the free oxygen radicals produced enhance the immune mechanisms of the organism [1].

The environment of the oral cavity developed a system of antioxidants due to which survival in the environment with high oxygen concentration is possible. ROS may be produced in cells by deliberate or accidental generation [1]. Reactive oxygen species that are produced in the course of deliberate synthesis have specific functions, e.g. they participate in phagocytosis. A major source of ROS originates from the NADPH (nicotine adenine dinucleotide phosophate) oxidase system, which is located in the cytoplasmic membrane of phagocytes. The enzymes are inactive when the organism is healthy, whereas in various pathological states they become activated by bacteria and their toxins, mitogens and cytokines. Activated NADPH oxidase catalyses the production of large amounts of $O_2^{\bullet-}$, which in turn may undergo spontaneous or enzymatic dismutation to H_2O_2. This process, called respiratory burst, takes up 90% of molecular oxygen assimilated by neutrophils, and its main task is to destroy phagocitized microorganisms [5]. On the other hand, accidental release of the reactive oxygen species leads to various lesions, both at cell and tissue level. The most common process generating ROS in this way is mitochondrial oxidative phosphorilation combined with complete oxygen reduction to the water molecule [1, 2, 3].

The reactive oxygen species play an important role in numerous basic processes of the organism. However, apart from physiological functions, it may also have some side-effects harmful to the organism. ROS react with virtually all cell components, affecting lipids, proteins, nucleic acids and carbohydrates causing damage to cells, which often results in cell death [1, 6, 7, 8, 9, 10, 11, 12].

In order to counteract the harmful influence of ROS on cells, aerobes have developed a special antioxidant defence system, which is able to deactivate ROS of both physiological and pathological origin [1, 2, 8].

The antioxidant defence system involves three stages:
- the first line of defence is to prevent ROS formation or their reaction with biologically-active compounds (prevention);
- the second line of defence is provided by so-called ROS scavengers, acting by scavenging ROS in hydrophilic (L-ascorbic acid, uric acid, glutathione) or hydrophobic environments (tocopherols, carotenoids, ubihydrochinone).
- during the third line of defence, products of the reaction between ROS and biomolecules are removed (elimination or repair) [1, 8, 12, 13].

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Oxidative stress is a condition of increased ROS activity, which results from a disturbed balance between production and elimination of toxic derivatives of oxygen. [3, 4, 6, 7, 8]

Saliva is a secretion easily obtained and due to its amount and composition in may be a valuable diagnostic and research material. It seems to be a better material than blood serum, since its collection is non-invasive and does not require any special equipment, which in turn allows less expensive access to screening for a larger populations. Whole saliva is often used in an additional examination for diagnosis of hereditary, autoregressive, infectious, neoplastic diseases, as well as in the evaluation of the therapeutic level of medicines in the organism or the presence of drugs. Using saliva as a diagnostic tool is becoming increasingly more common, since it is an attractive alternative for other invasive, time-consuming and complicated diagnostic methods. Moreover, it is suitable for comparison with other biological materials, e.g. plasma [13, 14, 15, 16, 17].

Dental caries is defined as a local lesion to the hard dental tissue. It results from the demineralization of enamel and dentine by sour products of the dental plaque bacteria, which develop as a result of fermentation of carbohydrates present in food.

Disturbances in the balance between free oxygen radicals and antioxidants in saliva is an important aetiological and developmental factor of numerous inflammatory processes in the oral cavity [13, 18, 19].

Objective. The objective of the study was analysis of the level of antioxidants TAS (Total Antioxidant Status) in stimulated and unstimulated saliva, according to the number of active carious lesions in generally healthy, non-smoking individuals aged 15–17.

MATERIALS AND METHOD

The study covered 113 adolescents aged 15–17 years whose state of dentition was evaluated with the use of the DMFT index and its components DT, MT and FT, and state of oral hygiene evaluated according to the Green and Vermilion Oral Hygiene Index – Simplified (OHI-S), with the use of the Plaque Test.

The total antioxidant status (TAS) in saliva was determined by spectrophotometric analysis using a test kit from Randox Laboratories Ltd. Using this method, 2,2'-azino-di-(3-ethyl-benzothiazoline 6-sulphonate) (ABTS) incubates with peroxidase (metmyoglobin) and H_2O_2, which produces a cation form of ABTS ($ABTS^{\bullet+}$), which results in a blue and green sample dye. The dye intensity is measured spectrophotometrically at 600 nm wavelength. Antioxidants contained in saliva inhibit development of the dye dependent on the $ABTS^{\bullet+}$ concentration, proportionally to their amount in the sample.

Examination of the total antioxidant status was performed for each subject on both unstimulated and stimulated saliva.

All the studied adolescents were in a good general health, with no gum inflammation and stated that they were non-smokers. Both unstimulated and stimulated saliva was collected for the study in the morning hours. Two hours prior to the examination, the subjects could not clean their teeth nor eat any meals.

The subjects were divided into five groups, depending on the DMF index:
- Group I consisted of those with healthy dentition DMFT 0, DS 0.0 (25);
- Group II – with healed carious foci DMFT 4.52, DS 0.0 (25)
- Group III – DMF 4.52 and DS 2.33, 1–4 decayed teeth (21)
- Group IV – DMF 10.0 and DS 8.67, 5–8 decayed teeth (21)
- Group V – DMF 13.38 DS 15.33, 9–14 decayed teeth, (21).

Statistical methods. The results of the studies were subjected to statistical analysis based on the Spearman's rank correlation coefficient. The analyses were carried out using Statistica version 7.0. The significance level was set at p=0.05.

RESULTS

Table 1 shows the DMF index and its components DT, MT, FT, DS, and FS, and the OHI- S index in particular groups.

Table 1. DMFT, DT, MT, FT, DS, FS and OHI- S indices in individual groups

Group Parameters		Group I n=25	Group II n=25	Group III n=21	Group IV n=21	Group V n=21
DMFT	M±SD	0	4.52±4.66	4.52±2.29	10.00±1.79	13.38±3.34
	(min; max)	0	1–20	1–11	7–15	8–22
DT	M±SD	0	0	2.0±1.05	5.76±1.04	10.62±2.05
	(min; max)	0	0	1–4	5–8	9–14
MT	M±SD	0	0.04±0.20	0.05±0.22	0.14±0.36	0.29±0.64
	(min; max)	0	0–1	0–1	0–1	0–2
FT	M±SD	0	4.48±4.66	2.90±2.23	4.1±1.81	2.5±2.94
	(min; max)	0	1–20	0–10	0–9	0–10
DS	M±SD	0	0	2.33±1.15	8.67±2.39	15.33±2.85
	(min; max)	0	0	1–4	5–11	12–22
FS	M±SD	0	6.92±9.16	4.04±4.32	5.95±2.65	3.95±4.78
	(min; max)	0	1–40	0–20	0–14	0–19
OHI-S	M±SD	0.44±0.51	0.89±0.50	0.94±0.70	1.61±0.61	1.91±0.53
	(min; max)	0.0–1.6	0.0–1.6	0.0–2.0	0.75–3.0	0.5–2.7

Table 2 presents the total antioxidant status in stimulated and unstimulated saliva in particular groups. TAS u in Group I – 1.084±0.144, Group II – 0.968±0.126, Group III – 0.932±0.148, Group IV – 0.803±0.167, and Group V – 0.734±0.164, whereas TAS s in Group I was 1.006±0.130, Group II – 0.902±0.141, Group III – 0.851±0.110, Group IV – 0.684±0.149, and Group V – 0.651±0.153. TAS u was higher than TAS s in all the studied groups.

Table 2. Total antioxidant status in stimulated and unstimulated saliva in individual groups

Group	n	TAS u unstimulated saliva		TAS s stimulated saliva	
		M	SD	M	SD
Group I	25	1.084	0.144	1.006	0.130
Group II	25	0.968	0.126	0.902	0.141
Group III	21	0.932	0.148	0.851	0.110
Group IV	21	0.803	0.167	0.684	0.149
Group V	21	0.734	0.164	0.651	0.153
Total	113	0.913	0.192	0.828	0.191
Kruskal-Wallis test		H=49.462; p=0.00001		H= 50.164; p=0.00001	

Figures 1–4 present TAS u (in unstimulated saliva) and TAS s (in stimulated saliva) in relation to the DMFT, DT, DS and OHI-S indices.

Legends
TAS u – total antioxidant status in unstimulated saliva
TAS s – total antioxidant status in stimulated saliva
n – number of adolescents
M – mean
SD – standard deviation

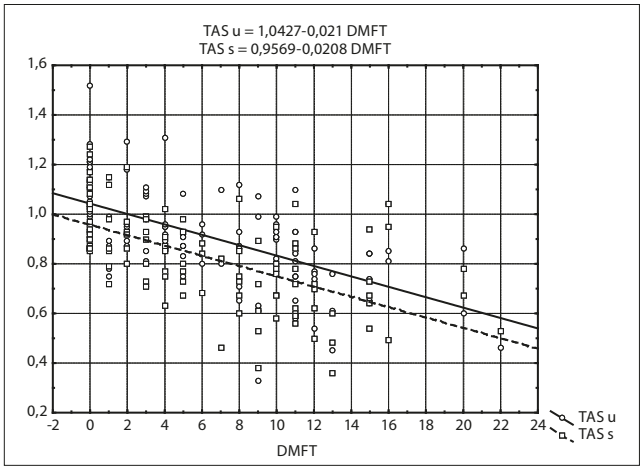

Figure 1. TAS u (in unstimulated saliva) and TAS s (in stimulated saliva) in relation to DMFT

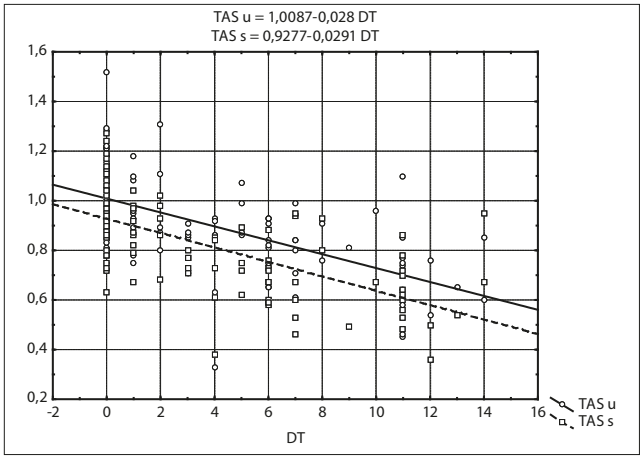

Figure 2. TAS u (in unstimulated saliva) and TAS s (in stimulated saliva) in relation to DT

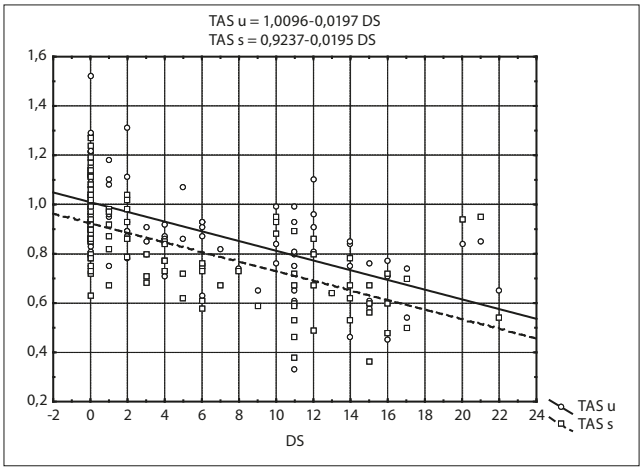

Figure 3. TAS u (in unstimulated saliva) and TAS s (in stimulated saliva) in relation to DS.

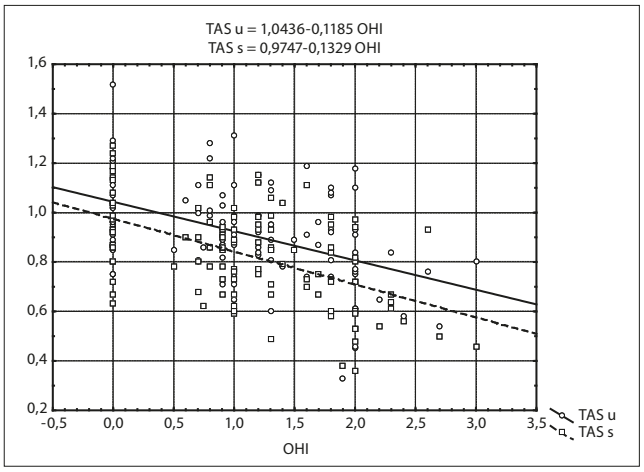

Figure 4. TAS u (in unstimulated saliva) and TAS s (in stimulated saliva) in relation to OHI

Analysis based on the Spearman's rank correlation coefficient revealed a significant correlation between TAS u and DMFT (R= -0.6385; p=0.0000001), and between TAS s and DMFT (R= -0.6166; p=0.0000001). There was a negative correlation between the values of DMFT and TAS u and TAS s in the studied adolescents, which means that increased DMFT was associated with reduced TAS u and TAS s. Analysis of the obtained results also revealed a negative correlation between TAS u and DT (R= -0.6516; p=0.0000001), FT (R= -0.3478; p=0.00016), DS (R= -0.6682; p=0.0000001), FS (R= -0.3259; p=0.000427), and between TAS s and DT (R= -0.6545; p=0.0000001), FT (R= -0.3368; p=0.000264), DS (R= -0.6661; p=0.0000001), FS(R= -0.3218; p=0.000510).

The highest correlation was observed between TAS u and TAS s, and DS, and the lowest – between TAS u and TAS s, and FS.

The Spearman's rank-order correlation was also used to asses the correlation between TAS u and TAS s, and the oral hygiene expressed in the OHI-S index. Analysis revealed a significant negative correlation, which means that an increase in OHI-S was accompanied by a decrease in TAS u and TAS s (TAS u R= -0.4368, p=0.000001, TAS s R=0.4841, p= 0.000001).

The chain index was used to describe dynamics of TAS u and TAS s changes in the course of an increase in the number of active carious lesions. A reduction in TAS u values between Groups I and II was 10.63%, chain index 0.894. The reduction between Groups II and III was the smallest, i.e. 5/0%, chain index 0.95, and it was the largest between Groups III and IV, 12.69%, chain index 0.87. The reduction between Groups IV and V was 8.59%, chain index 0.915. A reduction in TAS s values between Groups I and II was 10.37%, chain index 0.896, between Groups II and III – 6.37%, chain index 0.936, and was the largest between Groups III and IV – 19.03%, chain index 0.81. The reduction between Groups IV and V was the smallest – 4.74%, chain index 0.953.

DISCUSSION

Saliva plays a very important role in the health condition of the oral cavity and may be helpful in the diagnosis of numerous diseases, not only those related to the oral environment.

Studying various salivary parameters allows the diagnosing and monitoring of many pathological processes taking place in the human body. Determination of the total antioxidant status in saliva may be helpful in determining the antioxidant capacity of the whole organism. It is considered that an increase in the total antioxidant status is a sign of better protection against the reactive oxygen species. For example, the antioxidant level increases in chronic renal failure and decreases in cancerous diseases or diabetes [6, 7, 18, 20, 21]. Taking into account the fact that all the studied adolescents were healthy and did not have any inflammation of the soft oral tissues, it seems that the salivary antioxidant level may depend primarily on the advancement of the decaying process [19]

The results of the presented study concerning the total antioxidant status TAS were similar to those obtained by Sikorska-Jaroszyńska who examined adolescents aged 17 and observed the highest TAS level at D <5 (0.93±0.36), which gradually decreased to the D index between 5–10 (0.71±0.41) [18]. It was observed that further increase in the number of decayed teeth was accompanied by a slight TAS increase (D 10–15, TAS 0.73±0.35, D>15 TAS 0.75±0.34), whereas the presented study shows the smallest TAS decrease at D>9 in stimulated and less rapid TAS reduction in unstimulated saliva [21]

The results obtained by Sikorska-Jaroszyńska are compatible with the presented results, where the highest TAS value was observed in children free of decay. Tulungolu et. al. also obtained a higher TAS value in children free of decay compared to children with D=1–2. In their studies of children aged 7–15, Tulungolu et al. noticed a higher level of antioxidants in those with decay, compared to the group free of decay, although the differences were not statistically significant (p>0.05). Only in 11–15-year-old girls the antioxidant capacity of saliva was higher in those free of decay than in the children with active decay (total antioxidant mmol/l 0.6±0.15 in children free of decay and 0.57±0.18 in those with active decay) [22].

Different results, however, were obtained by Uberos et al. who studied children aged 4.5–14.5. They observed an increase in the total antioxidant status together with an increase in the number of decayed teeth. A clear linear increase was noted with regard to decay of the primary teeth (TAC in children with primary teeth free of decay 7.8 ± 4.0 1/IC50, TAC in children with decay 10.6 ± 11.1 1/IC50), whereas in the case of decayed secondary teeth, TAC was higher in children whose number of decayed teeth was more than 5 (TAC in children with no decay in secondary teeth was 7.8 ± 5.0 1/IC50 and in children with decay – 9.0 ± 8.0 1/IC50. In children with more than 5 decayed teeth, TAC was 11.48 ± 12.4 1/IC50, and with less than 5 decayed teeth, TAC was 7.79 ± 4.41 1/IC50). The researchers explained that in the studied age and ethnic group (126 patients from the Western Sahara, resident in a refugee camp at Tindouf, Algeria, the salivary antioxidant capacity favours decay development, especially with regard to primary teeth [23].

Own studies have revealed higher TAS values in unstimulated saliva than in stimulated saliva, and the results are compatible with those obtained by Moore at al. It is thought that unstimulated saliva is a better measure of antioxidative properties than stimulated saliva. [24]

In the presented study, a regular decrease in salivary TAS was observed which depended on the number of active carious lesions. This may be related to an increased oral activity of neutrophils and monocytes, which generate reactive oxygen species in the presence of bacteria in a so-called respiratory burst producing a bactericidal effect. On the other hand, increased ROS generation contributes to the intensification of oxidative stress as a result of reduction in the total antioxidant capacity of saliva.

Oxidative stress produced as a result of overproduction of free oxygen radicals is considered as an important factor causing many systemic diseases, such as diabetes, cardiovascular diseases, cancer, increased susceptibility to infections, and oral cavity diseases. The promotion of a healthy life style exerts a significant effect on the general health and health of the oral cavity among the young population [25].

CONCLUSIONS

On the basis of the conducted studies, the following observations can be made:

1. The education of adolescents should cover the promotion of a healthy life style from the aspect of both general health and health of the oral cavity.
2. Multiple causes of dental caries indicate that it is necessary to undertake wide health-promoting actions, mainly among the young population.
3. Elimination of harmful habits, such as cigarette smoking, decreases the risk of dental caries.

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