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Dental caries and total antioxidant status of unstimulated mixed whole saliva in patients aged 16-23 years

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ABSTRACT

Purpose: The purpose of the studies performed was an attempt to establish a potential relationship between total antioxidant status of unstimulated whole saliva, patients' ages, oral hygiene status and dental caries.
Material and methods: The study involved 120 non-smokers. Mean age of the study subjects was 18.40±1.74 years. Clinical examination was performed to evaluate the state of hard and soft oral tissues, and oral hygiene status. Additionally, biochemical tests and statistical studies were carried out. Laboratory examinations involved measuring the total antioxidant status level in supernatant using the Total Antioxidant Status (TAS) test from Randox. In statistical analysis, Spearman Rang correlation coefficient, Kruskal-Wallis test, Mann – Whitney test, logistic regression and ROC curve were used. The analysis was conducted using Statistica 9 software.
Results: Mean D, M, F and DMFT values were 4.04±3.96; 0.09±0.34; 5.00±3.90; 9.05±5.30, respectively. Mean OHI – S was 0.95±0.76. Mean TAS value in the studied population was 0.82±0.26 mmol/l. In the group of subjects without active dental caries, the level of the total antioxidant potential was higher, with a value of 0.89±0.16 mmol/l, and in the study group subjects who had tooth decay it was 0.80±0.28 mmol/l. It was found that TAS was higher in younger subjects.
Conclusions: The conducted studies seem to allow for the following conclusions:
TAS level in the supernatant of unstimulated whole saliva decreases with age; TAS level in the supernatant of unstimulated whole saliva is the highest in patients without caries, and the oral hygiene status does not have significant influence on TAS.

Key words: Total antioxidant status, dental caries, D value, age, OHI-S

INTRODUCTION

The oral cavity constitutes a unique, complex, open and dynamic environment. It plays an incredibly important role in the defense mechanisms of the organism, as well as in maintaining homeostasis between internal and external factors. The indispensable factor contributing to maintaining good oral health status is a heterogeneous systemic secretion, the saliva. Mixed unstimulated saliva includes 99.4% water and 0.6% organic and inorganic substances [1-4]. On the basis of results of studies performed by 3 study teams, 1,166 saliva proteins were catalogued. According to the authors, the healthy people saliva proteins catalogue will allow for the use of saliva as study material in the diagnostics of patients suffering, not only from oral cavity health problems, but also systemic diseases [5].

Saliva plays a range of roles in the human organism, among which one of the most important is the defense function, thanks to the specific and unspecific antibacterial factors included in it, as well as to the antioxidant defense system [1, 6-8]. The integrated antioxidant defense system provides a dynamic balance between prooxidants, that is the compounds possessing at least one unpaired electron, and factors characterized with antioxidant properties. It plays an extraordinarily significant role in protecting the organism against the influence of adverse factors and in the maintenance of systemic homeostasis. Due

Table 1. **Mean DMFT, DMF-S, D, M, and F values, Ds, Ms, Fs values, GI and OHI-S in reference to gender.**

Examined value	Men N = 66	Women N = 54	Total N = 120	Statistical significance
	$\overline{X}$ ± SD	$\overline{X}$ ± SD	$\overline{X}$ ± SD	
D value	4.13±4.58	3.92±3.08	4.04±3.96	H = 0.35 p = 0.55
M value	0.06±0.29	0.12±0.39	0.09±0.34	H = 1.78 p = 0.18
F value	4.15±3.40	6.03 ±4.23	5.00 ±3.90	H = 6.76 p = 0.009
DMFT value	8.27±5.48	10.00±4.961	9.05±5.30	H = 3.82 p = 0.05
Ds value	5.15±6.04	5.14±4.63	5.15±5.43	H = 0.53 p = 0.46
Ms value	0.30±1.48	0.64±1.95	0.45±1.71	H = 1.78 p = 0.18
Fs value	6.25±5.19	9.51±8.45	7.72±7.01	H = 4.46 p = 0.03
DMF-S value	11.71±8.52	15.31±9.30	13.33±9.02	H = 4.94 p = 0.02
OHI - S	1.13±0.72	0.72±0.75	0.95±0.76	H = 9.46 p = 0.002
GI	0.10±0.09	0.09±0.08	0.09±0.09	H = 0.81 p = 0.36

H – test function value when using Kruskal-Wallis test
$\overline{X}$ – mean, SD – standard deviation

to the fact that neither all the interactions taking place between the separate elements of the integrated antioxidant defense system nor all the compounds with antioxidant properties are believed to have been identified, the measurement of the total antioxidant status facilitates acquisition of more reliable information [8-11, quoted after 12-14].

It therefore seemed purposeful to attempt to confirm the potential existence of a relationship between the total antioxidant status of unstimulated mixed saliva, the age of patients, oral hygiene status and the progression of dental caries.

MATERIAL AND METHODS

163 people aged 16-23 years were randomly selected for the study. The following inclusion criteria were used: generally healthy patients without any topical lesions that could affect the salivary glands function, not taking any medicines and non-smokers. 15 subjects refused to have their mixed unstimulated saliva collected, 21 subjects refused to have their oral health status checked, and 7 subjects did not conform with the instructions received beforehand. Thus, the remaining 120 subjects were ultimately included in the study (54 women and 66 men from Lublin). The mean age of the subjects was 18.40±1.74 years. The subjects were divided into 2 groups – adolescents (aged 16-18) and young adults (age 19-23). At the time of the study the subjects were healthy. The condition of hard dental tissues was evaluated using mean DMFT (Decayed, Missing, Filled Teeth Value) and DMF-S (Decayed, Missing, Filled Surface Value) values, as well as the values of the separate components of these values, using a dental mirror in artificial light. Evaluation of the state of gingiva was conducted using the Gingival Index (GI) [15, 16]. The health status of the soft dental tissues was evaluated by visual examination of oral mucosa. Oral hygiene was examined using the Simplified Oral Hygiene Index according to Green and Vermillion (OHI-S). Plaque Test preparation was used in order to stain the bacterial plaque and dental deposits. The preparation was applied on the buccal and labial surfaces of teeth [16,26,11,31] and on the lingual surfaces of teeth [36, 46] using an applicator (in the case of a missing tooth the preparation was applied on an adjacent tooth). After rinsing with water, the stained bacterial plaque and dental deposits were visualized using halogen lamp emitting a visible spectrum of 470-510 nm wavelength. Clinical examinations and laboratory tests were performed and the obtained results statistically analyzed. Oral hygiene status was evaluated using OHI-S (Oral Hygiene Index Simplified) Unstimulated mixed whole saliva was collected in the morning at least 1.5 hours after breakfast or beverage or tooth brushing. The subjects were instructed not to chew gum and not to use mouth fresheners. The saliva was centrifugated and than frozen at the temperature of -70^{0}C. Total antioxidant status was evaluated using Total Antioxidant Status (TAS) test [17] from Randox, in accordance with the manual.

Table 2. **Mean DMFT, DMF-S, D, M, and F values, Ds, Ms, Fs value, GI and OHI-S in reference to age.**

Examined value	Adolescents (age 16-18 years) N = 60	Young adults(age 19 – 23 years) N = 60	Statistical significance
	$\overline{X}$ ± SD	$\overline{X}$ ± SD	
D value	3.33±3.68	4.75±4.12	Z=2.21 p=0.02
M value	0.15±0.44	0.03±0.18	Z= -0.79 p=0.42
F value	3.61±3.30	6.38±3.98	Z=4.18 p<0.001
DMFT value	6.93±5.19	11.16±4.54	Z=4.70 p<0.001
Ds value	4.80±5.57	5.50±5.30	Z=1.40 p=0.15
Ms value	0.75±2.22	0.16±0.90	Z=-0.79 p=0.42
Fs value	5.45±6.21	10.00±7.08	Z=4.36 p<0.001
DMF-S value	11.00±9.25	15.66±8.22	Z=3.39 p=0.0006
OHI – S	1.19±0.75	0.70±0.70	Z=-3.39 p=0.0003
GI	0.08±0.09	0.11±0.09	Z=1.36 p=0.17

Z – test function value when using Mann-Whitney test
$\overline{X}$ – mean, SD – standard deviation

Determination of TAS depends on the reaction involving ABTS (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid), hydrogen peroxide and peroxidase. The latter catalyzes the reaction of ABTS single electron oxidation by hydrogen peroxide. The result is the occurrence of green-blue $ABTS^{\bullet+}$ cation radical. Spectrophotometrically measured inhibition of the process of sample greening after the preset incubation time is used to evaluate the antioxidant content in the sample. [11].

The obtained results were analyzed using tests evaluating their normal distribution. In the course of evaluation of the correlations between the respective parameters, Spearman Rang correlation coefficient and t-Student test function value were established. Other statistical methods used for comparing results were Kruskal-Wallis test and Mann-Whitney test. Logistic regression was also used in statistical analysis, as well as ROC (Receiver Operating Characteristic) curve. Statistical significance was established at the level of $p<0.05$. Calculations were performed using Statistica 9 software (Polish version).

RESULTS

On the basis of the clinical examinations performed, it was observed that there were no pathological lesions of the oral mucosa. No features of inflammation, discoloration of gingival or bleeding were found in any subjects. In course of the analyses of the patients' state of dentition, the mean DMFT and DMF-S values were established, as well as the values of the separate components of these values. Oral Hygiene Index Simplified and Gingival Index were also calculated for men, women, and the whole study population (*Tab. 1*).

Statistical analysis did not reveal significant differences between the state of dentition of the studied population described by the D, M, F, DMFT, Ds, Ms parameters, GI and the subjects' gender. However, statistically significantly higher values of such parameters as F value, Fs value and DMF-S were observed in women.

Statistical analysis facilitated the calculation of the mean DMFT and DMF-S values, as well as the values of the separate components of these values in the 2 age groups. OHI-S and GI were also calculated for adolescents and young adults (Tab. 2).

Statistical analysis showed that D, M, F, DMFT, Fs and DMF-S values were higher in subjects aged 19-23 years, and the correlations were statistically significant. On analyzing the OHI-S value, it was observed that the value was statistically significantly lower in young adults, which means that the oral hygiene status was better in this subgroup.

Mean TAS value in the study population was 0.82±0.26 mmol/l. In men, the level of the total antioxidant status of supernatant of saliva was 0.84±0.29 mmol/l. In the case of women, the value reached the level of 0.80±0.22 mmol/l. TAS median average value was 0.85 mmol/l (in the range of 0.26 mmol/l - 1.76 mmol/l). In the evaluation of the correlation between the subjects' age and the level of TAS in supernatant

Figure 1. **TAS level in mixed unstimulated whole saliva in reference to subjects' age.**

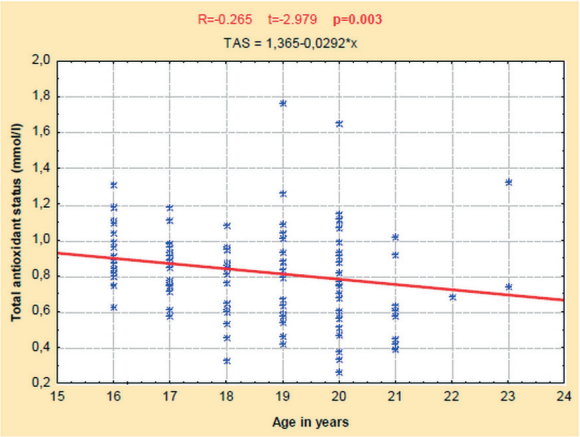


Figure 2. **TAS level and OHI – S in the study population.**

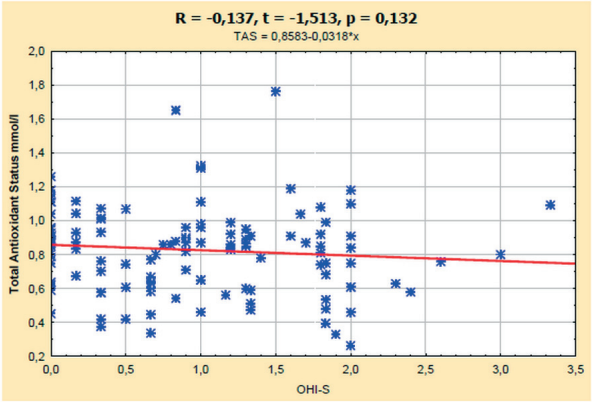


Figure 3. **Comparison of ROC curves for age, D index and OHI-S in the study population.**

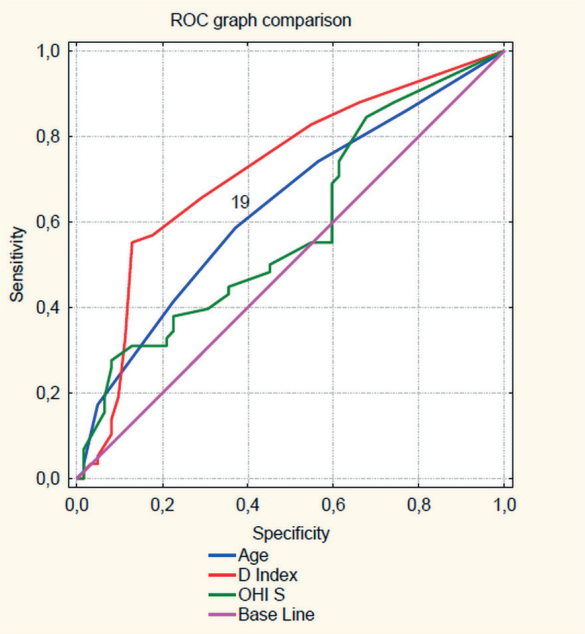
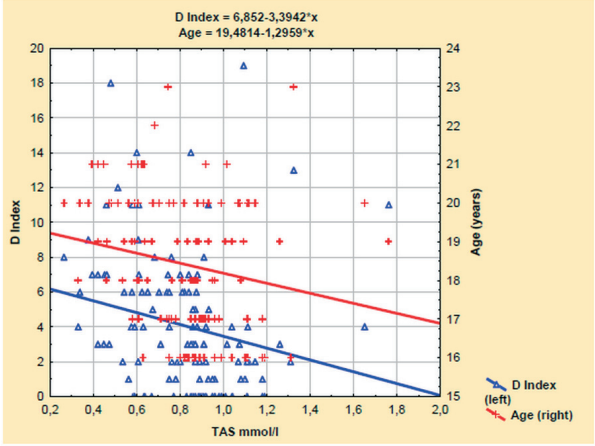


Figure 4. **The correlation between the total antioxidant status and D index and age.**



of saliva, the total antioxidant status value of supernatant of saliva was higher in the adolescent group. It was found that in the group of adolescents (age 16-18 years), TAS was 0.86±0.17 mmol/l, while in the group of young adults it was lower, with a value of 0.79±0.32 mmol/l. The correlation was statistically significant (H =4.93; p=0.02) (*Fig. 1*).

It was observed that with the increase of the OHI-S - the worsening oral hygiene status - TAS level was decreasing. However, statistical analysis did not show any statistically significant correlation between the TAS level and OHI-S (R = - 0.137, t = -1.513, p = 0.132) (*Fig. 2*).

In the group of subjects without tooth decay, the level of total antioxidant status was higher, with a value of 0.89±0.16 mmol/l, while in the group of subjects with tooth decay it was 0.80±0.28 mmol/l. It should be stressed that the correlation was statistically significant (H =6.40; p =0.011).

Logistic analysis allowed the establishment of a statistically significant correlation between age, D value, and the total antioxidant status. The chance prediction probability with TAS lower than median showed that the highest chance of prediction of the TAS level occurs first with reference to age, then the D value and OHI-S (Wald Chi-square = 4.56; p=0.03, Wald Chi-square = 3.48; p = 0.06; Wald Chi-square = 1.67; p = 0.19, respectively).

On the basis of ROC curves, it was observed that the highest specificity and sensitivity of the method of correct chance prediction for TAS in the range of 0 - 0.85 mmol/l (i.e. from 0 to median) was characteristic for subjects older than 19 years of age (postulated cut-off point), D value over 6 and OHI-S with a postulated cut-off point at the level of 1.832 (*Fig.3*).

Analysis of the correlation between the total antioxidant status (dependent variable) of supernatant of saliva and D value and age revealed statistically significant differences (F=4.433; p=0.013). The most influential feature of this model was the D value (*Fig. 4*).

DISCUSSION

The values of the total antioxidant status of saliva depend on the general health status of the patients, as well as the treatment used [11,12]. In our study group, none of the subjects was unhealthy, there were no active inflammatory processes of the marginal periodontium, or pathological lesions of the oral mucosa. Thus, it seems that caries was the main factor influencing the total antioxidant status of mixed unstimulated whole saliva. There are few publications concerning the correlation between the total antioxidant status of saliva and dental caries. The study by Tulunoglu *et al.*, consisted of 80 healthy children aged 7-15 years. The patients were divided into groups according to their age, gender, and the degree of activity of the carious process (a caries-free group and a group of patients with tooth decay). The authors found that the intensification of activity of the carious process coincided with the increase of antioxidant activity of saliva, with the exception of a group of girls aged 11-15 years [18]. In another study, Uberos *et al.* examined 126 patients with mixed dentition, among which 53.8% were girls (mean age 10.7±0.19 years) and 46.2% were boys (mean age 10.7±0.28 years). The authors measured Total Antioxidant Capacity (TAC) [19]. In literature, such terms as TAS (Total Antioxidant Status) and TAC (Total Antioxidant Capacity) are used interchangeably. It should be stressed that Total Antioxidant Status (TAS) is synonymous with Total Antioxidant Capacity (TAC) and describes the resultant ability of a given biological material to neutralize a given free radical [12]. In their study report, Uberos *et al.* presented separate levels of total antioxidant capacity for deciduous dentition and permanent dentition. The authors observed that in children who were caries-free the TAC was lower compared to patients with caries, although the difference was notatistically significant. The authors did not report the method they used for calculating TAC of mixed unstimulated whole saliva separately in relation to dmf (deciduous teeth) and DMF (permanent dentition) values. At the same time, it should be noted that the performed analysis did not specify the correlation between TAC and D value, but only between TAC and the values of dmf and DMF [19]. Studies performed by Preethi and Dodward *et al.* showed, that in children aged 7-14, the TAC level increased with intensification of dental caries activity. However, it should be pointed out that the majority of subjects in the study group had mixed dentition, which could have influenced the results [20,21]. Hedge *et al.* found, that the TAC level increased with the intensification of dental caries activity [22]. In studies conducted by Sikorska–Jaroszyńska *et al.,* it was established that in a group of adolescents in whom the D value was equal to or lower than 5, the total antioxidant status was the highest, and in the other 2 groups (with a D value in the range of 5 - 10 and with a D value in the range 10 - 15), TAS value decreased, while in the group of subjects with a D value over 15, the total antioxidant status was slightly higher [23]. In our study, the highest total antioxidant status was observed in the group of patients who had no tooth decay (D value = 0), and decreased in all the other groups. This phenomenon can probably be explained by the fact that dental plaque bacteria, and especially the components of the bacterial cell wall – liposaccharides - stimulate the immune system to produce inflammatory reaction mediators. Increased metabolism and a significant increase in oxygen consumption occurs in the neutrophil granulocytes and monocytes, a process referred to as 'respiratory burst'. The process is characterized by quick transformation of molecular oxygen into anion radical superoxide, from which hydrogen peroxide is derived. The latter compound is toxic, not only for bacteria, but also for the surrounding tissues and neutrophils. This, in turn, leads to a decrease of the total antioxidant status value [24-28].

Tulunoglu *et al.*, did not find a statistically significant correlation between total antioxidant status of saliva and subjects' age [18]. Hedge *et al.*, found that TAC increased with age [22]. In own studies, it was observed that TAS decreased with age (the difference concerned 2 age groups, 16-18 year olds and 19-23 year olds). Perhaps the difference between the study results can be explained by the fact that other age groups of patients were involved, and by the maturity of the integrated antioxidant defense system.

CONCLUSIONS

The conducted studies seem to allow for the following conclusions: TAS level in the supernatant of unstimulated whole saliva decreases with age; TAS level in the supernatant of mixed unstimulated whole saliva is the highest in patients without caries, and the oral hygiene status does not have significant influence on the total antioxidant status.

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