ORIGINAL RESEARCH

Oxidative Status of Saliva and Plasma in Diabetic Children

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ABSTRACT

Aim: The objective of this study was to determine the oxidative status of saliva and plasma in diabetic children, by analyzing advanced oxidation protein products (AOPPs) and total antioxidant capacity (TAC).

Materials and methods: Study included 60 patients with diabetes mellitus type I (DMT1) aged 12.45 ± 2.65 years, and 40 healthy age-matched controls. The AOPP and TAC of the plasma and saliva samples were determined using a commercial QuantiChrom™ Antioxidant Assay Kit (DTAC-100) for TAC determination, and Immunodiagnostic AG [enzyme-linked immunosorbent assay kit for AOPP].

Results: Values of salivary and plasma AOPP were lower in diabetic patients than in healthy controls, while value of TAC was clinically and significantly higher in plasma of controls, and clinically higher in saliva of healthy control group, compared with diabetic patients. Average value of hemoglobin A1c (HbA1c) was 7.58 ± 0.85%.

Conclusion: Results of this study showed that diabetes mellitus as a condition, with well-controlled HbA1c, has no influence on AOPP levels in saliva and plasma, while TAC levels of saliva and plasma are lower in diabetic patients, which means that DMT1 has an influence on the TAC.

Keywords: Advanced oxidation protein products, Children, Diabetes, Oxidative status, Plasma, Saliva, Total antioxidant capacity.

INTRODUCTION

Type I diabetes mellitus (DMT1) is a chronic illness characterized by the body's inability to produce insulin due to the autoimmune destruction of the beta cells in the pancreas. Onset most often occurs in childhood, but

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the disease can also develop in adults in their late 30s and 40s.[1]

Oxidative stress is central to the development of insulin resistance and diabetic complications. Oxidative stress plays a critical role in diabetic complications. Hyperglycemia leads to the overproduction of superoxides in mitochondria, and this increase in superoxide production activates several pathways that contribute to diabetic complications.[2] Hyperglycemia may directly cause increased generation of reactive oxygen species (ROS). Glucose undergoes auto-oxidation, which creates ∘OH radicals. Additionally, glucose nonenzymatically reacts with proteins, making Amadori products, which is followed by increased levels of AGEs.[3]

There is currently great interest in the potential role of increased oxidative stress in the development of diabetic complications. In patients with diabetes, oxidative stress seems to be caused by both increased production of plasma ROS and reduction of antioxidant defenses, with a consequent increase in lipid peroxidation and oxidized low-density lipoproteins (LDLs), which are more atherogenic than native LDLs.[4]

Plasma proteins, predominantly albumin and fibrinogen, undergo molecular modifications by oxidation, which can be measured as AOPPs, which were first identified by Witko-Sarsat et al as efficient oxidative stress biomarkers in uremic patients.[5]

The AOPPs are produced by the reaction between the chlorophora glycosides (HOCI/OCI-), neutrophil derivatives, and proteins, and are defined as cross-product dithyrosine protein. Increased AOPP levels indicate oxidative damage to protein.[6]

The TAC is a sum of endogenous and exogenous antioxidant systems. All systems involved in antioxidant activity act together and contribute to a better defense of the body toward oxidative stress. Because of this positive effect of some antioxidant systems, measurement of TAC gives a better insight into overall antioxidant activity.[7,8]

Research has shown that oxidative stress in diabetes is a cause, but, at the same time, the consequence of genesis and pathogenesis of diabetes. Oxidative stress has been demonstrated in the initial stage of diabetes and progressively increases with the development of the disorder.[9]

The aim of this study was to determine if there is a difference in salivary and plasma levels of AOPP and TAC in diabetic patients compared with healthy controls.

MATERIALS AND METHODS

The study included 60 children with DMT1 aged 8 to 18 years, and 40 healthy age-matched controls. The study was approved by ethical committee of Clinical Health Center of Zagreb, Croatia, where plasma and saliva samples were conducted. Written consent for the participation of children was obtained from their parents. Blood samples were collected from the cubital vein. Blood was centrifuged at 4,000 rpm for 10 minutes. Plasma samples were stored at −80°C. Tubes for vein collecting had heparin as anticoagulant (Vacuette, Greiner Bio-One, SAD). Samples of unstimulated saliva were collected and centrifuged at 4,000 rpm and stored at −80°C until the moment of determination of AOPP and TAC. Plasma and saliva AOPP concentration was determined by ELISA method, using commercial kits of Immunodiagnostic AG (ELISA Kit for AOPPs). The measurement principle is based on the spectroscopic analysis of modified proteins at a wavelength of 340 nm.

The absorbance was red on the microplate reader (STATFAX 2100, USA). The TAC of saliva and serum was determined by colorimeter, using QuantiChrom™ Antioxidant Assay Kit (DTAC-100). The method is based on Cu2 + reduction to Cu + by means of antioxidants in saliva or plasma samples. The resulting Cu + forms a colored complex whose intensity is read at 570 nm. The color intensity is proportional to the TAC of saliva or plasma. The absorbance is read on the Microplate Reader (STAT FAX2100, USA) reader. The values are determined in µm Trolox equivalents.[10]

Statistical Analysis

The results are presented as mean (x) and standard deviation (SD). Mann–Whitney's U test was used to test the significance of differences in continuous variables among the examined groups. The degree of correlation for the specified variables was determined by the Spearman method. A suitable multiple regression analysis model was used to determine the possible independent correlation of the examined variables. The value of $p < 0.05$ was taken as statistically significant. For all statistical analyses, Statistical Package for the Social Sciences for Windows, version 16.0 was used. Based on the interval and the level of reliability, in correlation with the population with DMT1 in the area where the study was conducted, all based on Prism Research, the size of sample 60:40 was statistically relevant.

RESULTS

The study included 100 examinees divided into experimental group (children with DMT1) and healthy control group. Experimental group covered 60 examinees, while control group had 40 examinees. Experimental group had 50% males and the same number of females.

The control group had the same statistical ratio. Using analysis of variance test, no statistically significant difference in the mean age of the examined subjects was established ($F = 0.014$; $p = 0.905$). The average age of the experimental group was 12.51 ± 2.78 years (the oldest 18 years, the youngest 8 years). The mean age of the control group was 12.45 ± 2.65 years (the oldest 17 years, the youngest 8 years). The mean value of hemoglobin A1c (HbA1c) in the blood of the experimental group was $7.58 \pm 0.85\%$.

There was no statistically significant difference in the average values of advanced plasma protein oxidation products compared with the investigated groups ($F = 2.272$; $p = 0.135$); however, a clinically significant difference was established. The mean plasma AOPP value of the experimental group was $8,801.02 \pm 2,373.94$ ng/mL, and in the control group, the clinically significant increase was $9,581.28 \pm 2,680.78$ ng/mL (Table 1).

There was statistically and clinically significant difference in the average values of advanced oxidation products of saliva protein compared with the investigated groups ($F = 4.628$, $p = 0.034$). The mean AOPP score of the subjects in the experimental group was $6,170.95 \pm 2,744.42$ pg/mL, and in the control group, subjects were statistically significant to a higher degree and it was $7,332.12 \pm 2,464.24$ pg/mL (Table 2).

Table 1: Average values of AOPPs in plasma

	N	X	SD	SEM	Minimun	Maximum
Experimental group	60	8801,02	2373,94	306,47	2910,20	14714,60
Control group	40	9581,28	2680,78	438,88	5094,10	14750,40
					F = 2,272; p = 0,135	

Table 2: Average values of AOPPs in saliva

	N	X	SD	SEM	Minimun	Maximum
Experimental group	60	6170,95	2744,42	357,29	1289,60	10000,00
Control group	40	7332,12	2464,24	389,63	1108,30	10000,50
					F = 4,628; p = 0,034	

Table 3: Average values of TAC in plasma

	N	X	SD	SEM	Minimun	Maximum
Experimental group	60	317,79	67,73	8,74	188,40	469,90
Control group	40	372,60	66,50	10,78	248,50	551,00
					F = 15,450; p = 0,001	

Table 4: Average values of TAC in saliva

|	N	X	SD	SEM	Minimun	Maximum
Experimental group	60	104,33	80,71	10,50	−45,32	328,23
Control group	40	115,91	46,20	18,37	−10,02	549,17
					F = 0,343; p = 0,560	

Table 5: Correlation between HbA1c values, age of respondents, and AOPP and TAC values in plasma and saliva of experimental group

Group			Age	AOPPp	AOPPs	TAKp	TAKs
Experimental	HbA1c	Rho	.414[a,**]	.123[a,**]	.777[a,**,*]	.304[a,**]	.173[a,**]
		p	.234	.735	.008	.392	.633
		N	60	60	60	60	60

"a"- level of significance (p < 0.05)
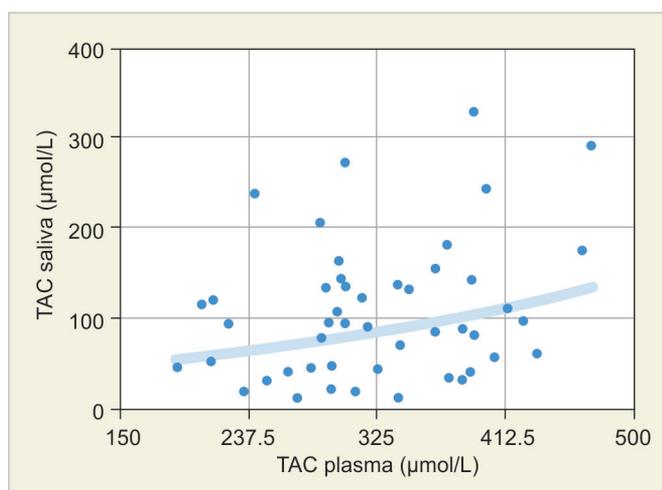
Graph 1: Correlation relations of TAC in saliva and plasma in subjects of the experimental group
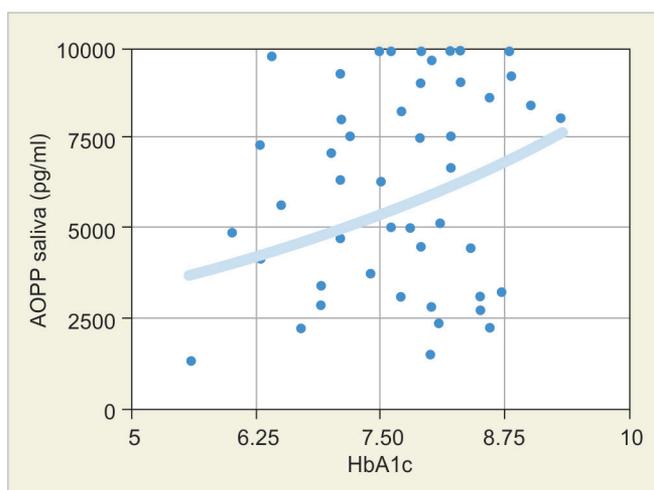
Graph 2: Correlation of HbA1c value and AOPP values in the saliva of experimental group

A statistically and clinically significant difference in the mean TAC in plasma compared with the investigated groups (F = 15.450; p = 0.001) was established. The mean value of TAC in the plasma of the subjects in the experimental group was 317.79 ± 67.73 µmol/L and in the control group, it was statistically significantly higher and was 372.60 ± 66.50 µmol/L (Table 3).

Although no statistically significant difference in the mean TAC in saliva compared with the investigated groups (F = 15.450; p = 0.001) was found, clinically, the control group respondents had a higher TAC value. The average value of TAC in the experimental group was 104.33 ± 80.71 µmol/L, and in the control group, the clinical significance was higher at 115.91 ± 46.20 µmol/L (Table 4).

Using Spearman's correlation with AOPP and TAC values in plasmas and pigs and the age of subjects in both study groups, a statistically significant positive correlation was found in TAC values in plasma and saline in the experimental group (rho = 0.671, p = 0.034), while in other parameters and in relation to both groups we did not establish a statistically significant correlation (Graph 1).

Statistical analysis of the correlation of HbA1c in the experimental group showed a statistically significant positive correlation between HbA1c and AOPP values in saliva, rho = 0.777; p = 0.008 (Table 5, Graph 2).

DISCUSSION

The lower values of AOPP in the experimental group in this study can be explained by the fact that most of the HbA1c subjects were within normal limits, so that the mean HbA1c score was 7.58 ± 0.85%; the fact was that children had diabetes mellitus or their parents/guardians were diabetic, and they, thus, paid more attention to the

quality of nutrition, and, in turn, had more input of anti-oxidants that affected plasma AOPP levels. In the studies available, higher values of plasma AOPP serum serotype in DMT1 patients and those of type II diabetes mellitus compared with healthy subjects were recorded.[11-14] What is characteristic of the above-mentioned research is that the respondents are older than the subjects in our research, and the HbA1c level in the investigators in the above-mentioned studies is also higher.

In the research of Pilar Martín-Gallán et al. From Barcelona who analyzed the plasma AOPP level in patients with DMT1 with and without complications, compared with the healthy control group, also recorded a higher plasma AOPP level in diabetic patients compared to the healthy control group and the correlation of HbA1c level And the height of AOPP was not recorded.[15]

The saliva is a combination of gingival cervical fluid, whose composition is similar to serum, and fluid released from the salivary glands. In correlation with the composition of saliva, the finding of lower values of AOPP in the experimental group was expected, since such finding was in plasma.[16]

Statistical analysis of correlation HbA1c in the experimental group showed a statistically significant positive correlation between HbA1c and AOPP in saliva, Diabetic hyperglycemia by the free radical production process, causing glication and oxidative degeneration of proteins. The emergence of alteration in function and structure of antioxidant protein enzymes can be due to non-enzymatic glitches, so detoxification of free radicals increases oxidative stress in diabetes.[17]

This study showed statistically and clinically significant difference in the mean values of TAC in plasma relative to the investigated groups. The results of our research correlated with the vast majority of available studies, where TAC in diabetic patients decreased compared to healthy subjects,[18] as well as the researches mentioned in, the review article, reduced plasma TAC values In persons with DMT1. In the research Đorđrvić et al, TAC values were not significantly correlated with glucose metabolism markers (glycemia and HbA1C), nor with the duration of the disease. This is explained by time-dependent changes in individual constituents of TAC, as described in litherature, especially when it comes to antioxidant enzymes, which can also be reflected in total antioxidant status.[19]

By analyzing the TAC in saliva, no statistically significant difference was found in mean values compared to the investigated groups, clinically, the control group respondents had a higher value of TAC. In Pendyal et al,[20] which describes increased salivary TAC in women with gestational diabetes but is also characterized by increased carious activity, which may be associated with increased

salivary TAC in this test group. Also in the research carried out by Reznick et al, The results showed that oxidative stress in diabetic patients was accompanied with increased TAC in bile and blood.[21]

Using Spearman's correlation with TAC values in plasma and stomach and on the age of subjects in both study groups, a statistically significant positive correlation was found in the TAC values in plasma and saline in the experimental group. Statistical analysis of the relationship between HbA1c and saline and plasma TAC showed no correlation. In the research of Pieme et al,[22] with the increase in glycolysis HbA1c, a decrease in TAC values was observed.

CONCLUSION

Results of this study showed that diabetes mellitus as a condition with well-controlled HbA1c has no influence on AOPP levels in saliva and plasma, while TAC levels of saliva and plasma are lower in diabetic patients, which means that DMT1 has influence on TAC.

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