Salivary oxidative analysis and periodontal status in children with atopy

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Abstract: *Background and aims:* Prevalence of atopic diseases is progressively increasing in children with unclear clinical picture in oral cavity. This study aimed to find correlation between the total antioxidant and periodontal status in the saliva of periodontally compromised with atopy. *Materials and methods:* The groups consisted of patients of atopic diseases and gingivitis (ADG), of atopic diseases without gingivitis (AD), and healthy controls (HC). The level of gingival inflammation was evaluated using the sulcus bleeding index (SBI) reported by Mühlemann and Son. Glutathione (GSH), malondialdehyde (MDA), and superoxide dismutase (SOD) were estimated in saliva. *Results:* The mean salivary MDA levels of group HC was significantly lower compared with group ADG and AD ($P < 0.05$). Similarly, in the case of salivary GSH, marker levels revealed a significant decrease ($P < 0.05$) when mean values in the control group (5.12 ± 0.66 μmol/L) were compared with the ADG (2.31 ± 0.44 μmol/L) and AD groups (2.69 ± 0.56 μmol/L). The SOD activity was significantly lower in the ADG and AD groups compared with HC. *Discussion:* As there was no significant difference in the level of SOD concentration between groups, we suggest that antioxidant imbalance is primarily explained by atopic disease. *Conclusion:* The results showed changes in antioxidant balance in children with atopy.

Keywords: gingivitis, antioxidants, atopy, oral cavity, children

Introduction

Prevalence of atopic diseases, such as bronchial asthma, allergic rhinitis, and atopic dermatitis, is progressively increasing in children all over the world. The International Study of Asthma and Allergies in Childhood has mapped out a significant variation and increase in the prevalence of childhood allergies across many countries [1–3].

On the other hand, periodontal problems are often observed in the age of children as consequences of different processes [4] and also atopic conditions [5, 6]. As a result, research of the pathogenesis of atopic changes becomes goal for allergists, internists, pediatricians, and other specialists.

Lipid peroxidation (LPO) has been implicated in the pathogenesis of several pathologic disorders, including periodontal disease. Studies investigating the use of saliva as a diagnostic fluid have a long history. This non-invasive approach is limited not only to the diagnosis of oral diseases, but also many systemic diseases, such as different types of cancers, cardiovascular diseases, immunologic syndromes, and hereditary deficiencies, can be studied with the aid of salivary diagnostics [7, 8].

Saliva is an accessible biofluid that contains components derived from the mucosal surfaces, gingival crevices, and tooth surfaces of the mouth. Saliva also contains microorganisms that colonize the mouth and other exogenous substances and so can potentially provide an insight into the relationship of the host with the environment [9].

Currently, there is growing interest in the linkage between antioxidants and periodontal disease. Antioxidants include superoxide dismutase (SOD), uric acid, ascorbic acid, α-tocopherol, glutathione (GSH), and albumin. SOD, an important antioxidant, catalyzes the dismutation of the superoxide anion, defending the cells against the hazardous effects of reactive oxygen species [10]. SOD has also been localized within the human periodontal ligament and may represent an important defense mechanism within gingival cells against superoxide release [11].

Malondialdehyde (MDA) is one of many low molecular weight end products of LPO [12]. As MDA is a highly toxic aldehyde molecule, it is considered to be an ideal marker of LPO [13].

This study aimed to find correlation between the total antioxidant and periodontal status in the saliva of periodontally compromised and not compromised patients with atopy and healthy controls (HC) to assess diagnostic utility of MDA, GSH, and SOD.

Materials and Methods

A cross-sectional study was conducted on 141 subjects, who were in the age group of 12–18 years reporting to the Department of Pediatric Dentistry. Among the 141 subjects, 55 HC were formed (group HC). The atopy group consisted of 126 patients with the following conditions: 76 patients of atopic diseases and gingivitis (group ADG) and 50 patients of atopic diseases without clinical manifestations of gingivitis (group AD). Patients with systemic illness except bronchial asthma, atopic dermatitis, allergic rhinitis, previous history of malignancy, or history of antioxidant medication were excluded. Ethical approval was obtained from the Ethical Committee of Kharkiv National Medical University and informed consent was obtained from all the study subjects (their parents). Unstimulated saliva was collected from the study subjects between 9:00 a.m. and 12:00 p.m. to avoid diurnal variation. The subjects were requested not to eat, drink, perform oral hygiene activities, or chew 60 min prior to the saliva collection procedure. The subjects were then seated on the dental chair and asked to spit in a graduated container every 1 min till 5 mL of saliva was obtained. During saliva collection, subjects were instructed not to speak or swallow. The salivary samples were stored at a temperature of −20 °C. MDA, the marker of LPO, was estimated as thiobarbituric acid-reactive substances [14]. To 1 mL of sample, 1.5 mL of 0.8% thiobarbituric acid (TBA) was added. Then, 1.5 mL of acetic acid and 0.4 mL of 8.1% sodium dodecyl sulfate were added. Distilled water was added to make the mixture up to 5 mL, and it was then placed in a hot water bath at 95 °C for 1 h. The mixture was allowed to cool, and 5 mL of pyridine and n-butanol (15:1, v/v) along with 1.0 mL of distilled water were added. The mixture was vortexed and centrifuged at 4,000 rpm for 10 min. With a spectrophotometer, absorbance of the upper layer was measured at 532 nm against distilled water. When allowed to react with TBA, MDA formed a colored complex that was measured using the spectrophotometer.

GSH was estimated using the method of Beutler et al., based on reduction of 5,5′-dithiobis(2-nitrobenzoic acid) (DTNB) by GSH. The technique employs meta-phosphoric acid for protein precipitation, and the supernatant obtained on reaction with DTNB resulted in a yellow colored derivative that was assayed with a spectrophotometer at 412 nm [15].

SOD activity was analyzed by the reduction of nitroblue tetrazolium by superoxide, which formed formazan and spectrometrically detected at 560 nm using Genesys 10 UV and expressed in terms of U/mL [16].

The level of gingival inflammation was evaluated using the sulcus bleeding index (SBI) reported by Mühlemann and Son. The SBI was recorded on six tooth surfaces (mesiobuccal, buccal, distobuccal, mesiolingual/palatal, lingual/palatal, and distolingual/palatal). The scores for the SBI were: 0 – no bleeding; 1 – bleeding on probing with no change in color and no swelling; 2 – bleeding on probing with a change in color and no swelling or macroscopic edema; 3 – bleeding on probing with a change in color and edematous swelling; 4 – bleeding on probing with a change in color due to inflammation, edematous swelling with ulceration; and 5 – spontaneous bleeding, changes in color and marked swelling with ulceration.

Statistical analysis of the data was performed by using the Microsoft Office software. Data were statistically analyzed by Microsoft Excel using unpaired *t*-test for significance of differences between each group and regression analysis by least squares method, which is a statistical process for estimating the relationships among variables. *P* values of less than 0.05 were considered to be statistically significant. The normality of data was checked before the statistical analysis was performed.

Results

The mean salivary MDA levels of group HC was 3.81 ± 0.83 μm/L. The mean salivary levels of group ADG was 6.87 ± 0.91 μm/L and group AD was 5.96 ± 0.79 μm/L.

The mean salivary MDA levels of group HC was significantly lower compared with group ADG and AD ($P < 0.05$). The mean salivary MDA levels of group ADG and group AD did not have significant difference *(Table I)*.

Similarly, in the case of salivary GSH, marker levels revealed a significant decrease ($P < 0.05$) when mean values in the control group (5.12 ± 0.66 μmol/L) were compared with the ADG group (2.31 ± 0.44 μmol/L) and AD groups (2.69 ± 0.56 μmol/L) *(Table I)*, but the change was not significant ($P > 0.05$) when AD and ADG patients were compared *(Table I)*.

The SOD activity was significantly lower in the ADG and AD groups compared with healthy children. In saliva, the SOD concentration was equal to 3.3 ± 0.41 U/L in group of atopy- and gingivitis-infected children and 3.27 ± 0.4 U/L in group of atopy children. There was no significant difference in the level of SOD concentration between these two groups.

Table I Evaluation and comparison of mean ± SD values for salivary MDA and GSH in ADG, AD, and HC patients

Groups	MDA (μmol/L)	GSH (μmol/L)	SOD (U/L)
ADG	6.87 ± 0.91	2.31 ± 0.44	3.3 ± 0.41
AD	5.96 ± 0.79	2.69 ± 0.56	3.27 ± 0.4
HC	3.81 ± 0.83	5.12 ± 0.66	5.26 ± 0.57

AD: atopic diseases without gingivitis; ADG: atopic diseases and gingivitis; GSH: glutathione; HC: healthy controls; MDA: malondialdehyde; SD: standard deviation; SOD: superoxide dismutase
$P < 0.05$ were considered to be statistically significant

The mean SBI index of group ADG was 3.42 ± 0.42. The mean SBI level in AD group was equal to 0.55 ± 0.52. The HC group level was 0.57 ± 0.6.

The comparison of mean values of SBI shows significant higher level of inflammation in periodontal tissues in ADG group of patients compared with AD and HC groups *(Table II)*.

For estimating the relationships between SBI index and level of MDA in the ADG group, regression analysis was done *(Table III)*. As R^2 is equal to 0.432358, there is very weak correlation between level of MDA level and periodontal problems.

The regression analysis method demonstrates that regression function cannot be built in the group of ADG patients. The value of R^2, generalized R^2, and multiple R prove that an inflammation in periodontal tissues that is measured by SBI index does not correlate to the level of MDA *(Table III)*.

The similar situation is observed, if SBI index (in regression function Y) and MDA (in regression function X) level are analyzed in AD group. Multiple R is 0.012526, R^2 is 0.000157, and generalized R^2 is −0.02067.

The regression statistics in AD group shows that there is no correlation between SBI index and MDA level. In clinical practice, it means that level of MDA cannot be used for SBI prognosis. Increased level of MDA is not always revealed in patients with clinical signs of gingivitis, but can be observed in preclinical stage of development of gingivitis.

There was no correlation between SBI (in regression function Y) and GSH (in regression function X) level in AD and ADG groups of patients *(Table IV)*.

In AD group, R^2 is equal to 0.031482, generalized $R^2 = 0.011304$, multiple $R = 0.177431$, which means that there is no correlation between inflammation in periodontal tissues and level of GSH. In ADG group, we observed similar situation. Multiple R was equal to 0.090662, $R^2 = 0.00822$, and generalized $R^2 = 0.00518$.

As results of regression analyses showed there was no correlation between the SBI and the SOD activity *(Table V)*.

Table II Evaluation and comparison of mean ± SD values for SBI index

Groups	SBI
ADG	3.42 ± 0.42
AD	0.55 ± 0.52
HC	0.57 ± 0.6

AD: atopic diseases without gingivitis; ADG: atopic diseases and gingivitis; HC: healthy controls; SBI: sulcus bleeding index; SD: standard deviation
$P < 0.05$ were considered to be statistically significant

Table III Regression analyses of SBI and MDA correlation in AD and ADG group

	Regression statistics	
Group	AD	ADG
Multiple R	0.012526	0.657539
R^2	0.000157	0.432358
Generalized R^2	−0.02067	0.424687
Standard deviation	0.527224	0.317763
Observations	50	76

AD: atopic diseases without gingivitis; ADG: atopic diseases and gingivitis; MDA: malondialdehyde; SBI: sulcus bleeding index

Table IV Regression analyses of SBI and glutathione correlation in AD and ADG group

	Regression statistics	
Group	AD	ADG
Multiple R	0.177431	0.090662
R^2	0.031482	0.00822
Generalized R^2	0.011304	−0.00518
Standard deviation	0.518899	0.420024
Observations	50	76

AD: atopic diseases without gingivitis; ADG: atopic diseases and gingivitis; SBI: sulcus bleeding index

Table V Regression analyses of SBI and SOD correlation in AD and ADG groups

	Regression statistics	
Group	AD	ADG
Multiple R	0.107837008	0.326201613
R^2	0.01162882	0.106407492
Generalized R^2	−0.008962246	0.094331918
Standard deviation	0.524190256	0.398690311
Observations	50	76

AD: atopic diseases without gingivitis; ADG: atopic diseases and gingivitis; SBI: sulcus bleeding index; SOD: superoxide dismutase

In the ADG group, R^2 is equal to 0.106407492, multiple $R = 0.326201613$, and generalized $R^2 = 0.094331918$. Almost similar situation is observed in the AD group: R^2 is equal to 0.01162882, multiple $R = 0.107837008$, and generalized $R^2 = -0.008962246$.

Discussion

Regression analysis is widely used for prediction where its use has substantial overlap with the field of machine learning. Regression analysis is also used to understand which among the independent variables are related to the dependent variable, and to explore the forms of these relationships. In restricted circumstances, regression analysis can be used to infer causal relationships between the independent and dependent variables [17].

Of the many biological targets of oxidative stress, lipids are the most commonly involved class of biomolecules. Lipid oxidation gives rise to a number of secondary by-products. MDA is the principal and most widely studied product of polyunsaturated fatty acid peroxidation. Its interaction with DNA and proteins has often been referred to as potentially mutagenic and atherogenic [8, 18, 19].

The changes in the salivary antioxidant enzymes suggest that saliva may be appropriate marker for the prognosis of oral diseases compared with the conventional invasive serum antioxidant enzyme [20].

In this study, a significantly higher salivary MDA level was observed in the AD and ADG groups, when compared with the HC group. This is in accordance with recent studies where scientists found significantly elevated levels of LPO products when compared with controls [21–24].

Chapple et al. [25] showed that patients with compromised periodontium had a low total antioxidant status. Other group of scientists [26] reported that the level of antioxidants was lower in the peri-implant disease group than HC. There are studies [27] that proved absence of difference in the antioxidant level in the saliva between patients with periodontal disease and HC.

As there was no significant difference in the level of SOD concentration between groups of atopy children with gingivitis and health periodontium, we suggest that antioxidant imbalance is primarily explained by atopic disease. The changes in antioxidant balance in connection with nitric oxide synthase metabolism disturbance [28] could be an important link of pathological process appearance in oral cavity in atopic diseases that could be used for creation of adequate methods of treatment [29] for improvement of dental status population [30].

This study suggests that salivary MDA and GSH could serve as a potential diagnostic marker in children with atopy. However, regression analysis showed that there is no correlation between inflammation in periodontal tissues and level of antioxidants. The obtained data prove that there is an oxidative misbalance in children with atopy. The level of MDA is increased and the level of GSH is decreased in group of patients with atopy and it does not depend on the presence of gingivitis in these groups.

Conclusions

This study has been done to evaluate salivary LPO and periodontal status and to find the correlation between antioxidant level in unstimulated saliva of children and inflammation in periodontal tissues. The results showed changes in antioxidant balance in children with atopy. The regression analysis, which was done, showed absence of correlation between level of antioxidants and inflammation in periodontal tissues.

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