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Total Salivary Antioxidant Capacity in a group of Egyptian Children under Active Orthodontic Treatment

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Abstract:

Objectives: *To determine and compare Total Antioxidant Capacity (TAC)of saliva in children undergoing active orthodontic treatment with removable and fixed orthodontic appliances before and after treatment.*

Methods: *Total antioxidant capacity of saliva was investigated in 46 healthy children aged 8-14 years. The children divided into three groups. Group A treated with fixed orthodontic appliance , group B treated with removable orthodontic appliance and group C receive no treatment. Saliva samples(2mm) were collected. in different time intervals: before the orthodontic treatment, three months and six months after treatment. Salivary total antioxidant capacity was measured by spectrophotometric method.*

Results: *The results indicated that TAC values in the saliva of the children treated with fixed and removable orthodontic appliances had statistically higher values after treatment than before . The average values of TAC values in the saliva of the children treated with fixed appliances after treatment were significantly higher than those treated with removable appliances.*

Conclusion: *There was a statistically significant positive correlation between total antioxidant capacity and active orthodontic treatment.*

Key words: *Total Antioxidant Capacity, Fixed orthodontic appliances, Removable orthodontic appliances.*

I. Introduction:

Cells in the human body use oxygen as a fuel to breakdown proteins and fats to produce energy. It also utilizes oxygen to help the immune system, destroys foreign substances and combats diseases. The byproduct of this process and other metabolic process can lead to development of molecular agents that react with body tissues in a process called oxidation that can damage healthy cells of the body. The by-product of this natural process of energy generation is called free radicals[1].

Free radicals are electrically charged molecules, i.e., they have an unpaired electron, which causes them to seek out and capture electrons from other substances in order to neutralize themselves. Antioxidants are capable of stabilizing, or deactivating, free radicals before they attack cells.[2] An antioxidant is a molecule capable of inhibiting the oxidation of other molecules. Oxidation is a chemical reaction that transfers electrons from a substance to an oxidizing agent. Free radicals produced from oxidation reactions can start chain reactions that damage cells. Antioxidants terminate these chain reactions by removing free radical intermediates, and inhibit other oxidation reactions. [3]

Antioxidants are absolutely critical for maintaining optimal cellular and systemic health and well-being. Hence body maintains complex system of enzymatic antioxidants such as catalase, SOD, peroxidases etc. and non enzymatic antioxidants such as Vit C, E & glutathione etc. Oxidative stress occurs as a result of increased oxidative metabolism.[4]

In normal condition, our cell can be capable of preventing free radical induced diseases by generating its own endogenous antioxidants or by taking them from food[5].

Saliva is rich in antioxidants, mainly uric acid, with lesser contributions from albumin, ascorbate , glutathione and salivary urate [6].

The antioxidant defense system are highly complex. It is essential to evaluate the amounts and/or the activities of the different antioxidants when assessing antioxidant status, several methods have been developed for estimating the antioxidant activity in saliva, for these reasons, research is now directed towards assays that evaluate the so-called ‘‘Total antioxidant capacity’’ of biological fluids, including saliva[6,7].

Aim

The aim of this study was to assess:

1.Determination of total antioxidant capacity in children undergoing orthodontic treatment with two different orthodontic appliances over 3 month and six month evaluation periods.

2. Evaluation of total antioxidant capacity changes between group A and group B after treatment.

II. Material and methods:

Study design

This is a prospective clinical study comparing the TAC levels in children treated with removable orthodontic appliances and children treated with fixed orthodontic appliances before, three and six months after treatment and the TAC levels between both groups after treatment.

The study was designed and carried out in a private clinic in collaboration with Department of Medical Biochemistry in National Research Centre.

Study population

A total of forty six children were selected from those attended the Outpatient Clinic of a private orthodontic clinic. The age group of the children ranges from eight to fourteen years. Sex distributed in groups (6 female & 8 male in group A and B,10 female and 8 male in group C). All children included in this study were free from any apparent genetic disorders or dental anomalies, apparently healthy, free from any systemic or chronic diseases. They were caries free and have good oral hygiene.

The children were divided into three groups (two study groups and one control group) :

1- Group A containing 14 children treated with fixed orthodontic appliances.
2- Group B containing 14 children treated with removable orthodontic appliances.
3- Group C containing 18 children receive no treatment.

Collection of saliva:

Subjects were informed in advance not to eat or drink (except for water) or chew gum for two hours before saliva collection. Obtaining an informed consent from the parents , unstimulated saliva was collected from each child in the morning between 10 to11 AM.

The samples were taken in different time intervals: Before treatment, 3 month after treatment and 6 month after treatment.

All salivary samples were collected in sterile containers, saliva was collected by passive drool method; the participant was asked to rinse with water, accumulate the saliva in the floor of the mouth and then spit it into a pre-labeled sterile container. Then 2 ml of saliva was taken by a dropper and stored in test tubes. Salivary samples were stored on dry ice and were carried immediately to the Department of Medical Biochemistry in National Research Centre where they kept frozen at the deep freezer (Samsung RZ90EERS) at -20^{o} C.

Methods of detection of TAC in saliva

Salivary total antioxidant capacity was estimated by an adaptation of the ABTS assay(2,2-azino-di(3-ethylbenzthiazoline-6-sulphonate))using spctrophotometer[8].

Statistical methods

The collected data were tabulated, and statistically analyzed using IBM SPSS statistics (Statistical Package for Social Sciences) (V. 22.0) software version 22.0,IBMCorp., USA, 2013.

Descriptive statistics were presented for quantitative data as mean ± (standard deviation SD), minimum, maximum and range, while it was presented for qualitative data as number and percentage.

Inferential analyses were done for quantitative variables using independent t-test in cases of two independent groups with parametric data and paired t-test in cases of two dependent groups with parametric data. In qualitative data, inferential analyses for independent variables were done using Chi square test for differences between proportions.

The level of significance was taken at P value < 0.050 is significant, otherwise is non-significant. The p-value is a statistical measure for the probability that the results observed in a study could have occurred by chance.

The sample size was calculated based on the results of previous studies [3] where the significance level was set at $p < 0.05$ and the confidence level (power) to be 0.90, a number of 14 subjects in each group would be sufficient.

III. Results:

Mean value of TAC in saliva increased significantly after 3 and 6 month of treatment in group A and group B compared to before (0.54±0.10,1.45±0.30 and 1.53±0.31),(0.54±0.10,0.81±0.13 and 0.89±0.12)mMol/L

respectively. While mean value of TAC in group C didn't show any significant changes at the three time intervals (0.54±0.10, 0.55±0.11and 0.54±0.10) mMol/L respectively.

Table (1): Comparison between groups regarding TAC (mMol / L)

Group	Measure	Group A (N=14)	Group B (N=14)	Group C (N=18)	^$P_{A/B}$	^$P_{A/C}$	^$P_{B/C}$
Before	**Mean±SD**	0.54±0.10	0.57±0.12	0.54±0.10	0.435	0.946	0.368
	Range	0.41–0.70	0.41–0.79	0.41–0.71			
3 months after treatment	**Mean±SD**	1.45±0.30	0.81±0.13	0.55±0.11	**<0.001 ***	**<0.001***	**<0.001***
	Range	1.00–1.93	0.50–1.00	0.39–0.70			
6 months after treatment	**Mean±SD**	1.53±0.31	0.89±0.12	0.54±0.10	**<0.001***	**<0.001***	**<0.001***
	Range	1.03–1.92	0.67–1.13	0.40–0.70			
Difference between 3ms and Before	**Mean±SD**	0.91±0.23	0.24±0.10	0.01±0.04	**<0.001***	**<0.001***	**<0.001***
	Range	0.57–1.30	-0.01–0.36	-0.04–0.09			
	#$P_{Before/3m}$	**<0.001***	**<0.001***	**<0.001***			
Difference between 6ms and Before	**Mean±SD**	0.99±0.25	0.31±0.11	0.00±0.03	**<0.001***	**<0.001***	**<0.001***
	Range	0.60–1.32	0.02–0.46	-0.07–0.04			
	#$P_{Before/6m}$	**<0.001***	**<0.001***	**<0.001***			
Difference between 6ms and 3ms after treatment	**Mean±SD**	0.08±0.09	0.07±0.06	-0.01±0.04	0.924	**0.002 ***	**<0.001***
	Range	-0.19–0.19	-0.04–0.17	-0.09–0.07			
	#$P_{3m/6m}$	0.239	0.680	0.469			

Negative values indicate reduction, ^Independent t-test, #Paired t-test, *Significant.

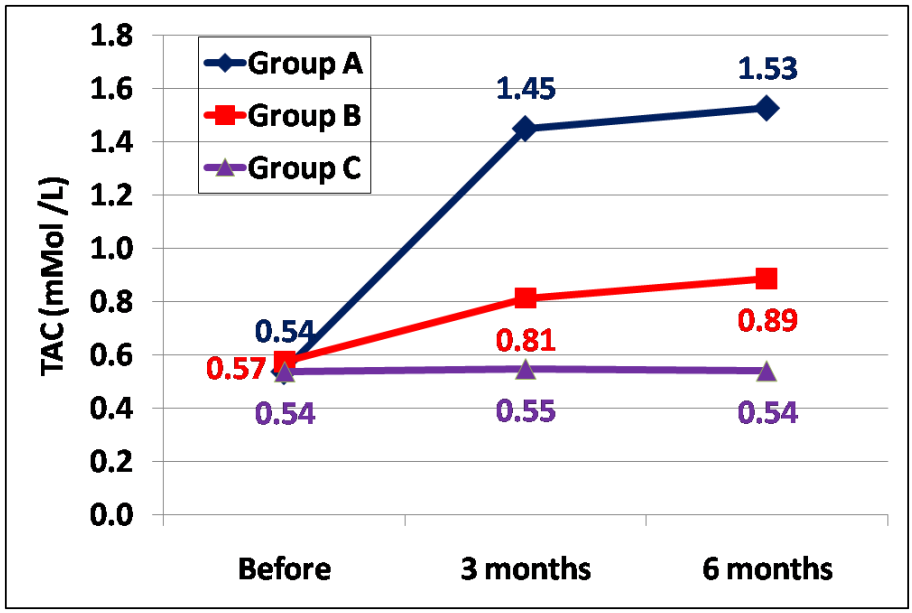

Figure (1): Comparison between groups regarding TAC

Table (1) and figure (1) show that: No significant difference between groups regarding TAC before treatment. After treatment, Group A had the highest TAC level, followed by group B and least among group C. The mean value of TAC levels were significantly higher in both groups (A&B) 3 and 6 months after treatment as compared with before as well as with group C. Degree of elevation was significantly higher in group A than group B. After 6 month; there was no significant change in TAC from 3 month level in both groups (A and B) but there were significant change in TAC between these groups and group C.

Table (2): Comparison between groups regarding age (years), and sex

Group	Measure	Group A (N=14)	Group B (N=14)	Group C (N=18)	^$P_{A/B}$	^$P_{A/C}$	^$P_{B/C}$
Age (years)	**Mean±SD**	10.8±1.3	10.7±1.4	10.8±1.4	^0.890	^0.987	^0.899
	Range	7.0–13.0	8.0–13.0	8.0–13.0			
Sex (n, %)	**Male**	6 (42.9%)	6 (42.9%)	8 (44.4%)	#1.000	#0.928	#0.928
	Female	8 (57.1%)	8 (57.1%)	10 (55.6%)			

^Independent t-test, #Chi square test

There was no statistically significant correlation between total antioxidant capacity and age and sex as shown in table (2) **.**

IV. Discussion:

Antioxidants are an important part of our diet, and these systems, together with intracellular antioxidants and enzymatic systems, impede inflammation, infections, and tumor formation.[9] Some types of inflammation, especially periodontal disease, have been associated with reduced salivary antioxidant status and increased oxidative damage within the oral cavity.[10] Oxidative stress has been defined as a disturbance in the oxidant-antioxidant balance, resulting in potential cell damage. It is known that oxidative stress plays a role in the pathogenesis of cancer and many inflammatory diseases, such as diabetes mellitus, atherosclerosis, hypertension, and obesity.[11] Antioxidant defense systems have been found to prevent the formation of free radicals and to reduce cell damage.

Children were examined clinically and divided into three groups according to type of orthodontic appliance treatment to clarify the relationship between this active treatment and total antioxidant capacity of saliva.

Saliva was collected by the passive drool method, passive drool is highly recommended because it is both cost effective and approved for the use with almost all analytes also it was easy to obtain the child's cooperation. Unstimulated saliva was collected because stimulated saliva increases the salivary flow rate which in turn decreases the concentration of TAC[12].

Each subject was asked to accumulate the saliva in the floor of the oral cavity and asked to spit it into a pre-labeled sterile container and 2 ml saliva was collected. Subjects were informed in advance not to eat or drink (except for water) two hours before saliva collection as this period has been reported to have less variations in the flow rate and composition of saliva and to minimize possible food debris and stimulation of salivary secretion.[13,14].

Total antioxidant capacity of saliva was evaluated rather than individual antioxidant which was expensive as well as misleading and less representative of the whole antioxidant status.[6,15] Thus the total salivary antioxidant capacity was determined colorimetrically by using the spectrophotometer.

To´thova´ et al.[16] found that salivary antioxidant status in children was related to oral hygiene and periodontal status. To assess the results with accuracy, all of the patients who were selected for this evaluation had no fillings, no caries, and no periodontal disease. We found that the levels of TAC in control group didn't change over time "no statistical differences between the three time points of treatment". These may be related to a lack of fillings, lack of caries and good oral hygiene. Also they didn't expose to any orthodontic treatment that can affect TAC directly or indirectly.

The results showed that there was no statistical significant correlation between total antioxidant capacity with age and gender which may be explained by the elevated free radical generation with aging so salivary antioxidants would be exhausted in reaction with the elevated free radicals which come in accordance with Parvinen and Lama.[17], Heft and Baum.[18], Hershkovich et al.[19] and Dipanshu et al.[20]

In the current study, there was no significant differences of the average values of TAC for group A , group B and Group C before treatment. The average of TAC values become significantly elevated with time in the study groups only. There were significantly higher values of TAC for both groups (A and B) 3 and 6 months after treatment. After treatment the average values of TAC were significantly higher in group A than the values recorded in group B at the two different time points after treatment.

This finding can be explained by the effect of using orthodontic appliances in the treatment of the various dento-maxillar anomalies which most frequently presume the application of high intensity forces, non-physiological, which always will produce an inflammatory response localized around the tooth or the teeth subjected to displacement. The presence of an inflammatory process at this level will produce an increased synthesis of free radicals, secondary followed by the oxidative stress[21].

The appliances in orthodontic treatments are fabricated from different alloys. These alloys include nickel, cobalt, and chromium. These metallic ions and monomers released from removable and fixed orthodontic appliances have harmful effects on the adjacent oral tissues. In addition, it has been reported [22] that the mucosa of the mouth may absorb residual monomers and that these monomers may be taken into the digestive system by means of the saliva.

Orthodontic appliances includes bands, brackets and wires which are made up of stainless steel with the composition of roughly nickel and chromium and other trace metals. Corrosion of orthodontic appliances can have a toxic effect on surrounding oral tissues [23]. It has been proved by many research studies that orthodontic appliances release metal ions through discharge of electrogalvanic currents with saliva acting as medium for continuous erosion over period of time. [24] Leached metal ions from orthodontic appliances can lead to metal mediated generation of free radicals which causes various chemical alterations in DNA bases, increased lipid peroxidation and altered homeostasis of calcium and sulfhydryls [25]. Lipid peroxides formed by assault of

radicals on polyunsaturated fatty acids residues of phospholipids can further react with metals finally producing mutagenic and carcinogenic malondialdehyde, 4- hydroxynonenal and other exocyclic DNA adducts (ethanol or propane adducts) [26]. Nickel and chromium can yield free radical species straightaway from molecular oxygen to produce highly toxic radical. [27]. The placement of orthodontic bands, brackets and wires increases the risk of plaque accumulation, periodontal disease, caries and endogenously synthesized antioxidants [28].

During stress conditions the cell turns on the gene expression of certain anti stress enzymes and antioxidants molecules like Glutathione and Metallothionein as defense mechanisms to neutralize free radicals and prevent the highly reactive molecules from further damaging the cell and its component.[29]

Orthodontic composites are often used by orthodontists for the bracket-banding process in children with both primary and permanent dentition. However, some components of the orthodontic composites may be released into the oral environment and saliva during fixed-appliance treatment ,and even following polymerization.[30] The release of these components and their diffusion may cause various adverse effects in the organism,such as allergic reactions, systemic toxicity, cytotoxicity, mutagenicity, and carcinogenicity.[31]

Orthodontic appliances also may change the oral microbiota,and produce physical, chemical and biological changes in it mainly by the introduction of new retentive areas which facilitate the micro;organisms colony.Removable orthodontic appliances favor a fast microbial colonization due to the hydrophobicity and high content of surface free energy in acrylic resin (polymethylmetacrylate) [32] .

However, these materials are liable for microbial adhesion, greatly inhibit oral hygiene and create new retentive areas for plaque and debris, which in turn pre- disposes the wearer to increased microbial burden and possibility of subsequent infection.[33]

In our study we succeeded to provide significantly the mean TAC levels increased due to active orthodontic treatment.

V. Conclusions:

Levels of total antioxidant capacity in the saliva of children of group A and group B increased significantly after treatment than before. Salivary TAC level increased significantly in group A compared to group B after treatment.

The utilization of orthodontic appliances affect the Total Antioxidant Capacity in the oral cavity. There is a significant positive correlation between TAC and active orthodontic treatment.

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