RESEARCH ARTICLE

Comparison of Salivary Antioxidants in Children with Primary Tooth Abscesses before and after Treatment in Comparison with Healthy Subjects

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Abstract

Aim: The aim of this study was to compare the total antioxidant capacity (TAC) of children with primary molar abscesses before and two weeks after extraction of the infected tooth. **Materials and methods:** Forty one children aged between 3-6 years participatesd in this cross sectional study. The antioxidant activity of saliva was investigated in 20 patients with tooth abscesses affecting one of the first primary molars before and after tooth extraction in the case group and once in 21 children with no caries or dental problems in the control group. The FRAP (ferric reduction antioxidant power) method was used to measure the antioxidant power of salivary samples and TAC values were calculated. Data were analyzed with SPSS Version 16 using the paired t-test at a significance level of 0.05. **Results:** The mean antioxidant index increased in children after (509.2 ± 138.4) treatment (before 483.4 ± 183.6), but this difference was not significant (P=0.61). Also, there was no difference in the mean antioxidant index in control group (494.5±147.9) compared the study group before (P=0.83) and after (P=0.75) treatment. **Conclusion:** Under the conditions of this study the total antioxidant capacity of saliva was not compromised in children with abscessed teeth.

Keywords: Total antioxidant capacity- saliva- dental infection- primary tooth- FRAP

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Introduction

Dental or dentoalveolar abscess is a denomination used to describe local accumulation of pus in the alveolar bone near the root apex of the affected tooth. It usually occurs secondary to dental caries, trauma, deep restorations or failed root canal treatments (Shweta, 2013).

After entering the microorganisms from the root canal to the periradicular tissues these bacteria capable to induce an acute inflammation leading to pus formation. The main cause of tissue destruction is the host's response to periodontal pathogens, which caused by the production of free radicals that is the main host response tool against infectious agents.

Polymorphonuclear cells (PMNs), monocytes and other inflammatory cells, such as endothelial cells, fibroblasts and osteoclasts, release free radicals to meet bacterial challenges (Battino et al., 2002). The respiratory burst in the neutrophils, which is the central pathway of phagocytosis, indicates that inflammation is a booster factor in local oxidative stress. Ultimately, tissue degradation is caused due to the collapse balance of active radicals and antioxidants. These radicals react in the tissues and affect its structure, function and biological behaviors (Novakovic et al., 2014)

The main biological concept in the treatment of dental abscess is based on the reduction of causative agents, followed by stimulation and support of the regeneration of damaged tissues.

Cells, tissues and body fluids are powerful defensive systems against oxidative damage. These include superoxide dismutase, catalase, glutathione peroxidase, uric acid, ascorbic acid, glutathione, lipoic acid, carotenoids, vitamin E, and Uobicquinol (Baser et al., 2015).

It has been noted that the imbalances between the levels of free radicals and ROS (reactive oxygen species) and antioxidants may have an important role in the onset and development of various inflammatory oral diseases (Battino et al., 2002).

Saliva, is a heterogeneous fluid comprises proteins, glycoproteins, electrolytes, small organic molecules and compounds transported from the blood, constantly surrounded the teeth and oral mucosa. Its functions are cleansing, an ion reservoir, a lubricant and a buffer. In addition saliva could constitute a first line of defense against free radical mediated oxidative stress (Battino et al., 2002).

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Total antioxidant capacity (TAC) is an additive parameter reflecting the combined effect of mainly non-enzymatic antioxidants in plasma and body fluids. (Baser et al., 2015)

Saliva compounds, both systemically and locally, indicative of infectious conditions and the state of the body's defense in the oral cavity and on the other hand, it's possible to measure the changes in its antioxidants (Takane et al., 2005; Miricescu et al., 2011)

Studies have shown that low levels of peroxidases cause and increase oral diseases, including oral cancers, and the levels of antioxidants in blood samples and tumor tissues in Squqmous Cell Carcinoma (SCC) patients, has shown a significant decline (Fiaschi et al., 2005).

Several studies conducted on oral antioxidants of people with various oral diseases have reported that the level of antioxidants in both the saliva and the gingival sulcus fluid of the patients changes in comparison with healthy subjects(Miricescu et al., 2011; Rai et al., 2012; Darczuk et al., 2016; Jurczak et al., 2017).

TAC measurements can provide value information about the balance between the oxidant -antioxidant systems so that the inadequate amount of antioxidants is contributed to excessive tissue damage associated with bacterial infections.

Since no study has been conducted on the measurement of salivary antioxidants in children with dental abscess, and it can be helpful in improving community awareness and better service (Ebrahimipour et al., 2013; Akbari et al., 2015), thus the aim of this study was to compare the TAC of saliva in children with dental abscess before and after treatment.

Materials and Methods

In this experimental cross-sectional study, saliva samples were collected from children in the primary dentition period (between 3-6 years old) who referred to the dental clinic of Birjand University of medical sciences using simple random sampling.

The exclusion criteria for the case group were children with more than one tooth abscess, periodontal or systemic diseases or intake of any drug or antioxidant complementary.

Informed consent was obtained from parents. This research was approved by the Ethics Committee of Birjand University of Medical Sciences with the ethics code 13.

The sample size was estimated 19 in each group based on:

$$n = \frac{\left(Z_{1-\frac{\alpha}{2}} + Z_{1-\beta}\right)^2 (S_1^2 + S_2^2)}{d^2}$$

Before sampling, the children were asked to wash their mouth with water to remove exfoliated cells and foreign substances.

Unstimulated saliva samples from 20 children in the case group who have an abscess in one of the primary first molar (teeth D) were collected at 9-11 am with a sampler and transferred in two 1.5 mm microtubes to the research laboratory of Birjand University of Medical Sciences. Sampling was done in two steps before and two weeks after tooth extraction that the wound of extraction site was healed.

Saliva samples from 21 caries free children with good oral hygiene were collected as control group. The samples were stored at minus 70 ° C.

To measure the antioxidant strength of salivary samples, FRAP (Ferric Reducing Antioxidant Power) method was used such as Zarban et al., (2007) study . In this method, the saliva's ability to reduction the ferric ions and conversion into Fe 2+ ions in acidity PH and in the presence of TPTZ (Tri Pyridyl-S-Triazine) was measured, [Fe (II)-TPTZ] complex is formed which has an intense blue color and the intensity of the obtained color can be measured at a wavelength of 593 nm spectrophotometrically. This reaction is non-specific, and any molecule that has the ability to reduce the ferric ion under the above conditions will enter in this reaction.

TPTZ was prepared by the Fluka Company (Buchs, Switzerland) and chloride acid, sodium acetate, glacial acetic acid, iron chloride and iron sulfate were purchased from Merek (Merck's CP reagent) Company.

The working FRAP reagent was prepared by mixing 1 volume of 10 mmol/L TPTZ, 10 volumes of 300 mmol/L acetate buffer, PH 3.6, in 40 mmol/L HCL (3.31 ml of HCL to prepare a liter) and 1 volume of 20 mmol/L FeCl3.

1.5 ml of freshly prepared FRAP agent added to the proper amount of saliva samples (50 µl) in test tubes and incubated at 37° C for 5 min, then reading was taken at 593 nm versus the control (1.5 ml FRAP solution and 50 µl distilled water).

The standard curve was drown using aqueous solution of iron sulfate (FeSO4. 7H2O) as calibration and immediate absorbance of FRAP (µM Fe (II)) values of samples.

Data were collected and analyzed using SPSS version 16 software.

For data analysis, descriptive and analytical statistics including frequency, percentage, mean and standard deviation were used Paired t-test at significant level of 0.05 was used for comparison of the groups.

Results

This study was performed on 41 under 6 years children including 20 children with dental abscess as case and 21 cares free and good oral hygiene children as control groups.

The mean antioxidant indexes before and after treatment in the case and control groups is shown in Table 1.

Table 1- Average amounts of antioxidants before and after treatment in the case and control groups.

Based on the data of table 1, the mean antioxidant indexes of children increased after treatment compared to pre-treatment, but this difference was not statistically significant. (P = 0.61).

There was no significant difference in mean antioxidant index in healthy children and children with dental abscess before (p = 0.81) and after treatment (p = 0.75).

The mean antioxidant index in girls was higher than that of boys, but this difference was not significant (p =

Table 1. Average Amounts of Antioxidants before and after Treatment in the Case and Control Groups

Groups	N	SD ± Mean	Paired t test (Pvalue)
Case(before TX)	20	483.4± 183.6	p=0.61
Case (after TX)	20	509.2±138.4	
Control	21	494.5±147.9	P=0.83
Case(before TX)	20	483.4± 183.6	
Control	21	494.5±147.9	P=0.75
Case (after TX)	20	509.2±138.4	
Female	21	508.6±150.7	P=0.44
Male	20	468.7±179.1	

0.44).

Discussion

Various methods have been designed to evaluate antioxidants in biological systems. The measurement of each antioxidant individually or evaluation of TAC is very common, although direct measurements of free radicals are less common due to expensive to measure, instability and short life span of free radicals (Battino et al., 2002). Of these FRAP, is a quick, sensitive and cheap method (Del Pino-García et al., 2015).

The present study is the first study to compare the total antioxidant capacity of saliva in children under 6 years of age with dental abscess before and after treatment. Children without caries in the same age group were included as control group, so caries and its related factors did not act as confounding factors (Muchandi et al., 2015).

Unstimulated saliva of children were used because stimulate saliva contains a lower concentration of antioxidants. Unstimulated saliva is a more valid criterion for assessing intraoral condition (Miricescu et al., 2011), in addition stimulation of saliva may increase the expulsion of gingival cervicular fluid from periodontal pocket during mastication. This may artificially increases the concentration of salivary antioxidants (Sculley and Langley-Evans, 2002)

The study by Suzanne Moore et al. Showed that the antioxidant capacity of saliva in patients with periodontal disease does not change and not endangered (Moore et al., 1994). Azizi et al., (2012) showed no significant difference in the TAC of saliva in patients with oral aphthous ulcer and healthy subjects.

Also, the results of Maleki Rad et al. Showed that TAC of saliva in patients with type 2 diabetes is not significantly differ from that of the control group (Malekirad et al., 2005).

In the study by Ulku Baser et al., (2015) TAC of saliva was not significantly different between two groups of patients with progressive periodontitis and healthy subject.

Both mechanisms of dental caries and consequently tooth abscess as well as periodontal diseases, lead to host immune response and the production of free radicals and ROS.

Lovaf et al. compared the TAC of saliva and serum in patients with TMD (temporomandibular joint disorders) and healthy subjects. Their results showed that mean plasma TAC in TMD patients was significantly lower than that in the control group but no significant difference was detected in salivary TAC among the groups (Lawaf et al., 2015).

However, in Novakovik et al., (2014) the TAC of saliva in 42 patients with chronic periodontitis was increased after treatment (scaling), which contradicts our study results.

In the study of Jurczak et al., (2017) the levels of salivary antioxidants were significantly affected by the stage of decay progression.

The reason for this difference can be found in the method used to determine the antioxidant strength and the sample size of this study.

Darczuk et al., (2016) Showed that TAC of saliva in patients with oral lichen planus is significantly less than that of healthy group. The reason for this difference can be related to the extent of involved areas. Dental abscess is localized and affects less areas. The difference in age and sample size can also be a factor.

Mahjoub et al., (2014) showed that TAC of children with severe early childhood caries were higher than caries-free children, which contradicts the results of other studies, in which antioxidant levels are more in illness condition. They stated that significant increase in antioxidant levels in progressive caries is a compensatory mechanism against the oxidative stress created in these conditions.

Miricescu et al., (2011) evaluated the TAC of 20 patients with periodontitis, 20 patients with lichen planus and 20 cigarettes. Their results showed significant decreases in each of the three case groups compared to the control group. It seems that the generalized nature of these disease and more risk factors are involved. In addition, the average age of the subjects of the study and the different testing methods are also had an impact (Ebrahimipour et al., 2015).

All methods of antioxidant assay, regarding the chemical conditions the reactions and its mechanism, only represent a total estimation of the antioxidant capacity of the sample under laboratory conditions and have some limitations that make a difference in the results of various studies.

Antioxidants are essential for health, but their main mechanism, especially in the oral pathology, is unknown. Therefore, more studies are needed to understand their roles as well as their functions.

Moreover, since GCF (gingival cervicular fluid flow) increases during gingival inflammation and changes the saliva composition with products from the inflammatory response, a study is recommended to evaluate the antioxidant levels of GCF in abscesses.

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