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Original Research Article

Salivary Alpha Defensin 1-3, Total Protein and Total Antioxidant in Children with Gingivitis

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ABSTRACT

Background: Saliva is a complex oral fluid, can be used to monitor inflammatory diseases. Several salivary biomarkers may serve as an important biochemical parameter of gingival inflammation.
Objective: The aim of this study is to evaluate the concentration of salivary total protein, total antioxidant and α- defensin 1, 2 and 3 (HNP1-3) in relation to gingival condition of school children.
Methods: Unstimulated whole saliva was collected from 80 caries free school children, 40 children with healthy gingiva (control group) and 40 children with gingivitis to compare the salivary concentration of total protein, total antioxidant and α-defensin in both groups.
Results: The concentration of total protein, and α- defensin were highly significant in gingivitis compared to control group. However, the difference in total antioxidant between gingivitis and control groups was insignificant. Additionally, there were significant positive correlations between total protein versus both total antioxidant and α- defensin.
Conclusion: Children with gingival inflammation had high levels of total protein and α-defensin which may serve as an important biochemical parameter of gingival inflammation.

Keywords: Gingival disease, Alpha defensin, Total protein, Total antioxidant.

INTRODUCTION

Saliva plays a crucial role in oral health and any changes in quantity or quality of saliva may lead to oral diseases. Whole human saliva is unique body fluid contains arrays of immunoglobulin and non immunoglobulin defense factors. The major components of the non immunoglobulin group are antimicrobial peptides (AMPs), antioxidant, lysozyme, lactoferrin, agglutinins, histidine rich protein and anionic protein. It seems likely that testing methods can be developed and used in dental practice to provide a foundation for the recognition of potential biomarkers of the gingival disease. [1]

Previous studies have investigated the correlation between concentrations of the predominant protein components in saliva and gingival inflammation. These studies have shown a significant rise in the salivary total protein concentration in gingivitis and periodontitis patients compared to healthy individuals. [2,3]

The recruitment of polymorphnuclear leukocytes (PMNs) and other inflammatory cells to gingival inflammation is an important feature of the inflammatory process in oral disease. After stimulation by bacterial pathogens, PMNs produce free radicals. The disparity between free radicals and antioxidants levels in saliva may modulate the role of total antioxidant in the pathogenesis of oral diseases. Antioxidants combat the adverse effects of any reaction that cause excessive oxidations by neutralizing the toxicity of free radicals and cytokines, and reduction in antioxidant levels leads to oxidative stress. [4] Antioxidant alterations in some inflammatory and pre cancerous diseases have been assessed and confirmed [5] but the correlation between antioxidants and gingival diseases is still less clear.

PMNs kill bacterial pathogens in the periodontal pocket by oxygen radical- and non-oxygen-dependent mechanisms. [6] The non-oxidative antibacterial mechanisms involve a various types of antimicrobial peptides which contribute to the killing of microorganisms in the extracellular environment. [7] The most abundant of these antimicrobial peptides are the so-called defensins. [8-10]

Defensins are important antimicrobial peptides in innate and adaptive immune response pathways. [11] Defensins not only have the ability to strengthen the innate immune system but also enhance the adaptive immune system by chemotaxis of monocytes, T-lymphocytes, dendritic cells and mast cells to the infection site. In addition, defensins improves the capacity of macrophage phagocytosis and might serve as immune-modulators to activate the immune system suppressed by infection and inflammation. [12-14] Moreover, defensins can activate the classical complement pathway and have the potential to modulate the inflammatory response through the regulation of cytokine and adhesion-molecules expression. [15] α-defensins in particular, are able to up-regulate IL-8 expression, which is known to improve neutrophils recruitment to effector sites. [16]

The salivary α- defensins, a mixture of HNP1–3, are elevated in patients with oral inflammation. [12] Levels of HNP1–3 vary in healthy individuals ranging from undetectable to ~12 μg/ml. [12,17] The presence of α- defensins in saliva is most likely derived from neutrophils and is a reflection of gingival or mucosal inflammation and loose or exfoliating teeth. [18,13]

A greater understanding of how these salivary biomarkers act in the healthy, gingivitis and periodontitis conditions would definitely open new opportunities for identification, prevention and treatment of gingival diseases. There have been scarcity studies which assess the association between salivary biomarkers and gingival inflammation in children. The present study was designed to investigate the relationship between gingival inflammation and different salivary biomarkers including total protein, total antioxidant and α- defensin.

MATERIALS AND METHODS

Subjects

After receiving the written approval from the concerned school authorities and informed consent from the parents, a total of eighty healthy caries free school children, from both genders (equal distribution), with an age ranging from 6 to 12 years old were selected to participate in this study. Children with systemic diseases, or those who were using any medications or mouth rinses during the last two months before saliva collection were excluded from the study. The study population consists of 40 children with gingivitis (cases) and 40 children with healthy gingiva (control). The intra-examiner calibration was done by the researcher according to WHO Basic Method 1997 to reduce the intra-examiner variability. [19] Gingival inflammation was scored according to the National Institute of Dental Research (NIDR) criteria.

NIDR – Gingival Inflammation Index (Bleeding index)

0=No bleeding
1 = Bleeding after probe is placed in gingival sulcus up to 2 mm and drawn along the inner surface of the gingival sulcus.
Children in cases group had at least six areas with gingival inflammation while controls were completely free from gingival inflammation.

Saliva collection

Unstimulated whole saliva samples were collected and stored as described previously by Chiappin *et al*. [20] to determine the levels of salivary total protein, total antioxidant and α-defensin.

Total protein and antioxidant assessment

The total salivary protein level was measured by an autoanalyser (Technicon RAXT, USA) according to Biuret method. [21] The determination of the total antioxidant of saliva was performed by the reaction of antioxidants in the sample with a defined amount of exogenously provide hydrogen peroxide (H_2O_2) according to manufacturer's instructions (Biodiagnostic, Dokki, Giza, Egypt).

α- defensin (HNP1-3) assessment

α- defensin level of the samples was measured by ELISA according to manufacturer's instructions (Hycult Biotechnology, Uden, Netherland).

Statistical analysis

The collected data was analyzed using SPSS software program version 22. All data in the present study was quantitative data. Data was presented as mean ± standard deviation and tested for normality distribution by Kolmogorov–Smirnov test and found to be of parametric distribution, so independent t test was used to compare between the two groups, Pearson correlation used to illustrate the correlation among salivary markers. $p \leq 0.05$ was considered to be statistically significant. [22]

RESULTS

The study results showed an increased concentration of salivary total protein and α- defensin in gingivitis patients compared to healthy individuals, the differences were statistically significant (p=0.001 and 0.010 respectively). In addition, the level of total antioxidant was higher in children with gingivitis compared to healthy children but the difference was statistically insignificant (p=0.095) [Table 1/ Fig.1].

Table1: The concentration of salivary markers in relation to gingival condition

Groups / Variables	Gingivitis (cases) Mean ± SD	Normal (control) Mean ± SD	p value
Age	9.560 ± 1.822	9.763 ± 2.812	0.703
Total protein g/dl	1.049 ± 0.961	0.596 ± 0.483	0.001
Total antioxidant mmol/l	1.493 ± 0.715	1.236 ± 0.641	0.095
α- defensin µg/ml	7.140 ± 3.364	5.046 ± 3.750	0.010

SD = Standard Deviation, p = value of significance.
Analysis done by independent t test at CI=95% and level of significance at p≤0.05.

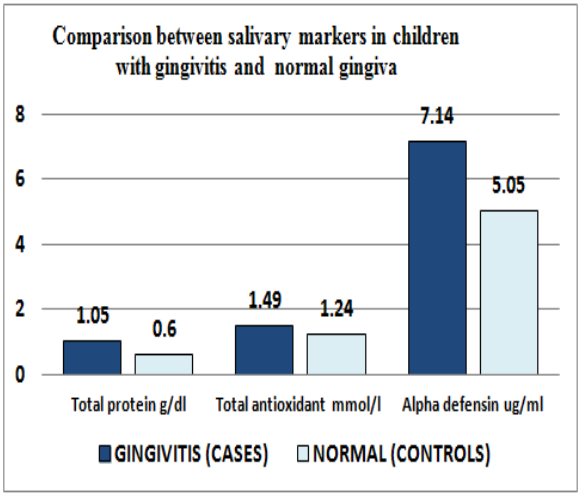

Figure 1: Comparison between salivary markers of children with gingivitis and normal gingiva

A statistically significant positive correlation was found between salivary total protein versus both total antioxidant and α-defensin levels in all children (cases and control) [Table 2/Fig- 2 and 3].

Table2: The correlation between total protein versus total antioxidant and α- defensin.

Pearson's correlation	r(p)
Total protein vs antioxidant	0.452** (0.000)
Total protein vs α- defensin	0.345** (0.002)

**correlation is significant at the 0.01 level (2-tailed)

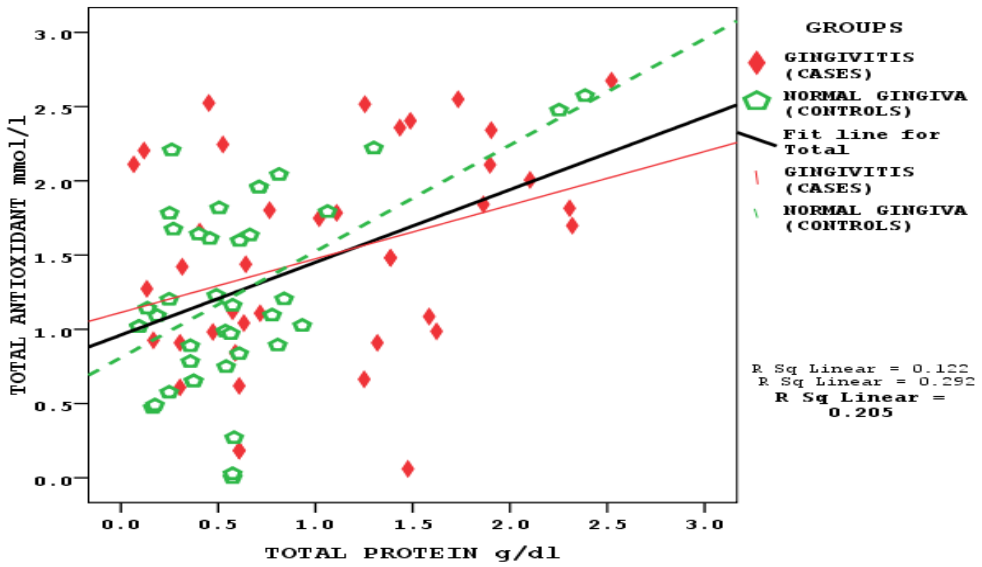

Figure 2: The correlation between total protein and total antioxidant

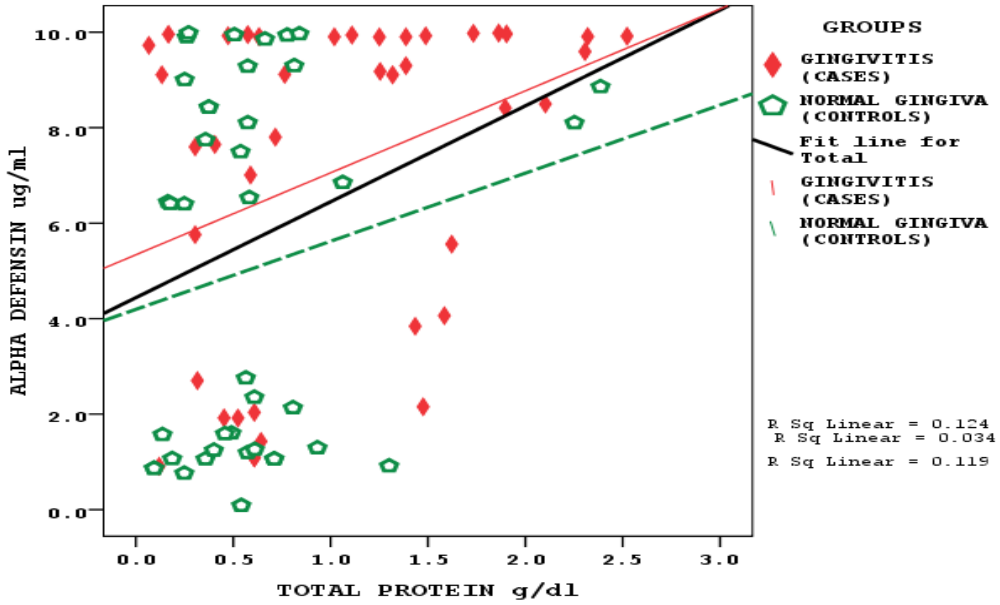

Figure 3: The correlation between total protein and α- defensin

DISCUSSION

Several salivary markers may be correlated to different oral diseases; therefore, more attention has been given to this topic lately. The present study was carried out on caries free children to overcome the effect of dental caries on salivary biomarkers as different studies indicating increased total protein and total antioxidant with increased caries activity [23,24] and others indicating increased α-defensin in caries free children. [25,26]

The results of this study showed statistically significant higher levels of total protein in gingivitis group compared to healthy children [Table 1 and figure 1]. These results are in agreement with other studies that showed similar results. [27,2]

The increased total protein levels in the gingivitis patients could be due to the inflammatory process that causes leakage of plasma proteins into saliva and activates the sympathetic system to enhance the synthesis and secretion of proteins thereby increasing the protective potential of saliva. [27, 28]

In the current study, salivary total antioxidant was not significantly higher in children with gingivitis compared to healthy

control. These results were similar to the results of other studies. [29,30] Moore et al. [29] showed that there was no difference in amount and activity of antioxidants in cases and healthy control groups. In addition, Chapple et al. [30] also showed that, the amount of antioxidants is similar in patients with mild or severe periodontitis and healthy people. In contrast, a positive correlation was demonstrated between salivary total antioxidant and periodontitis [31] and the salivary concentration of total antioxidant was dependent on the clinical severity of periodontitis. [32] Other studies disagree with our results and showed that there is a reduction in total antioxidant activity of saliva for patients with periodontitis, [33-35] this disagreement may be explained by the difference in age of participants and disease level in the present study and other studies.

The results of the present study showed statistically significant higher concentration of α-defensin in children with gingivitis. The salivary HNP1-3 may be released from neutrophils at the time of inflammation and then disappear with the resolution of inflammation. [36] The results of the present study support the previous studies which concluded that, α- defensin are abundant and widely distributed peptides involved in host defense. [6,7]

In the present study, a significant positive correlation was observed between total protein versus both total antioxidant and α- defensin (table 2, figure 2 and 3) indicating that they are implicated in host response. This is in accordance with other studies which also evaluated the role of these salivary markers in patients with caries or periodontitis. [37-39]

CONCLUSION

The results of the present study showed increased levels of salivary total protein and α- defensin in children with gingivitis. Thus, salivary total protein and α- defensin may be used as risk assessment tool for early prediction of gingival and periodontal diseases.

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