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Assessment of salivary Interleukin (IL)-6, IL-10, Oxidative Stress, Antioxidant Status, pH, and Flow Rate in Dental Caries Experience patients in Tikrit Province

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Abstract

Background: Dental caries is an infectious and communicable disease and multiple factors influence the initiation and progression of the disease. Imbalance between oxidative stress and saliva antioxidants plays a major role in initiation and spread of dental caries.

Objective: The aim of this study was to evaluate physicochemical properties of saliva such as flow rate, pH, buffering capacity, interleukin(IL)-6, IL-10, and malondialdehyde (MDA),and total antioxidants capacity (TAC), and catalase (CAT) levels in caries free and caries active individuals.

Material and Method: Fifty consecutive subjects with dental caries experience (27 M & 23F) and fifty caries free subjects (24 M & 26 F) were recruited. The dental caries status was assessed depending on the WHO oral assessment form. Stimulated saliva samples were collected at the morning from both groups. The MDA, TAC, and CAT were evaluated using spectrophotometric assay. SPSS 13 analyzed data using Student's t-test.

Results: There was a statistically significant increase in salivary T A C, CAT, and IL-6 in group with dental caries compared with control group. However, the salivary pH, flow rate, buffering capacity, and IL-10 levels were statistically lower in caries active patients. Person's correlation coefficient indicated significant negative correlations between the IL-6 with IL-10, pH, MDA, and flow rate. Whereas positive correlation with TAC, and buffering capacity. In addition, there was negative correlation between IL-10 with TAC, MDA, and buffering capacity, While positive correlation with pH and Flow rate.

Conclusions: Alterations in salivary MDA, TAC, CAT, and IL-6 levels were significantly higher whereas Salivary pH, flow rate, buffering capacity, and IL-10 were significantly lower in the study groups when compared to control groups.

Keywords: Dental caries, interleukins; Antioxidants; saliva; Oral fluids.

Introduction

Dental caries and periodontal diseases are known as the top oral health burden. In both developing and developed nations affecting around 20–50% of the population of this planet, and is the uppermost reason for tooth loss[(1)]. it is a complex, multifactorial and dietary carbohydrate-modified infectious disease. It is a process that may take place on any tooth surface in the oral cavity where dental plaque is allowed to develop over a period of time[(2)].

Microbial products initiate an inflammatory response leading to local tissue destruction including connective tissue and bone support loss, which may ultimately result in tooth loss[(3)].

In salivary glands, reactive oxygen species (ROS) are involved in alteration of their functions. Oxidative stress which is formed by the breakdown of the balance between free radicals and antioxidants, due to the excessive production of ROS and the reduction in the rate of its removal by the antioxidant defense system [(4)].

Cytokines are small polypeptides with a wide range of inflammatory, metabolic and immunomodulatory properties. They are manufactured by macrophages, lymphocytes, monocyte, dendritic cells, neutrophils, endothelial cells and fibroblasts. Cytokines are the mean

of communication between immune and non-immune cells[5]. There is different types of cytokines including interleukinthat play a critical role in mediating inflammatory processes and tissue homeostasis underlying periodontitis[6-7].

The inflammatory and immune responses to the bacteria as well as viruses that colonize the periodontal and associated tissues involve the systemic circulation and ultimately the peripheral systems of the body. Loss of balance between ROS and antioxidant defense has also been implicated as an etiologic factor for oral diseases[8].

The aim of this study was to analyze the interleukin (IL)-6, IL-10, oxidative stress, and antioxidant status of saliva in individuals with dental caries.

Materials and Methods

Study Population

The students of college of dentistry/ Tikrit University of all levels was corroborate in this study, which take place, between May 2018 and February 2019.

The study group included 50 (27 M & 23F) with a mean age of 20.46 ± 1.95 year, and control group comprised of 46 (24 M & 26 F) healthy individuals with a mean age of 21± 1.47 year.

Dental Caries Assessment

The diagnosis of dental caries was recorded according to decayed, missing and filled index (DMFS), which allow recording decay lesion by severity, depending on the WHO oral assessment criteria [9].

Collection of Saliva

Stimulated saliva was collected between 9-11 AM, following the washing of mouth with distilled water for 5 minutes under relaxed conditions and the patients asked to spit un-stimulated saliva for 5 minutes. The salivary sample was centrifuged at 3000 r.p.m. for 10 minutes. Salivary supernatant was stored at (-20ºC) in polyethylene tubes for subsequent chemical analysis.

Estimating the time of collection of 5mml saliva to obtain the flow rate. The pH of the saliva sample was measured with the help of a single electrode digital pH meter (Ottawa, Canada).

IL-6 and IL-10 were analyzed by Enzyme Linked ImmunoSorbent Assay (ELISA) test using special kits according to the manufactured instructions. MDA, TAC, CAT levels were measured by spectrophotometric kit.

The statistical analysis was performed using statistical software (SPSS version 16). Data are represented as means ± SD unless otherwise stated. The significant level considered as P value less than 0.05.

Results

The results of table (1) indicate the mean of salivary T A C (48.15±3.07), MDA (1.6730±0.0798), CAT (6.969±0.763), and IL-6(14±0.914) levels were significantly higher in subjects with dental caries respectively than in control group (39.38±3.23), (0.6400±0.0877), (2.087±0.151),and (3.071 ± 0.367) respectively.

On other hand. the results showed a significant decrease in salivary buffering capacity (8.760 ± 0.827), pH (5.870 ± 0.400), flow rate(1.120 ± 0.133), IL-10 (1.710 ± 0.313) in subjects with dental caries in comparison with caries free group (11.00 ±1.01), (7.260 ± 0.686), (1.530 ± 0.177),and (2.000 ± 0.146), respectively.

Table 1. Physicobiochemical parameters of study and control groups:

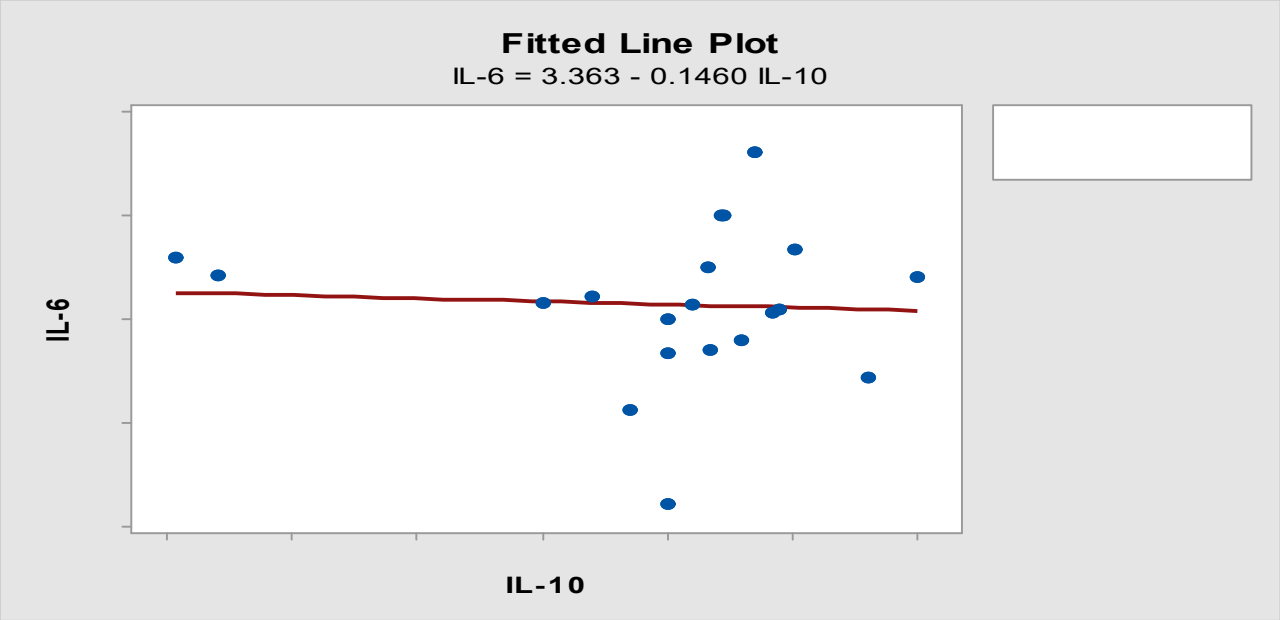
Parameter	Study group	Control group
Buffering capacity	8.760 ± 0.827	11.00 ±1.01
p H	5.870 ± 0.400	7.260 ± 0.686
Flow rate	1.120 ± 0.133	1.530 ± 0.177
T A C	48.15±3.07	39.38 ±3.23**
M D A	1.6730±0.0798	0.6400±0.0877
CAT	6.969 ±0.763	2.087±0.151
I L - 6	14±0.914	3.071 ± 0.367
I L – 10	1.710 ± 0.313	2.000 ± 0.146

Correlation within Parameters

The results revealed that there was negative correlation between IL-6 with IL-10 (-0.058), pH (-0.010), MDA (-0.444), and flow rate (-0.314), whereas positive correlation with TAC (0.197), and, buffering capacity (0.115).

In addition, there was negative correlation between IL-10 with TAC (-0.296), MDA (-0.396), buffering capacity (-0.359), While positive correlation with pH (0.166), Flow rate (0.101).

Fig. 1. Correlation between IL-6 and IL-10 in subjects with dental caries.



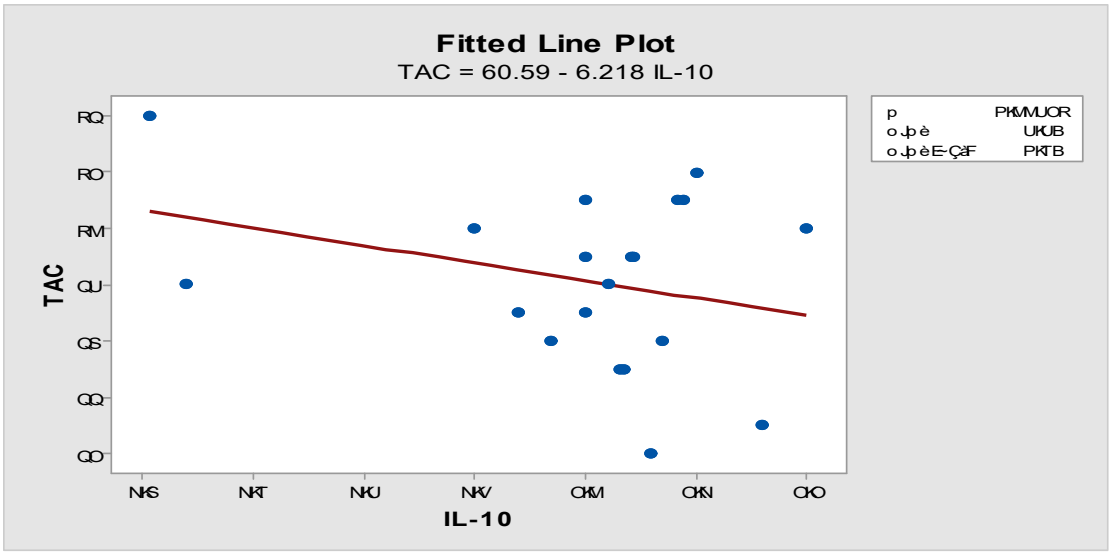


Fig. 2. Correlation between TAC and IL-10 in subjects with dental caries

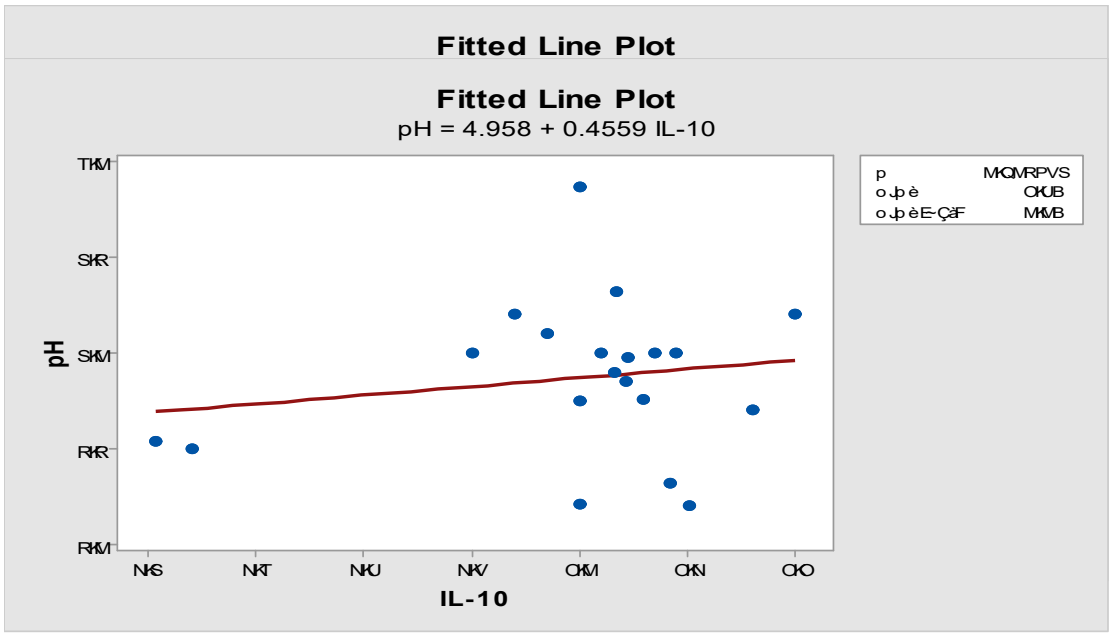


Fig. 3. Correlation between MDA and IL-10 in subjects with dental caries

Fig. 4. Correlation between pH and IL-10 in subjects with dental caries

Discussion

Human saliva is a biological fluid with myriad of biological functions important for the maintenance of oral and general health, it is considered as the gold standard in biochemical assays and analysis[10,11]. Similarly, saliva was used as the diagnostic fluid for IL-6, IL-10, MDA, TAC, and CAT estimation in this study.

Perfluoroalkyl acids (PFAAs) may interfere with hormones that affect salivary gland function, which in turn alters salivary rate in the oral cavity. The quantity and quality of saliva in the mouth is an important factor associated with caries incidence, and the endocrine-disrupting properties of PFAA may have altered the functioning of salivary glands[12].

It was observed in the present study that the Buffering capacity, pH, and Flow rate level in saliva decreased with caries activity. Decreased salivation leads to dryness in the mouth and poor oral clearance, thereby facilitating caries formation.

The decrease in Buffering capacity, pH, and Flow rate could be explained by the fact that the cariogenic bacteria such as Streptococcus mutans, Streptococcus sobrinus, and lactobacilli produce acids following an individual's sugar consumption[11].These acids, mainly lactic acid, diffuse through the dental calcified tissues and drop the local pH to below 5.5, which in turn leads to a dissolution of the mineral crystals and cause caries initiation in enamel [14], Also the proteins have some effect on the buffer capacity at acidic pH values below 5.

IL-6 Interleukin-6 (IL-6) is a multifunctional inflammatory cytokine that is secreted by immune cells, adipose tissues and muscles that plays an important role in the response to environmental stress which regulates the immune reactivity also has functioning in haematopoiesis, bone metabolism, and tissue regeneration[15]. The results of current study revealed that the concentration of salivary IL-6 among study group was higher than that among control group. The Explanation for this result could be that the IL-6, can be produced not only by activated macrophages /monocytes, but also by fibroblasts and activated endothelial cells in inflamed tissue via proteases, osteoclasts, and

methylation changes[16].

Our results confirm those of Gabay *et al* [17], which showed that Higher levels of TNF-a and IL-6 in dental caries patients lead to a lower number of osteoblasts and fibroblasts, and they support the demineralization of teeth and development of the dental caries process , IL-6, with other factors, causes the resorption of bones and stimulates the synthesis of chemocines.

IL-10 is a pleiotropic cytokine known for its immunosuppressive properties. It has a dual role; it plays a major role in suppressing immune and inflammatory responses as well as B cell activation[18], it can restore balance by inhibiting synthesis of pro-inflammatory cytokines (IL-1, IL-6, TNF-alpha) and stimulating protective antibody production. For this reason, IL-10 gene polymorphism might contribute to periodontitis development[19].

In present study, the dental caries experienced group had a higher level of IL-10 compared to caries free group, this may due to the severity of the inflammatory process going on. Contrary to our results, Fine, *et al*. [20] found in their study that dental caries was not significantly correlated with salivary or serum concentration of IL-10.

MDA is a reactive aldehyde and is one of the many reactive electrophile species that cause toxic stress in cells[21].Oxidative stress, a pathophysiologic imbalance between the generation of reactive oxygen/nitrogen species and the capacity of cells to neutralize them by the antioxidant defence([22].

The level of salivary MDA which considered one of the final products of lipid peroxidation is higher in patients with dental caries experience than control group, due to the direct impact on the basic mechanisms of signal response and transduction, which leads to an increased growth and differentiation of cariogenic bacteria and their increased potency[23,24]. In consistence with our findings Rai *et al*., [25] demonstrated that salivary MDA levels in dental caries experience groups was elevate significantly compared with their levels in the control group. Interestingly, in another study found no difference between patients and controls in salivary MDA [26]. Salivary antioxidant system was found to reduce the susceptibility to dental caries.

The specific role of antioxidants is to neutralize rampaging free radical and thus reducing its capacity to damage. They act as radical scavenger, hydrogen donor, electron donor, peroxide decomposer, singlet oxygen quencher, and synergist. Large numbers of free radicals are produced during the process of dental decay. The numbers of free radicals vary directly with the degree of activity of the caries [27].Catalase protects cells from hydrogen peroxide generated within them. Even though CAT is not essential for some cell types under normal conditions, it plays an vital role in the acquisition of tolerance to oxidative stress as an adaptive response of cells. H_2O_2 is enzymatically catabolized in aerobic organisms by CAT and several peroxidases[28].

Total antioxidant capacity, is an indicator of oxidative stress, reflecting the redox balance between oxidation and antioxidation [29].Similar results were obtained in our previous study where we had compared the total antioxidant capacity levels in caries-free and caries-active groups. [30], who concluded that this TAC elevation can be related to more oxidative stress due to caries in study group than control group. As well as TAC may be increase with increasing salivary protein level because salivary proteins interfere with bacterial colonization when bacterial load were elevated [31].

Conclusions

Within the limitations of this longitudinal study, it can be suggested that there is alterations in salivary MDA, TAC, CAT, and IL-6 levels, were significantly higher whereas Salivary pH, flow rate, buffering capacity, and IL-10 were significantly lower in the study groups when compared to control groups.

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