Original
ISSN 2521-8492

Comparison of Some Indicators in Serum and Saliva on Periodontitis

Araz M Yousif[1*], Parween A Ismail[2], Narmeen A Ismail[3], Namam A Ismail[1]

[1]Department of Basic Science, College of Dentistry, Hawler Medical University, Erbil, Iraq.
[2]Department of Chemistry, Education College, Salahaddin University, Erbil, Iraq.
[3]Department of Biology, Faculty of Science, Soran University, Erbil, Iraq.
*Correspondence to: Araz M Yousif (E-mail: araz.zangana@hmu.edu.krd)
(Submitted: 08 September 2021 – Revised version received: 16 September 2021 – Accepted: 02 October 2021 – Published online: 26 December 2021)

Abstract
Objectives: To assess the serum indicators level in healthy and periodontitis subjects and correlate the levels in the terms of changes of serum and salivary parameters in periodontal disease.
Methods: The assay comprised 85 subjects, enrolled of both genders in the age range of 33–56 years old. Volunteers were categorized into two groups: 40 healthy individual, other groups (periodontal disease) embraced 45 patients. The salivary and blood samples were collected and sent for biochemical analysis to measure the nitric oxide (NO), superoxide dismutase (SOD), glutathione, catalase, Interleukin 6 (IL-6), Interleukin 4 (IL-4), and interferon-γ (IFN-γ) levels in periodontitis patients.
Results: Based on the statistical evaluation of bio-indicator parameters, the following conclusion can be shown from this revision: Serum and saliva SOD ($P < 0.001$), glutathione ($P < 0.001$), and catalase ($P < 0.001$) activates were significantly reduced in periodontitis as compared with control groups, the remaining biochemical assessed in periodontitis patients [NO ($P < 0.001$), IL-4 ($P < 0.0001$), IL-6 ($P < 0.0001$), and IFN-γ ($P < 0.0001$)] indicated significantly high levels in both serum and saliva samples.
Conclusions: The NO, SOD, catalase, glutathione and cytokines may be used as bio-indicator for periodontitis exposure, medical conduct and severity. This recognition offers early diagnosis of disease and progression. Also there was a direct positive correlation between the salivary and the serum bio-markers levels, which it was proved that the salivary non-invasive examination had a significant association with the serum investigation.
Keywords: Nitric oxide, enzymes, glutathione, cytokines, periodontitis

Introduction

Periodontitis is a widespread inflammatory disease; they influence the supporting tissues of the teeth.[1] It is started by precise bacteria in the plaque biofilm and evolutions due to abnormal inflammatory- immune reaction to those bacteria, including the excretion of extra proteolytic enzymes and reactive oxygen species,[2] categorized by gingival bleeding, periodontal pocket creation,[3] frequently leading to damage of the bone.[4] Presently, the diagnosis of periodontal diseases clinically includes primarily the examination of clinical measures of tissue damage and signs of tissue inflammation.[5] The advances in the diagnostic articles of periodontal diseases are moving to the methods, whereas the periodontal threat can be recognized and quantified by indicators. An indicators or biomarker is a matter that is measured and assessed as a marker of the normal biologic progressions and pathogenic developments, or the pharmacologic reactions to a therapeutic involvement. Saliva and Serum can be easily collected so they might be considered as the locally and systemically derived indicators that can be used for periodontitis and other systemic disease assessments.[6] A noninvasive diagnostic fluid is saliva, it could be used as a biomarker to measure indicators released during disease beginning and progression such as periodontitis.[1] Host cells such as polymorphonuclear leukocytes relief reactive oxygen species (ROS) as response of the immune system in periodontitis. ROS act fundamental roles in regulating of physiological progressions and it represents significant pathogenic mechanism for tissue destruction and diseases.[7] Example for ROS such as nitrous oxide, superoxide anion, hydroxyl radical and hydrogen peroxide are formed via the bacteria-host mediated cycle involving tissue damage. The subtraction of ROS are done by antioxidant[8] such as enzymes includes superoxide dismutase (SOD),[9] catalase (CAT), glutathione reductase and etc., or thiol-containing peptides e.g glutathione and thioredoxine,[8] which all acted as resistance systems. The equilibrium is sustained by the collaboration of oxidants and antioxidants in healthy organisms, while in various pathological situations, the balance may be altered to the oxidative side.[10] The messenger that transfers signals to other cells called cytokines which are soluble proteins and released by the cells that transmits signals to further cells. They recruit, facilitate and regulate immune and inflammatory replies; they also control growth and variation of cells.[11] Host cells are secreted pro-inflammatory cytokines when stimulated by bacterial pathogens as an action of the immune reactions.[12] These cytokines recruit to the location of infection. Many cytokines are produced from Gingival epithelial cells such as interleukin-1a (IL-1a), interleukin-1b (IL-1b), tumour necrosis factor-a (TNF-a), interleukin-6 (IL-6) and interferon-γ (IFN-γ) are the pro-inflammatory cytokines while the anti-inflammatory cytokines are interleukin-4 (IL-4) and interleukin-10 (IL-10).[13]

Materials and Methods

Unstimulated whole saliva and blood samples were collected in clinics from a total of 85 volunteer were recruited to this clinical trial, enrolled of both genders in the age range of 33–56 years old. 40 healthy controls included 25 males with 15 females, and 45 advance stage periodontal patients (29 males and 16 females). The serum was prepared by collecting 5 ml of the blood in a test tube without anticoagulant, and plasma was prepared by adding 2 ml of the blood in a test tube contain anticoagulant (EDTA) both samples were separated by centrifugation. Unstimulated saliva was collected from the same

patient's after the diagnosis, under resting conditions between 8.30–10.0 am. Saliva patients and controls were collected by spitting (spitting method) into a test tube (10 ml), and then it was centrifuged. The salivary and blood samples (serum and plasma) were collected and sent for biochemical analysis immediately to measure the NO, SOD, glutathione, catalase, IL-6, IL-4, and IFN-γ levels in periodontitis and healthy subjects. The ELISA (sandwich enzyme linked immunosorbent assay) was used to asses NO, IL-6, IL-4, and IFN-γ levels by SunLong Biotech company ELISA kit, while, SOD, glutathione, and catalase (Biolabo kit) were determined by using spectrophotometer. The levels of the biochemical parameters in the samples were obtained by instructions supplied by the manufacturer. The program of SPSS 16.0 was used for all statistical analyses. Biochemical analyses were achieved to evaluate and compare the salivary and the serum parameters levels of the above groups.

Results

The clinical data of 40 healthy subjects and 45 advance periodontitis patients were enrolled for two different samples in this revision. The results of salivary parameters levels have been present in Figures 1 and 2, while the blood parameters levels (serum and plasma) have been shown in Figures 3 and 4. The results displayed that the levels of salivary levels Figure 1 of SOD ($P < 0.001$), glutathione ($P < 0.001$), and catalase ($P < 0.001$) activates were significantly reduced in advance stage periodontitis as compared with control groups, the remaining biochemical indicators exhibited a significantly greater mean in advance stage periodontitis as compared with healthy subjects as seen in Figure 2 [NO ($P < 0.001$), IL-4 ($P < 0.0001$), IL-6 ($P < 0.0001$), and IFN-γ ($P < 0.0001$)]. Figures 3 and 4 indicated the results in serum for the same

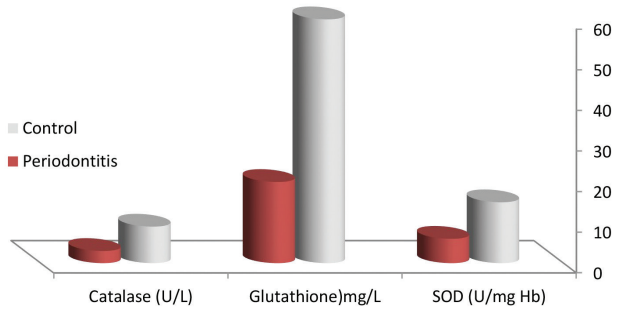

Fig. 1 **Salivary SOD, glutathione and catalase levels of healthy controls compared with advance periodontitis (Values are expressed in mean ± SD).**

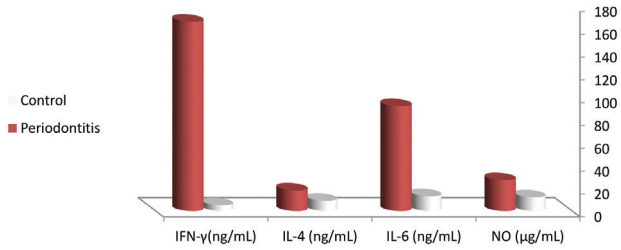

Fig. 2 **Salivary NO, IL-4, IL-6 and IFN-γ levels of healthy controls compared with advance periodontitis (Values are expressed in mean ± SD).**

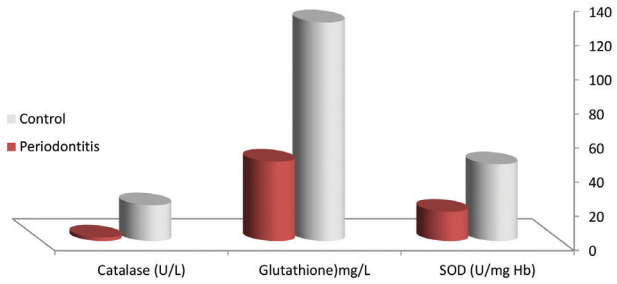

Fig. 3 **SOD, glutathione and catalase levels in blood samples of healthy controls compared with advance periodontitis (Values are expressed in mean ± SD).**

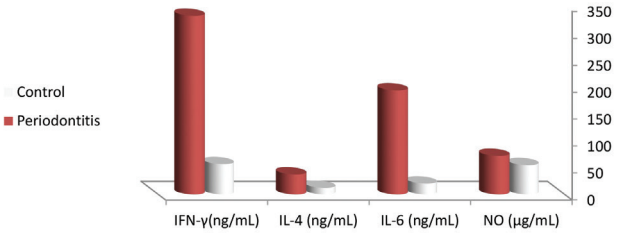

Fig. 4 **NO, IL-4, IL-6 and IFN-γ levels in blood samples of healthy controls compared with advance periodontitis (Values are expressed in mean ± SD).**

salivary parameters levels, which shown significantly lowering [Figure 3] of SOD ($P < 0.001$), glutathione ($P < 0.001$), and catalase ($P < 0.001$) levels in advance stage periodontitis, whereas significantly higher levels [Figure 4] of NO ($P < 0.001$), IL-4 ($P < 0.0001$), IL-6 ($P < 0.0001$), and IFN-γ ($P < 0.0001$) in advance stage periodontitis as compared with control groups.

Discussion

Polymorphonuclear leukocytes are capable of generating ROS naturally in periodontitis, and they are believed to be functionally activated and thus lead to improved ROS production. Tissue damage cause by ROS in different mechanisms, which involved protein destruction due to reversible or irreversible protein folding or unfolding, protein destruction and polymerization metabolisms, and protease degradation. Another path is by forming lipid peroxidation which causes to creation of different conjugated dienes, lipid peroxides, and aldehydes formation of which intracellularly, disrupt the cell membrane integrity and causes breakdown of cells, all these reactions are informed to be induced by ROS. Enzymatic removal to eliminate ROS by some preventive antioxidants like SOD, catalase, glutathione peroxidase etc.[14] In the current research, focused to assess the roles of both the saliva and serum bio-indicator parameters as inflammatory markers in periodontitis. In this article the influence of periodontitis on SOD levels in both blood and saliva's samples were significantly reduced ($P < 0.001$) as seen in Figures 1 and 3. Some articles such as Fenol et al.,[7] Patil et al.,[15] Singh et al.,[16] and Wei et al.,[17] informed lowering SOD activities in periodontitis in line with current study, and this due to primary acute host defense cells are triggered as a two phase process in periodontitis, ether present in gingival or in the blood circulation, involving of priming and full scale activation. Primed neutrophils establish raised amount of ROS which modified the ecological balance in turn to pro-oxidant species leading to elevated utilization

of SOD enzyme. Converse to present investigation, the SOD antioxidant activities have been significantly increased in chronic periodontitis. It was guessed that the scavenging of disproportionately synthesis of lipid peroxidation products at the inflammatory spots may be responsible for stimulating enzymatic antioxidant activity.[18] Another antioxidant enzyme which has been study in the present study is catalase activity. It was significantly decreased ($P < 0.001$) in the salivary and serum samples [Figures 1 and 3]. These results are in harmony with those recorded by Gharbi et al.,[19] and Thomas et al.,[20] In contrast, an article conducted by Panjamurthy et al.[18] observed the high levels of catalase and SOD antioxidants in periodontitis. Glutathione is an example of thiol-containing peptides antioxidants.[21] There were also statistically significantly ($P < 0.001$) low levels of glutathione levels among the serum and salivary advance stage periodontitis groups as founded in Figures 1 and 3 in this study which was consistent with the results of the revision which was performed by Dede et al.,[22] Koregol et al.,[23] and Bains et al.,[8] This is due to that glutathione has been measured as protagonist in cellular controling. Glutathione has also considered as antitoxic, pro-oxidant, and modulator in cellular homeostasis.[24,] Glutathione is contributed in the synthesis and preservation of protein disulfide bonds, as well as in the amino acids moment across cell membranes. Glutathione capably removes free radicals and other ROS by changing non-enzymatically to glutathione disulfide.[25]

A gaseous free radical with a short living half-life is named NO, which is formed enzymatically from L-arginine by the isoenzymes of NO synthase.[6] The results of this paper indicated significantly ($P < 0.001$) improved concentrations of NO in advance periodontitis, as compared to control group [Figures 2 and 4] in both serum and salivary samples. So many articles have revealed the alterations in the NO amounts, which agree with the present paper results such as conducted by Inasu et al.,[1] Sundar et al.,[6] and Menaka et al.,[26] The higher levels of NO had contributed to the production of NO by macrophages and polymorphonuclear leukocytes intra the isoform NO synthase cycle, which was stimulated in periodontitis.[27] Reversely, Aurer et al.,[28] reported that the NO levels were decreased in the saliva of the periodontitis patients. This may be due to the instability of NO in the existence of oxygen, which rapidly auto-oxidizes to alter to nitrogen oxides, or because of the difficult assessing of NO in the cells and tissues because of reactivity and short-life of NO. Various cytokines in serum and saliva have been planned for the diagnosis of periodontal disease in this article such as IL-4, IL-6, and IFN-γ levels, which elevated significantly ($P < 0.0001$) as shown in Figures 2 and 4 in the presence investigation. The significant increase in these cytokines levels in periodontitis patients is probably related to the consequence of the host immune reply to these bacteria, which trigger the immune system to produce pro-inflammatory cytokines.[29] This was in agreement with the studys of Zong et al.,[30] and Tsai et al.,[31] which demonstrated that the high level of blood serum IFN-γ and IL-4 in patients with chronic periodontitis. Xu et al.,[32] Loo et al.,[11] observed with enlarged levels of IFN-γ and IL-6 in chronic periodontitis comparison to healthy individuals. Maulani et al.,[33] reported that the levels of IFN-γ (pg/mL) were correlated with the severity of the periodontal disease. Saliva can be a prized cradle for the detection of the oral and general diseases together.[34]

Conclusion

Based on the statistical evaluation of these biochemical parameters, the following conclusion can be drawn from the study, that NO, SOD, catalase, glutathione and cytokines may be used as bio-indicator for periodontitis exposure, medical conduct and severity. This recognition offers early diagnosis of disease and progression. The data of this article showed that there was a direct positive correlation between the salivary and the serum bio-markers levels, which it was proved that the salivary non-invasive examination had a significant association with the serum investigation.

Acknowledgments

Thanks to Erbil Hospital in Erbil/Iraq (for the all staffs) assist, which let me to collect samples of saliva and blood only without any funding source.

Conflicts of Interest

In this research, there is no conflict interest.

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