Measurement of salivary Tumor Necrosis Factor-alpha (TNF- α) in periodontitis patients with or without diabetes mellitus

ABSTRACT

Introduction: Tumor necrosis factor-alpha (TNF-α) is a proinflammatory mediator considered to be a soluble mediator released from immunocompetent cells. It exercises a wide range of proinflammatory and immunomodulatory effects in different cell populations, such as stimulating prostaglandin synthesis, promoting tumors in a variety of cancers, producing proteases, and activating osteoclastic function and therefore bone resorption. Its myriad functions suggest that TNF-α plays an important role in mediating the immune-inflammatory responses initiated by infection or other types of damage.
Aim: The aim of the present study is to evaluate the concentrations of Tumour Necrosis Factor- alpha (TNF- α) in the saliva of periodontitis patients with or without diabetes mellitus.
Materials and methods: A total of 30 participants were taken and divided into 3 groups with each group consisting of 10 saliva samples from patients with clinically healthy gingiva and patients with chronic periodontitis with diabetes mellitus and periodontitis patients only. Saliva sample was collected and salivary TNF- α was done using Enzyme Linked Immunosorbent Assay (ELISA). Statistically analyses was done by one way ANOVA test
Results: Salivary TNF- α concentration in periodontal health, periodontitis with diabetes mellitus (P+DM), Periodontitis only (P) were analysed. Salivary TNF- α concentration was found to be significantly higher ($p<0.05$) in periodontitis with diabetes mellitus (49±3.5 pg/ml) when compared with periodontitis only (29±3.6 pg/ml) and also when compared with healthy controls (17±1.4 pg/ml).
Conclusion: The present study showed elevated TNF- α in periodontitis patients with diabetes mellitus when compared to patients with periodontitis only and normal healthy patients. Further studies are required in a large scale to substantiate the role of TNF-α in the progression of periodontal diseases.

Keywords: Biomarker, Novel Study, Periodontitis, Saliva, TNF-α

1. INTRODUCTION

Periodontitis is the most common form of periodontal disease. It is more prevalent in adults but can occur in children. Although multifactorial in origin, three main factors are involved in periodontitis appearance and progression: accumulation of plaque and calculus; level of bacterial virulence; and cellular immune response[1] . Progression is slow to moderate, but more rapid periods of destruction can be observed, influenced mainly by systemic or environmental factors that can affect the normal interaction between host and bacteria [2]. Plaque accumulation and host response to it can be affected by local factors such as systemic diseases (e.g., diabetes mellitus, HIV), which can depress host defenses, and the environment.

Diabetes mellitus is a group of metabolic diseases characterized by hyperglycemia resulting from defects in insulin secretion, insulin action or both [3]. The prevalence of diabetes mellitus is increasing globally, as the number is expected to rise to 300 million by 2025 and 366 million by the year 2030. Currently, diabetes mellitus is diagnosed by evaluating blood glucose level, however, monitoring glycated haemoglobin levels is a frequent and relatively accurate measure of average glycaemic control[4]. Monitoring of biochemical parameters of diabetes mellitus such as fasting blood glucose levels and glycated haemoglobin levels, typically involves invasive techniques associated with pain and distress [5]. The development of non-invasive methods for frequent sampling of biomarkers is a growing area of research. Salivary biomarkers indicate the existence or the risk of developing a disease, as well as the response to a particular therapy[6]. Furthermore, saliva offers significant potential as a diagnostic tool for the monitoring the disease due to its similar proteomics with blood[7] and it is an inexpensive, simple and

non-invasive screening method. Salivary diagnostics is a rapidly emerging field that is dependent on the development of sensitive and specific biomarkers that can be employed in large scale clinical settings.

Tumor necrosis factor-alpha (TNF-α) is a proinflammatory mediator considered to be a soluble mediator released from immunocompetent cells. It exercises a wide range of proinflammatory and immunomodulatory effects in different cell populations, such as stimulating prostaglandin synthesis, promoting tumors in a variety of cancers, producing proteases, and activating osteoclastic function and therefore bone resorption[8]. Its myriad functions suggest that TNF-α plays an important role in mediating the immune-inflammatory responses initiated by infection or other types of damage [9]. During its initial production in the inflammatory response, TNF- α is also vital for maintaining chronic inflammation, angiogenesis, tissue remodeling, tumor growth, and metastasis; TNF- α blockers are therefore effective in treating a variety of acute and chronic inflammatory conditions [10].

TNF are grouped among the "major inflammatory cytokines", which are characteristically produced at the sites of inflammation by infiltrating mononuclear cells[11]. Tumor necrosis factor "family" includes two structurally and functionally related proteins, TNF-α or cachectin, mainly produced by monocytes and / or macrophages and TNF-β or lymphotoxin, a product of lymphoid cells [12].

It has also been reported that specific local blockage of IL-1 and TNF-α significantly reduced periodontal destruction in a monkey periodontitis model. In situ, hybridization and immunohistochemistry were used to show that TNF-α mRNA was abundant in macrophages and T-cells of the gingival tissues of patients with moderate to severe periodontitis[13]. These findings support the hypothesis that TNF-α could have some possible role in the inflammatory process and subsequent tissue destruction in periodontal disease. Evaluation of the contents of GCF is a promising, non-invasive method for determining the tissue changes in periodontium[14].

TNF-α production occurs in response to stimuli from cell types such as macrophages, neutrophils, keratinocytes, adipocytes, fibroblasts, and NK, T and B cells. High serum levels of this cytokine have been detected in patients with POD, suggesting it may be contributing to pathogenesis. Its activation also stimulates bone resorption by induction in osteoclast progenitor proliferation, and production of extracellular matrix metalloproteinases, cytokines, collagenase, and prostaglandins [15].

The biomarkers help in identifying susceptible patients and serve as surrogate endpoints for monitoring the end of therapy [16].It therefore meets the demands of being an inexpensive and noninvasive collection technique. Our team has extensive knowledge and research experience that has translated into high quality publications [17–29],[30–34] [35] [36]. The aim of the present study is to evaluate the measurement of Tumour Necrosis Factor-Alpha (TNF- α) in the saliva of periodontitis patients with or without diabetes mellitus.

2. MATERIALS AND METHODS

2.1. Study design

A total of 30 participants were taken and divided into 3 groups with each group consisting of 10 saliva samples from patients with periodontal health (Group a), patients with periodontitis without diabetes mellitus (Group b) and patients with periodontitis only (Group c).

2.2. Inclusion criteria

The criteria for the Periodontitis include: not >2 teeth missing in each quadrant; greater than or equal to 20% of periodontal sites with interproximal clinical attachment loss >2 mm; greater than or equal to 30% of periodontal sites with periodontal pocket depth greater than or equal to 4 mm; greater than or equal to 30% of sites showing bleeding on probing; and radiographic evidence of bone loss visible in posterior vertical bitewing films.

2.3. Exclusion criteria:

Exclusion criteria for periodontitis were individuals who had undergone periodontal treatment in the last 6 months; smoking or use of tobacco in any form; history of known systemic diseases that would alter the healing response of the oral tissues; acute periodontal conditions, such as periodontal abscess and acute necrotizing gingivitis; history of alcoholism; betel nut users; history of medications (antibiotics or anti-inflammatory drugs) in the last 6 months; and detection of any obvious oral mucosal lesion.

2.4. Saliva collection

Participants were instructed to refrain from drinking, eating and practicing oral hygiene procedures 12 hours before saliva collection. Whole unstimulated saliva was collected from all the patients using expectoration into sterile bulbs. Collected samples were immediately placed on ice and transported to the laboratory, where it was centrifuged at 5,000 rotations per minute for 15 minutes and the clear supernatants were stored in aliquots at -70°C. The samples were thawed and the assay was performed within 3 months of collection.

2.5 Statistical analysis

The triplicate analysis results of the experiments performed on control and test subjects were expressed as mean ± standard deviation. Results were analyzed statistically by one-way analysis of variance (ANOVA) and significant differences between the mean values were measured using Newman-Keuls multiple comparison test using Graph Pad Prism version 5. The results with the $p < 0.05$ level were considered to be statistically significant.

3. RESULTS AND DISCUSSION

Salivary TNF- α concentration in periodontal health, periodontitis with diabetes mellitus (P+DM), Periodontitis only (P) were analysed. Salivary TNF- α concentration was found to be significantly higher ($p<0.05$) in periodontitis with diabetes mellitus (49±3.5 pg/ml) when compared with periodontitis only (29±3.6 pg/ml) and also when compared with healthy controls (17±1.4 pg/ml).

The present study results indicate that good control of blood glucose in patients with periodontitis can directly influence expression of the TNF-α cytokine; however, low TNF-α concentrations are directly correlated with greater insertion loss and probe depth. Increases in TNF-α concentrations have been reported after nonsurgical periodontal treatment, with higher levels in healthy subjects than in unhealthy patients [7] [50]. According to Kadhiresan Rathinasamy et al., [37]patients with generalized chronic periodontitis, which was a common disease, were taken as the study group. Although the results yielded were not statistically significant, to promote the use of TNF-α as serum and salivary biomarkers in predicting the risk of development of systemic disease such as diabetes mellitus. When the TNF-α levels were compared between serum and saliva in the healthy group and diseased group, there was a marginal increase in the diseased group compared with the healthy group, but they were not statistically significant.

According to Teles et al., [37,38] when chronic periodontitis patients within an age range of 17–30 years were assessed for their salivary level of TNF-α, the periodontitis patients showed a higher level when compared with the control group, and the slight difference in the results was attributed to reasons such as different age groups, limited study samples, and dissimilar sampling and evaluation techniques.

According to Prince Jain et al.,[39] a positive association existed between periodontal disease and increased levels of TNF-α in serum. It can be concluded that there is a prospect of using the estimation of TNF-α in serum as a “marker” of periodontal disease in future. However, it remains a possibility that the absence or low levels of TNF-α in serum might indicate a stable lesion and elevated levels might indicate an active site but only longitudinal studies taking into account, the disease “activity” and “inactivity” could suggest the possibility of using TNF-α in serum as an “Indicator” of periodontal disease.

Table 1: Comparison of salivary caspase-9 levels among 3 groups (patients with periodontitis only- P, patients with periodontitis along with diabetes mellitus- P+DM, patients with periodontal health). The values are expressed in pg/ml.

GROUP	PERIODONTAL HEALTH (Group a)	P+DM (Group b)	P (Group c)	P- value
TNF-α	17±1.4	49±3.5	29±3.6	P<0.0041

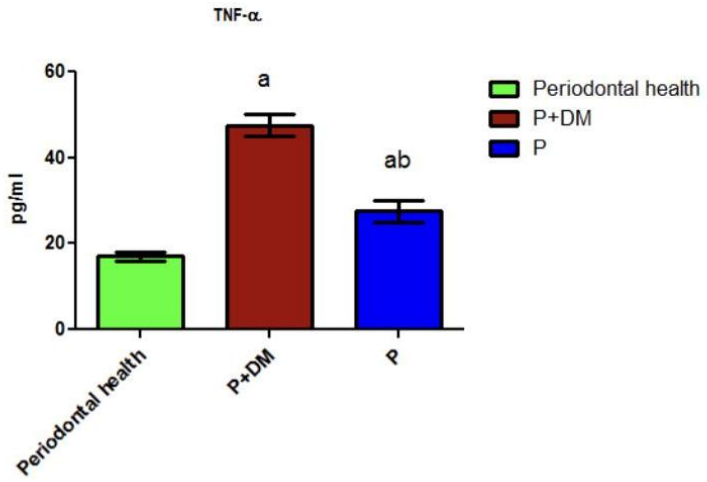

FIGURE:1 Assessment of salivary TNF-α levels among periodontal health, periodontitis only and periodontitis with diabetes mellitus. The levels of salivary TNF-α were assessed by the Enzyme Linked Immunosorbent Assay (ELISA) method. Significance at p <0.05, a- compared with the periodontal health group. b-compared with periodontitis along with diabetes mellitus.

4. **CONCLUSION:**

Salivary TNF-α levels were found in detectable quantities. They showed a marginal increase in chronic periodontitis patients with or without diabetes mellitus when compared with normal healthy patients in the absence of systemic diseases. Further studies are required in a large scale and with different methodologies to substantiate the role of TNF-α in the progression of periodontal diseases.

COMPETING INTERESTS DISCLAIMER:

Authors have declared that no competing interests exist. The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

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