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Transforming Growth Factor Beta as Salivary Biomarker in Periodontitis Patients with or without Diabetes Mellitus

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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Original Research Article

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ABSTRACT

Aim: To estimate the salivary TGF-beta levels in periodontitis patients with or without diabetes mellitus
Materials and methods: Thirty patients [15 males and 15 females] were included in this study and divided into three groups. Group a included 10 participants with periodontal health. Group b included 10 participants with periodontitis and diabetes mellitus. Group c included 10 participants with periodontitis only. Saliva samples were collected and TGF-beta levels were compared between the groups using Sandwich-enzyme linked immunosorbent assay by commercially available human TGF-beta 96 well ELISA kit. The data were statistically analysed by One-Way-ANOVA. Newman-Keuls multiple comparison test was used to test the significance at the levels of $P< 0.05$.
Results: TGF- beta level was found to be significantly higher ($p<0.05$) in periodontitis with diabetes mellitus (108 ±7.1pg/ml) when compared with periodontitis only (77±3.5 pg/ml) and also when compared with healthy controls (66±5.6 pg/ml).

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Conclusion: The results indicate that TGF beta level was found to be increased in the saliva of patients with periodontitis with diabetes mellitus than healthy controls, suggesting that diabetes mellitus and periodontitis show detrimental effects on each other through TGF beta pathway and thus estimation of salivary TGF beta levels may help to monitor the periodontal disease severity in diabetic patients.

Keywords: Diabetes mellitus; innovative technology; saliva; TGF-beta; periodontitis.

1. INTRODUCTION

Human periodontal diseases can lead from accumulation of bacteria that causes interaction of the biofilm formation within the periodontal structures, each of which is modulated by individual hosts' immune and inflammatory response [1]. Inflammatory cytokines play a significant role in initiation and progression of periodontal diseases by releasing upon the microbial colonies [2]. It also establishes the balance between catabolic and anabolic activities by maintaining the tissue hemostasis during the course of periodontitis [3]. These cytokines show evidence of considerable levels in other severe conditions such as diabetes mellitus and cancer [3,4].

Transforming growth factor beta [TGF-beta] is an anti-inflammatory cytokine produced by T-cells and macrophages. It has potent immune suppressant activity and plays a significant role in collagen metabolism [5]. On the other hand, Diabetes mellitus is a well-known risk factor for periodontitis, as several investigations have shown that both increase the prevalence, severity and progression of disease [6]. Reduced levels of TGF-beta may cause impairment of the wound healing process which is associated with diabetes mellitus. Multiple previous studies reported varied levels of TGF beta in diabetes mellitus patients. A study reported by Rivarola et al., concluded increased levels of TGF beta in urinary samples of diabetes mellitus patients compared to healthy individuals [7]. Also a study by Ahluwalia et al., reported TGF beta is one of the most common inflammatory cytokines seen in diabetes mellitus patients [8]. Our team has extensive knowledge and research experience that has translated into high quality publications [9–28]. Hence the current study analyses the salivary TGF-beta in periodontitis patients with or without diabetes mellitus.

2. MATERIALS AND METHODS

2.1 Study Design

Patients aged 20 to 50 years, visiting the Saveetha Dental College from July 2020 to November 2020 were examined. Thirty patients (15 males and 15 females) were included in this study and subdivided into three groups. Group a -10 participants with periodontal health. Group b - 10 patients with periodontitis and diabetes mellitus. Group c - 10 patients with periodontitis only.

2.2 Inclusion Criteria

Healthy periodontium of similar age, and gender who had <10% of sites with bleeding on probing, no sites with probing depth ≥4 mm, no clinical attachment loss >2 mm were included in group a. The inclusion criteria for periodontitis in group b and c were as follows: no more than >2 teeth missing in each quadrant; >30% of periodontal sites with PD >4 mm; >20% of periodontal sites with interproximal clinical AL >2 mm; >30% of sites showing BOP.

2.3 Exclusion Criteria

Patients with any other systemic diseases other than diabetes mellitus, smoking habit, history of periodontal treatment in the last 6 months, betel nut users, alcoholism.

2.4 Saliva Collection

Participants were instructed to refrain from eating, drinking, and practicing oral hygiene procedures 12 hours before saliva collection. Whole unstimulated saliva was collected from all patients using spitting into saliva containers. Collected samples were transported to the laboratory and assessed for TGF beta assay using ELISA method.

2.5 Measurement of TGF-beta Levels

TGF beta levels in saliva samples were measured in duplicate using a commercially available enzyme-linked immunosorbent assay kit, which was specific for human TGF beta. This assay used the quantitative sandwich enzyme immunoassay technique. The samples were diluted with the calibrator diluent provided with

the kit in the ratio of 1:4, and the assay was performed according to the manufacturers' instructions. Standards were included in each run and all results were reported within the linearity of the assay. The results of the colorimetric reaction were read as the value of the optical density directly on the automatic microplate reader set to 450 nm. The values obtained were multiplied by the dilution factor so as to obtain the actual concentration of TGF beta. The results were reported as concentration of TGF beta in picogram per milliliter of sample.

2.6 Statistical Analysis

Obtained results are tabulated into excel sheets and imported to Statistical Package of Social Sciences (SPSS version 17). The data were statistically analysed by One-Way -ANOVA. Newman-Keuls multiple comparison test was used to test the significance at the levels of P<0.05.

3. RESULTS AND DISCUSSION

From the study, Table 1 shows the mean values of TGF beta level (pg/ml) in all the thirty samples separately in patients with healthy periodontium, periodontitis with diabetes mellitus and periodontitis patients only. TGF- beta level was found to be significantly higher (p<0.05) in periodontitis with diabetes mellitus (108 ±7.1pg/ml) when compared with periodontitis only (77±3.5 pg/ml) and also when compared with healthy controls (66±5.6 pg/ml) with significance of *P*<0.0001 (p<0.05 which is statistically significant). Fig. 1 represents the levels of TGF beta in patients with healthy periodontium, periodontitis patients with diabetes mellitus and patients with only periodontitis. TGF-beta was shown to be higher in periodontitis patients with diabetes mellitus followed by patients with periodontitis only and in periodontal health.

Transforming growth factor-β1 [TGF-β1] are thought to play important roles in modulating the proliferation and/or migration of structural cells involved in inflammation and regulation of immune responses. The alteration in immune response is usually downregulated by TGF beta enzyme thereby plays a significant role in pathogenesis of systemic diseases [29]. Various structural cells such as neutrophils, monocytes, and lymphocytes are being modulated in such a way that altered proliferation and/or migration takes place to get involved in an inflammatory process which is being controlled by TGF beta [30]. TGF beta secretion leads to increased eradication of inflammation through apoptotic mechanisms [31].

Table 1. Comparison of TGF beta levels in all the three groups. (periodontitis patients- P, patients with Periodontitis along with diabetes mellitus- P+DM and patients with periodontal health). The values are expressed in pg/ml

Group	Periodontal health	P+DM	P	*P* value
TGF- beta (pg/ml)	66 ± 5.6	108 ±7.1	77 ±3.5	*P*<0.012

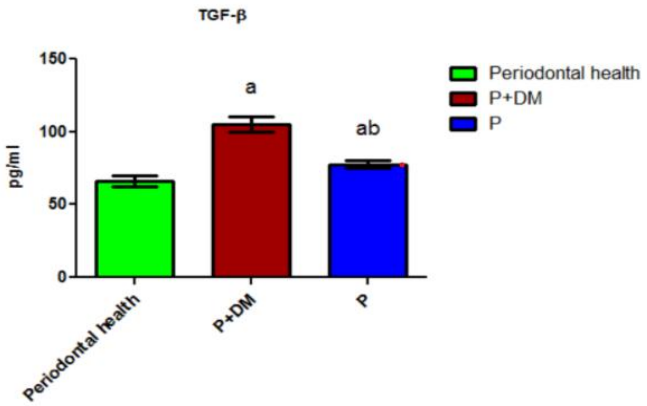

Fig. 1. Assessment of salivary TGF beta concentration among periodontal health, periodontitis with diabetes mellitus and periodontitis only. The levels of salivary TGF beta were assessed by Enzyme linked immunosorbent assay method. Significance at *P*<0.05, a - compared with periodontal health group. b - compared with periodontitis with diabetes mellitus

From the results of the current study, it is proved that TGF beta is higher in periodontitis patients with diabetes mellitus than the patients with only periodontitis and periodontally healthy gingiva. The result is found to be statistically significant. The similar results were obtained in a study conducted by Prasath et al., proving that TGF beta is elevated among periodontitis patients with diabetes mellitus [12]. In case of periodontally healthy gingiva, the response of the body's immune system is less due to minimal provocation of cytokine release following which no inflammation is established. In patients with only periodontitis, the TGF beta acts as immunosuppressive cytokine which stimulates the wound healing [32]. Though the glycemic control of diabetes is normal, periodontal tissues frequently manifest the changes in cytokine level because they are constantly wounded by substances emanating from bacterial biofilms present in and around periodontal ligaments [33]. A previous study proved that glucose control is inversely proportional to the severity of periodontal disease [34]. Also a study conducted by Steinsvoll et al., stated that the level of TGF beta is comparatively higher in regions of deep periodontal pockets than the region which is less severe [35]. Upregulation of the cytokine in inflamed gingiva may counterbalance for destructive gingival inflammatory responses that are simultaneously taking place in patients with associated systemic illness such as diabetes. This elevation of TGF beta is slightly higher in patients with diabetes than the patients with only chronic periodontitis. The main cause for destruction of alveolar bone in periodontitis is due to increased expression of RANKL which is affected by diabetes [36]. Moreover, the oral microbial flora is modified by supporting the inflammatory process and bone loss where certain bacteria are hyperresponsive to TGF beta release [37]. Genetic factors, local microbial quality, and contributions from other cytokines sharing similar biologic activity may also influence TGF beta levels [38].

Previous literature stated that various TGF beta genotypes were established by collecting samples from gingival crevicular fluid of patients affected with periodontitis and diabetes [20]. This brings into light the host response, which varies from patient to patient and the variation among patients in their ability to respond to a particular treatment. Also, the quality of bacterial challenge is known to influence TGF beta levels. Genetic factors, local microbial quality, and contributions from other cytokines sharing similar biologic activity may influence TGF beta levels. Since multiple studies enumerated the importance of TGF beta in periodontium, it must be taken into consideration in the field of assessing the periodontal health for providing an accurate diagnosis.

4. CONCLUSION

This study proved that TGF beta levels are high in periodontitis patients with diabetes mellitus followed by patients with only periodontitis and least in periodontally healthy patients. The interrelationship of periodontitis and diabetes mellitus is established through TGF beta as a salivary marker. Thus, estimation of TGF beta levels may potentially aid in distinguishing healthy state from diseased conditions and also monitoring the periodontal disease activity in diabetes patients. Further studies are required with larger samples to prove TGF beta as a standard salivary biomarker for periodontal disease.

DISCLAIMER

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.

CONSENT

Informed consent has been taken from the patients participating in this study

ETHICAL APPROVAL

As per international standard or university standard written ethical approval has been collected and preserved by the author(s).

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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