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Research Article

Relationship between Inflammation-Related Markers in Peri-Implant Crevicular Fluid and Clinical Parameters in Partially Edentulous Saudi Arabian Diabetic Subjects

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Abstract

Introduction: A high prevalence of diabetes mellitus and dental caries in Saudi Arabian population is overwhelmingly reported. The aim of the study was to assess the following: 1) clinical findings of PD, BOP, and amount of keratinized tissue present around dental implant and 2) PICF levels of IL-1α, IL-1β, IL-6, and IL-8 in diabetic and non-diabetic partially edentulous Saudi Arabian subjects.

Materials and Methods: Total of 32 partially edentulous subjects, 16 diabetic and 16 non-diabetic, were included in the study. PICF samples were taken from peri-implant sulcus using paper point and PCR essays for IL-1α, IL-1β, IL-6, and IL-8 was performed. Clinical parameters of peri-implant PD, BOP and amount of keratinized tissue were recorded.

Results: Diabetic subjects had more significant BOP than non-diabetic counterparts (p-<0.001). No significant difference was found between diabetic and non-diabetic patients in the amount of keratinized tissue ($P < 0.765$). A significant high level of IL-6 in diabetic subjects when compared to healthy subjects ($P < 0.033$). No significant difference between diabetic and non-diabetic subjects in the serum level of IL-1α ($P < 0.121$), IL-1β ($P < 0.171$) and IL-8 ($P < 0.223$). A positive correlation between increased serum level of IL-1α, IL-1β, IL-6 and IL-8 with increased PD was determined.

Conclusion: PD, BOP and PICF IL-6 levels were found to be significantly higher in Saudi Arabian diabetic subjects compared to Saudi non-diabetic subjects. Also, a positive relation found between deep PD and four inflammatory markers which are IL-1α, IL-1β, IL-6 and IL-8.

Keywords: Implant; Diabetes; Peri-Implant Crevicular Fluid; IL-1α; IL-1β; IL-6; IL-8; Diabetic Saudi Arabian

Introduction

Cytokines have been reported to be elevated in chronically inflamed gingival tissue, as well as in the gingival crevicular fluid from patients with periodontitis [1]. Interleukin1 (IL-1) family has a central role in triggering and perpetuating immune and inflammatory responses [2,3]. Interleukin-1β (IL-1β) is one of the most important cytokines in osteoclast formation and bone resorption and were therefore targeted by the majority of the reviewed studies [4]. Interleukin-6 (IL-6) induces bone resorption solely in conjunction with other bone-resorbing agents [5,6]. Presence of interleukin-8 (IL-8) has shown to be highly linked to increased susceptibility to periodontitis [6,7]. It has also been reported that inflammatory cytokines such as IL-1α, IL-1β, IL-6 and IL-8 are present in inflamed periodontal tissue and their significant production seems to facilitate chronic leukocyte recruitment and tissue destruction [2]. Monitoring cytokines may allow us to diagnose the status of periodontal disease and patients' susceptibility to it [2].

Type 2 diabetics are 2.8 times more likely to have periodontal disease characterized by clinical attachment loss and 3.4 times more likely to express radiographic bone loss [8]. Hyperglycemia could be an important factor in the development of biological complications for dental implants especially in poorly controlled diabetes [9]. The peri-implant crevicular fluid (PICF) is an osmotically mediated inflammatory exudate originating from the vessels of the gingival plexus. Its composition is similar to that of the gingival crevicular fluid (GCF) by containing host-derived enzymes and their inhibitors, inflammatory mediators, host-response modifiers, and tissue breakdown products [10,11].

Prevalence of diabetes mellitus type 2 (T2DM) in Saudi Arabia is 23.1% [12]. The World Health Organization has reported that the Saudi Arabia diabetes mellitus rate ranks as the second highest in the Middle East and seventh worldwide, which corresponds to 7 million of the Saudi Arabia population as diabetic and almost 3 million are pre-diabetic [13]. From indexed literature up to date, there are no published reports assessing the cytokine presence in PICF in Saudi Arabian diabetic patients.

Aim of the Study

The aim of the present study was to: 1) assess clinical findings of probing depth, bleeding on probing, and amount of present keratinized tissue around dental implants and 2) examine PICF levels of IL-1α, IL-1β, IL-6, and IL-8 among diabetic and non-diabetic Saudi Arabian subjects. The research hypothesis is that diabetic and non-diabetic Saudi Arabian individuals will exhibit the same level of cytokine in the PICF.

Materials and Methods

Ethical approval

Ethical approval for this study was obtained from the Ethics Committee of the Research Center and the study was done in compliance with the National Institute of Health guidelines for the care and use of laboratory animals (NIH Publication #85-23 Rev.1985). The exclusion criteria were: 1) systemic disease such as cardiovascular disorders and renal disorders, 2) systemic antibiotic or steroid intake within the last three months, 3) history of periodontal treatment within the previous 3 months, 4) radiation and/or chemo therapy during the last six months and 5) tobacco smoking. This was a parallel study design where each group has 16 subjects. The groups are: Group 1 consisted of diabetic patients and Group 2 of non-diabetic patients. Diagnosis of T2DM was confirmed by patient physician and a report of their last HA1C. Subjects voluntarily enrolled in the study and signed a written consent form.

Clinical examination

All clinical and radiographic evaluations were performed by one investigator. Intracalibration for the examiner was recorded and calculated kapa index was 0.92. Peri-implant probing depth (PD) was measured at six sites, and those sites were mesiobuccal, midbuccal, distobuccal, distolingual, midlingual, and mesiolingual. Bleeding on probing (BOP) and amount of keratinized tissue at midfasial were measured (Table 1). Clinical examination was measured using University of North Carolina probe (UNC-15, Hu-Friedy, Chicago, IL, USA).

Collection of peri-implant crevicular fluid samples

All examination sites were swabbed with 2 x 2 sterile gauze to clean the surrounding tissue from debris. PICF samples were collected using sterile paper point #35 (Meta Biomedm, Chungcheongbuk-do, South Korea). The sterile paper point was inserted in the peri-implant pocket and held in place for 60 seconds; then the paper point was transferred to sterile tubes containing 500 µL of sterilized elution buffer (0.05% polysorbate 20 in phosphate-buffered saline pH 7.4). Samples were subjected to centrifugation (HERMLE Labortichnik GmbH, Wehingen, Germany) at 13,000 rpm for 15 minutes.

Biochemical analyses

IL-1α, IL-1β, IL-6 and IL-8 were measured using the Milliplex Map kit (Millipore, Billerica, MA, USA) in the FlexMAP 3D (Luminex Corp, Austin, TX, USA) according to the manufacturer's instructions. Minimum detectable concentrations were as follows: IL-6, 0.9 pg/ml; IL-1α, 9.4 pg/ml; IL-1β, 0.8 pg/ml; and IL-8, 0.4 pg/ml. The intra-assay variation was as follows: IL-6, 2.0%; IL-1α, 3.3%; IL-1β, 2.3%; and IL-8, 1.9%, and inter-assay variation of: IL-6, 18.3%; IL-1α, 12.8%; IL-1β, 6.7%; and IL-8, 3.5%.

Statistical analysis

The data analysis was performed using Statistical Packages for Social Sciences (SPSS) version 21 (SPSS, Chicago, IL, USA). Descriptive statistics were presented using mean and standard deviation or counts and proportion (%) whenever appropriate. The statistical association between the characteristics of each study group (diabetic and non-diabetic) were conducted using an independent t-test or Chi-square test whenever applicable. Binary regression analysis was also conducted to predict the influence of PD, cytokine and bleeding on probing among diabetic patients where the odds ratio as well as 95% CI were also being reported. A P value of < 0.05 was considered statistically significant and a P value of <0.01 was considered highly statistically significant.

Results

Results show a higher and significant probing depth in diabetic group around dental implants (Table 1, $p < 0.004$). BOP was the most significant clinical measurement in diabetic subjects (Table 1, $p < 0.001$). There was no significant difference in the amount of keratinized tissue between diabetic and non-diabetic subjects (Table 1, $p < 0.765$). PICF IL-6 was the only inflammatory marker present with significantly elevated levels in the diabetic group when compared to non-diabetic patients (Table 2, $p < 0.033$). All remaining cytokines had no significant difference between the two groups (Table 2, IL-1α ($p < 0.121$), IL-1β ($p < 0.171$) and IL-8 ($p < 0.223$, Figure 1). IL-1α and IL-6 were significantly higher in subjects who had BOP (Table 3, ($p < 0.010$), ($P < 0.02$), respectively). IL-1β and IL-8 did not have any significant correlation with BOP. A positive significant correlation was found between PD and the following markers: IL-1α ($p < 0.006$), IL-1β ($p < 0.001$), IL-6 ($p < 0.018$) and IL-8 ($p < 0.002$) (Table 4). Current results suggest a correlation between increased serum levels of IL-1α, IL-1β, IL-6, and IL-8 with increased PD.

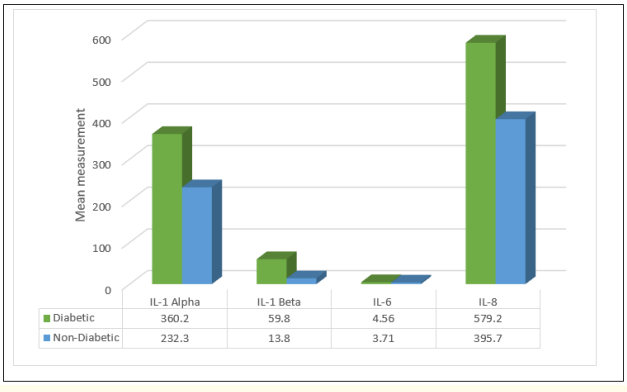

Figure 1: Mean comparison of IL-1 alpha, IL-1 beta, Il-6 and IL8 among diabetic and non-diabetic patients.

Factor	Diabetic (n = 16) Mean ± SD	Non-diabetic (n = 16)Mean ± SD	P-value
Keratinized tissue (mm)[a]	2.94 ± 1.48	2.81 ± 0.75	0.765
Probing Depth (mm)[a]	3.09 ± 0.66	2.51 ± 0.32	0.004**
bleeding on probing (%)[b]	87.5%	37.5%	< 0.001**

Table 1: Statistical association between Keratinized tissue, probing depth and bleeding on probing among diabetic and non-diabetic patients (n = 32).

[a]: P-value has been calculated using independent t-test.

[b]: P-value has been calculated using Fischer exact test.

** Significant at $p < 0.05$ level.

Cytokine parameters	Diabetic (n = 16) Mean ± SD	Non-diabetic (n = 16) Mean ± SD	P-value §
IL-1α (pg/mL)	360.2 ± 282.7	232.3 ± 152.2	0.121
IL-1β (pg/mL)	59.8 ± 130.7	13.8 ± 9.61	0.171
IL-6 (pg/mL)	4.56 ± 1.17	3.71 ± 0.95	0.033**
IL-8 (pg/mL)	579.2 ± 546.8	395.7 ± 220.6	0.223

Table 2: Statistical association between cytokine parameters among diabetic and non-diabetic patients (n = 32).

§: P-value has been calculated using independent t-test.

**: Significant at $p < 0.05$ level.

Cytokine	Bleeding on probing (n = 16) Mean ± SD	P-value §
IL-1α	375.4 ± 253.8	0.010 **
IL-1β	53.1 ± 116.9	0.210
IL-6	4.49 ± 1.07	0.021 **
IL-8	569.3 ± 497.4	0.158

Table 3: Correlation between bleeding on probing and cytokine markers among diabetic and non-diabetic patients (n = 32).

§: P-value has been calculated using independent t-test.

** Significant at $p < 0.05$ level.

Factor	PD R-value	P-value
IL-1α	0.479	0.006**
IL-1β	0.564	0.001**
IL-6	0.416	0.018*
IL-8	0.537	0.002**

Table 4: Correlation (Pearson-R) between probing depth and cytokine markers among diabetic and non-diabetic patients (n = 32).

**: Significant at $p < 0.01$ level (2-tailed).

*: Significant at $p < 0.05$ level (2-tailed).

Discussion

Present study showed PICF concentration of IL-6 was significantly higher in diabetic than in non-diabetic Saudi Arabian individuals. These results are consistent with previous studies [14-19]. Immunohistochemical studies have observed that a higher level of IL6 was expressed in inflamed gingival tissue than in healthy control tissue [20]. Previously published reports used a reverse transcription polymerase chain reaction (RTPCR) and ELISA to demonstrate mRNA and protein expression which have shown that IL6 serum levels were high in patients with periodontal diseases [21,22]. Also, our results confirmed the positive significant correlation between IL-6 and BOP in Saudi diabetic patients. Significant elevation of IL-6

usually associated with peri-implantitis [23]. Diabetic patient has increased odds of increased bio gingival biofilm with increase IL-6 [24]. This could suggest close maintenance program for T2DM patients with dental implants.

In our study, PICF concentrations of IL-1α, IL-1β, and IL-8 showed no significant difference between diabetic and non-diabetic Saudi Arabian patients. Others have confirmed the high overexpression and serum level of IL-1α, IL-1β, and IL-8 in periodontitis patients around natural teeth [25-27]. Some factors such as subjects' ethnic background, age, gender, how well controlled their diabetes is, and the onset of diabetes may make the comparisons impossible to do between these studies [27,28].

Our results confirm the correlation between elevated PICF concentrations for IL-1α, IL-1β, IL-6 and IL-8 with increased PD. This positive correlation is a proportional one. Similar findings have been reported that the increased levels of IL-1β correlate positively with increasing mean of probing depth around dental implants [29]. Another study showed that increased levels of IL-6 and IL-8 are associated with chronic periodontal disease and peri-implantitis sites in patients with T2DM [30]. Because of implant platform relative to the crestal bone position may create some crestal bone loss which may favor the colonization of anaerobic gram-negative species which may be involved in triggering pro-inflammatory responses [29,31].

This study identified a correlation between elevated PICF concentrations of IL-1α and IL-6 with BOP in Saudi Arabian diabetic subjects. Similar results reported elevated concentrations of IL-1β and IL-6 in PICF sites associated with mucositis [32]. Profuse bleeding and suppuration in untreated peri-implantitis can be associated with higher PICF concentrations of IL-1β, IL-8, TNF-a, and VEGF [33]. IL-6 was found to be more sensitive to the severity of the inflammation in peri-implant tissue [34]. IL-6 is one of the most investigated pro-inflammatory cytokines between healthy and diseased peri-implant tissues [34-36].

Conclusion

Up to date, there are no published report studying the cytokine presence of PICF in Saudi Arabian diabetic patients. PD, BOP and PICF IL-6 levels were found to be significantly higher in Saudi Arabian diabetic subjects compared to Saudi non-diabetic subjects. Also, a positive relation found between deep PD and four inflammatory markers which are IL-1α, IL-1β, IL-6, and IL-8. The main limitation of this study was the small sample size. Future studies on the Saudi Arabian diabetic population with a larger sample size representing all different regions in Saudi Arabia. Also, it is important to investigate the impact of cytokine in PICF and its impact on the glycemic level of Saudi Arabia diabetic patients.

Conflicts of Interest

The authors have no conflicts of interest to declare.

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