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Research Article

Candida Species Around Dental Implants in Saudi Arabian Diabetic Patients

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Abstract

Introduction: Reports confirm high prevalence of diabetes mellitus and dental caries associated with early tooth loss often replaced by dental implants in the Saudi Arabian society. This study aims to (1) assess clinical periodontal parameters of probing depth (PD), bleeding on probing (BOP) and amount of keratinized tissues (KT) around dental implants and (2) identify and type the fungal colonization present in the peri-implant crevicular fluid (PICF) in diabetic and non-diabetic Saudi nationals.

Materials and Methods: Thirteen individuals were divided into two groups: diabetics (Group I, n = 6) and non-diabetics (Group II, n = 7). PICF Serum samples were collected using paper points. Fifty-two agar plates were prepared: Group I with 24 and Group II with 28. Plates of agar without collected serum served as positive control and plates without *C. albicans* served as negative control. Microbiological analysis for *Candida* species included: 1) clinical strains of *Candida albicans*, *Candida albicans* ATCC 10231, *Candida parapsilosis* ATCC 22019, *Candida glabrata* ATCC 2001 and *Candida tropicalis* ATCC 750. All plates were incubated at 37°C for 1, 24, 48, 72 and 96-hours' periods. At each time period, the presence of fungal colonies was assessed and recorded. Clinical periodontal parameters of PD, BOP, and amount of KT around dental implants were recorded.

Results: Mean PD in Group I (4.83 ± 0.75 mm) was significantly higher than Group II ($P \leq 0.001$). BOP in Group I (83.30%) was significantly higher than Group II ($P < 0.029$). No significant difference was found in the amount of KT between the two study groups ($P > 0.960$). Fungal growth colonies in both groups were similar ($P > 0.05$).

Conclusion: Significantly higher BOP, PD in Saudi diabetic subjects compared to Saudi non-diabetic subjects. Candida species are present in PICF of Saudi Arabian subjects with slightly more frequent observation seen in diabetic subjects.

Keywords: Candida albicans; Dental Implants; Diabetic Saudi Arabian; Peri-Implant Crevicular Fluid; Fungi Around Dental Implants

Abbreviations: BOP: Bleeding on Probing; C. albicans: Candida albicans; DM: Diabetes Mellitus; KT: Keratinized Tissues; PD: Probing Depth; PICF: Peri-Implant Crevicular Fluid

Introduction

Fungus derived from biofilm can notoriously form on the surface of implanted medical devices and dental implants [1-3]. It was reported that *Candida albicans* (*C. albicans*) has high adhesion to dental implants [4]. Gökmenoğlu., *et al.* [3] have shown that *C. albicans* biofilms can adhere firmly to implant material surfaces. Dental

implant surface biofilm differs between healthy and failing dental implants [5-8]. A report [8] confirmed similarities in subgingival microbiota in periodontal pockets around natural teeth and dental implants.

Diabetic individuals are vulnerable to fungal infections, including *Candida* species, owing to compromised host immunity [9,10]. *C. albicans* have been detected in peri-implant pockets, and they are relatively opportunistic in nature [11]. Fungi are opportunistic microorganisms that can enhance their virulence through increased hyphae formation, providing a site of adhesion for countless pathogenic bacteria and upregulating hydrolytic enzymes [12,13]. Furthermore, *C. albicans* can alter the microbiota around dental implants by increasing the number of anaerobic bacteria in the biofilm, which in turn may result in greater soft tissue inflammation and further loss of supporting bone around dental implants [12,14].

Current prevalence of type-II diabetes mellites (DM) in Saudi Arabia is 23.9% [15]. The World Health Organization reported Saudi Arabia's DM rate as second highest in the Middle East and seventh in the world. Seven million people in Saudi Arabia are diabetic, and almost three million Saudi citizens are pre-diabetic [16]. To date, there are no published reports in the literature assessing fungal presence in the peri-implant crevicular fluid (PICF) of Saudi diabetic patients. The aim of the current study is to: 1) assess clinical periodontal parameters of probing depth (PD), bleeding on probing (BOP), and amount of keratinized tissues (KT) around dental implants, and 2) identify and type the fungal colonization present around dental implants in diabetic and non-diabetic Saudi nationals. Our hypothesis is that the two study groups have similar fungal colonization around dental implants.

Materials and Methods

Ethical approval

Ethical approval for this study was obtained from the Ethics Committee of the Research Center at Riyadh Elm University, Riyadh, Saudi Arabia. The study was conducted in compliance with the Declaration of Helsinki, as revised in 2013 for experiments involving human patients. Participants voluntarily enrolled in the study and signed a written consent form. Exclusion criteria were as follows: (1) subjects with systemic disease, such as cardiovascular disorders and renal disorders; (2) subjects who had antibiotic or steroid intake within the previous three months, (3) history of periodontal treatment within the previous three months, (4) radiation and/or chemotherapy during the previous six months and (5) tobacco smoking. We employed a parallel study type in which 13 subjects where enrolled: diabetics (Group I) and non-diabetics (Group II). Diagnosis of type 2 DM was confirmed by patients' physicians, along with a report of the most recent HbA1c level within the last six months.

Clinical examination

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

Collection of serum peri-implant crevicular fluid samples

All examined sites were swabbed with a 2 x 2 sterile gauze to clean debris from the surrounding tissue. 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 crevice, held in place for 60 seconds, and then placed in a sterile tube with 2 ml of Sabouraud dextrose broth (SDB) (Lab M®, Lancashire, UK). Samples were mixed for one minute using a vortex mixer, followed by centrifugation (HERMLE Labortichnik GmbH, Wehingen, Germany) at 12,000g for five minutes. The samples were then sent for microbiological analysis for *Candida* species clinical strains.

Microorganisms and agar plates preparation

Stock culture of *C. albicans*, *C. parapsilosis*, *C. glabrata* and *C. tropicalis* were obtained from American Type Culture Collection (ATCC). The *Candida* species selected for the study are *Candida albicans* ATCC 10231, *Candida parapsilosis* ATCC 22019, *Candida glabrata* ATCC 2001 and *Candida tropicalis* ATCC 750. Cells were grown in the plates for 24 hours at 37°C (Kord-Valmark, Ontario, Canada) using the Sabouraud dextrose agar (Winlab, Leicestershire, England). The agar compound was thoroughly combined into a uniform mixture, poured into sterile Petri dishes (Kord-Valmark, Ontario, Canada) with a diameter of 90 mm, and allowed to set completely.

Quantitative measurement

The fungal growth of the microorganisms was evaluated using an antimicrobial screening program, as recommended by the National Committee for Clinical Laboratory Standards (NCCLS) [17]. Fresh inoculation of the fungi was prepared by growing a medium culture overnight. The medium was diluted in a pre-warmed broth to reach a final density of 10^4 CFU/spot, as recommended by the NCCLS. The compound was declared active when complete suspension of growth was observed. Fifty-two agar plates were prepared and divided into 24 and 28 plates for the diabetic and control groups, respectively. Ten microliter aliquots of the test *C. albicans*, *C. parapsilosis, C. glabrata* and *C. tropicalis* were taken from the stock culture and plated in duplicate in a radial pattern on the agar compound of the diabetic and non-diabetic groups. The agar plates of all study groups were incubated aerobically at 37°C and evaluated at 1-, 24-, 48-, 72-, and 96-hour time intervals. At each interval, the presence or absence of *C. albicans*, *C. parapsilosis*, *C. glabrata*, and *C. tropicalis* colonies were assessed and recorded.

Results

Statistical analysis results

Statistical Packages for Social Sciences (SPSS) version 22 (Armonk, NY: IBM Corporation) was used for data analysis. Data were presented as mean ± standard deviations (SDs). Independent t-test or Chi-square test was used to analyze the statistical association between the characteristics in each study group whenever appropriate. The results were considered significant if the P value ≤ 0.05.

Clinical results

Mean peri-implant probing depth in the diabetic group was 4.83 ± 0.75 mm, which was found to be statistically significant as diabetic subjects had higher peri-implant PD than non-diabetic counterparts (P ≤ 0.001; Table 1). A significant BOP of 83.30% was higher for diabetic subjects, compared to non-diabetic subjects (P > 0.029; Table 1; Figure 1). No significant difference was found in the amount of KT between the two study groups (P > 0.960; Table 1).

Microbial results

Fungal growth colonies were more common in diabetic subjects but with no significant difference from healthy subjects (P > 0.05). All samples showed fungal growth of all *Candida* strains in all the time intervals investigated (Table 2).

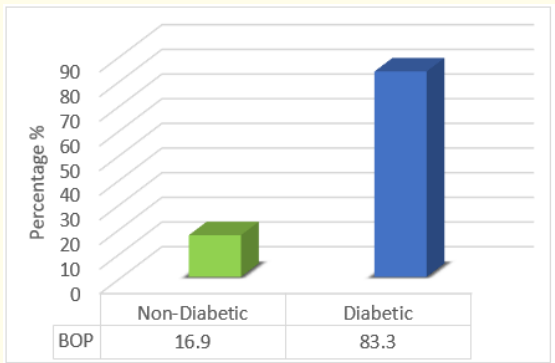

Figure 1: Mean bleeding on probing (BOP+) measurements for diabetic and non-diabetic subjects.

Parameter	Diabetics Mean ± standard deviation (SD)	Non-Diabetics Mean ± standard deviation (SD)	P-Value
Number of individuals (n)	6 (Male = 2, Female = 4)	7 (Male = 4, Female = 3)	-
BOP (%)	83.3%	16.9%	P < 0.029 a) **
KT (mm)	3.5 ± 1.5 mm	3.5 ± 3.1 mm	P > 0.960 b)
PD (mm)	4.83 ± 0.75 mm	3.28 ± 0.48 mm	P ≤ 0.001 b) **

Table 1: Statistical association between clinical parameters measured for the study subjects, (n = 13).

BOP: Bleeding on Probing , KT: Keratinized Tissue; PD: Probing Depth.

a) P-value calculated using Fischer exact test.

b) P-value calculated using independent t-test.

** Significant at p ≤ 0.05 level.

Time (hr)	Diabetic				Non-Diabetic			
	Candida albicans	*Candida glabrata*	*Candida* parapsilosis	*Candida tropicalis*	*Candida albicans*	*Candida glabrata*	*Candida* parapsilosis	*Candida tropicalis*
1	+	+	+	+	+	+	+	+
24	+	+	+	+	+	+	+	+
48	+	+	+	+	+	+	+	+
72	+	+	+	+	+	+	+	+
96	+ +	+ +	+ +	+ +	+ +	+	+	+

Table 2: *Candida* species (*C. albicans*, *C. glabrata*, *C. parapsilosis*, and *C. tropicalis*) growth observed on agar plates of diabetic and a non-diabetic subjects at different time periods (1, 24, 48, 72 and 96hr).

Candida growth is represented by (+), no growth (-) and more growths observed (+ +).

Discussion

Peri-implant diseases present challenges to clinicians [18]. Nearly 40% of all implant sites may develop peri-implant disease [19]. Peri-implant PD and BOP are considered reliable indices for the assessment of peri-implant health or disease [20,21]. These parameters are routinely used for the clinical monitoring of the soft tissue around dental implants [22]. Increased PD is frequently observed in peri-implant mucositis due to a decrease in resistance to probing owing to inflammation and collagen breakdown of the peri-implant tissues [23]. Present investigation found PD significantly higher in the diabetic group, which is in agreement with previous studies [24,25]. Elevated advanced glycation end products (AGEs) levels in diabetics could be good marker in the PICF examination of diabetic patients [26]. It was speculated that significant AGEs levels are reliable peri-implant indices to assess inflammation in diabetic patients [24]. However, authors did not expound on the subjects' ages, gender, ethnicity, type of diabetes, duration of diabetes, and associated systemic conditions, such as obesity or smoking, which are significant factors may impact the outcomes.

The current study found a significant BOP in diabetic subjects; they had 3.5 times more BOP compared to healthy subjects. Although studies have shown that AGEs play an important role in peri-implant inflammation [24,25], they did not confirm the diagnosis of diabetes, describe the calibration performed by the investigators, and clarified the subjects' ethnicity, gender, age, type of diabetes and associated systemic disease, which are relevant as Saudi Arabia has a high rate in some systemic diseases, such as obesity. Another report affirmed that AGEs interact with their cell surface receptors (RAGE), and the activation of RAGE can exaggerate inflammation and periodontal breakdown in diabetics through the expression of destructive inflammatory cytokines, including interleukin (IL)-6, IL-1β and tumor necrosis factor-alpha (TNF-α) [25-28]. Our present analysis revealed that both BOP implant sites and peri-implant PD were significantly higher in type-II DM, which confirms the findings of previous studies [24,25,29-33]. Our results and others' findings [34] suggest that diabetic subjects might be at higher risk for developing peri-implant diseases in the future. Nevertheless, other studies have failed to find a significant difference between diabetic and non-diabetic subjects as regards BOP and PD [35,36].

The present investigation was conducted on Saudi nationals. A previous study reported that *Candida* in diabetic patients were higher than in non-diabetics [14]. The present study found no significant differences in the number of *Candida* present in PICF between the two study groups. A possible explanation for this non-significant difference is the fairly good glycemic control in our enrollees. Hammad., *et al*. [37] concluded that poor glycemic control favors subgingival *Candida* growth in diabetic patients. A study conducted to evaluate oral *Candida*l carriage in Saudi Arabian subjects found that diabetes was significantly associated with greater *Candida* colonization [38].

Conclusion

Within the limitations of the present study, it was concluded that among the clinical parameters investigated, BOP and peri-implant PD are significantly higher in Saudi diabetic patients compared to healthy controls. Fungal growth, predominantly of *Candida* species, was found in the PICF of Saudi subjects, with slightly more

growth seen in the diabetic group but with no significant difference between diabetics and non-diabetics. Obesity and prediabetes in the healthy group could be important factors which we did not investigate them further in this study. Future studies are warranted.

Conflict of Interest

No potential conflict of interest relevant to this article was reported.

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