Bacterial Plaque around Dental Implant in Smokers and Non Smokers

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ABSTRACT
Background: To determine the prevalence of periodontal pathogens before and six months after implant placement in smokers and non smokers. Methods: Study subjects were 32 patients, where bacterial samples were taken with sterile paper points placed for 10 seconds, then sent to the laboratory within 1-2 hours. The specimens were cultured and incubated aerobically and anaerobically. Prophyromonas gingivalis, Aggregatibacter actinomycetemcomitans, Fusobacterium spp. and other organisms were identified by Gram stain, colony morphology, aerobic control, and specific identification by API 20 system. Results: All studied clinical parameters revealed reduction after six months period. The difference was significantly only in plaque index in both groups (smokers and non smokers). Aggregatibacter actinomycetemcomitans was found to be 16% of examined sites at baseline (T1) and was reduced to 7% after six months (T2) in smokers. In the same group Prophyromonas gingivalis was isolated from 13% of sites and increased up to 17% after six months. Fusobacterium spp. was isolated in 2% of sites and raised to 4% at T2. As for Streptococci, it was 7% at T1 and 18% at T2. The non smoker group showed occurrence of Aggregatibacter actinomycetemcomitans in 13% of sites, decreased to 10% after six months. While Prophyromonas gingivalis was 17%, it slightly decreased to 16% after six months. Conclusions: Healthy periodontal sites harbor Putative Periodontal Pathogens. Smokers have higher incidence for colonization of Prophyromonas gingivalis.

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INTRODUCTION

The role of bacteria in peri-implant infection was debated already in the early era of dental implant.[1] It was reported that the peri-implantitis as the most local factor cause for implant failure.[2] Peri-implantitis is a chronic, progressive, marginal, and inflammatory reaction affecting the tissues surrounding osseointegrated implants that results in the loss of supporting bone. Provetella intermedia and Actinobacillus actinomycetemcomitans were found to be the most predominant bacteria causing failure of dental implants isolated by culture, while Prophyromonas gingivalis was the most predominant bacteria detected by PCR.[3] Tannerella forsythia, Porphyromonas gingivalis, Treponema denticola, and Aggregatibacter actinomycetemcomitans found within the peri-implant sulcus were related to the peri-implant crevicular fluid volume.[4] The adverse effects of cigarette smoking on implant treatment are well documented. A longitudinal study found more failure of implant in smokers.[5] It was suggested that when considering all implant failures up to 5 years after loading, significantly more failures (5.5%) occurred in smokers compared with non-smokers (2.9%).[6] On the other hand, the long term success of implants replacing a single molar showed no relation among failure, complications, timing of implant placement, and smoking habits.[7] No clear association was found between smoking and change in subgingival flora.[8] Also, no difference in microbiologic pathogens was found between smokers and non smokers in relation to implant failure.[9] While, microbial profile of smoking related periodontitis was stated as distinct from that of non smokers.[10] More differences were observed in abundance than in prevalence of species, suggesting that smoking enriches an indigenous microbial community of bacterial colonization compared to non smokers.
Accordingly our plan was to explore if smoking will have influence of bacterial colonization around implant through culturing bacterial plaque sample before and 6 month after placement.

**MATERIALS AND METHODS**

Thirty two participants were selected from patients receiving dental implant in Riyadh Colleges of Dentistry & Pharmacy (RCsDP) after getting approval from ethical committee research center. All Participants signed informed consents. They were of both genders, free from any systemic disease with no history of antibiotics, anti-inflammatory medications. They were selected to be equal numbers of smokers and non smokers. Complete history, vital signs and examination of Temporo-Mandibular Joint and lymph nodes were performed. The following clinical parameters were recorded: Bleeding index,[11] Plaque index,[12] and Probing pocket depth measurement using standard Williams graduated probe (Hu-Freidy,Chicago, IL,USA).All patients received the same oral hygiene instructions.

*Bacterial sampling:*

First sample at the day of surgery: bacterial sample was taken from the distobuccal pocket of the tooth mesial to implant. Another sample was taken from mesiobuccal pocket of the tooth located distal to implant site. Second sample was taken after six months of placing implant from the same sites that were taken previously. Colony forming units were counted, and identified by colonial morphology, Gram stain, anaerobic control, susceptibility to special potency discs.[13] The isolated *Porphyromonas spp* (suggested *Provetella intermedia, Porphyromonas endodontalis* and *Porphyromonas gingivalis*) have been identified according to pigmented and non pigmented strains as anaerobic culture with a selective media called Scheduler Agar Media. Detections of *Porphyromonas spp*, a Gram-negative, non-spore-forming, anaerobic, rod-shaped bacteria that produce porphyrin pigments (dark brown/black pigments) (Figure1). The black pigmentation of *Porphyromonas gingivalis* is from the accumulation of hemin used as an iron source for bacterial growth. Selective media, Brain Heart Infusion Agar (BHIA), was used for isolation of other pathogenic bacteria accompanied as *Actionomyces spp, Aggregatibacter actinomycetemcomitans* was identified by its coccobacilli shape with Gram-negative rods, (Figure 2). Identification of Yeast was done according to the oval shape, (Figure 3).

**Fig. 2:** *Agrigatibacter actinomycetemcomitans* coccobacilli with G-ve rods.  

**Fig. 3:** Oval shape from *Yeast* growth in smoker patients.

*Isolation and identification of streptococci (Fig.4):*

API 20 A: system for the rapid identification of anaerobes Principle: The API 20A strip consists of 20 micro tubes containing dehydrated substrates. These tests are inoculated with a bacterial suspension which reconstitutes the media during incubation, metabolism produce color changes that are either spontaneously or revealed by the addition of reagents. The API 20A system enables 21 tests to be carried out quickly and easily for the biochemical identification of anaerobic microorganisms.(Figure 5)
RESULTS AND DISCUSSIONS

Clinical findings of the present research revealed no statistical differences in clinical parameters between smokers and non smokers at base line, when comparing clinical parameters at base line(T1) and 6 months late(T2), all parameters revealed reduction at T2. The difference was significant only in plaque index in both groups (Figure 5 & 6). Microbiological results revealed that aerobic microorganisms increased from T1 to T2 in both groups; the same was observed in anaerobic bacteria. In both groups the difference was not statistically significant. (Figure 6 & 7)

Regarding differential bacterial counts in smoker group, *Aggregatibacter actinomycetemcomitans* was found to be in 16%of examined sites at T1, and it reduced to 7% after 6 months. In the same group *Prophyromonas gingivalis* was isolated from 13% of sites and increased up to 17 % after 6 months. *Fusibacterium spp.* was isolated in 2% of sites and raised to 4% at T2. As for Streptococci it was 7 % at T1 and 18% at T2, (Figure 10 & 12). Non smoker group showed occurrence of *Aggregatibacter actinomycetemcomitans* in 13 % of sites, decreased to 10% after 6 months. While *Prophyromonas gingivalis* was 17%, and slightly decreased to 16% after 6 months, as shown in (Figure 11&13).

Clinical findings of this study revealed reduction of all clinical parameters studied (PI, GI, and PD). Although, reduction was significant only in PI in both groups, this could be explained by the fact that all participants were selected to be free from periodontal diseases. In addition patients enrolled in the study received intense oral hygiene instructions. In spite of presence of putative periodontal pathogens in healthy sites, this result agrees with literature.[14] Also, it was reported that half of their study population carried putative
periodontal pathogens at above-threshold levels, but without clinical signs of active periodontitis.\textsuperscript{15} In addition, it was concluded that in healthy and peri-implant mucositis conditions, partially edentulous patients showed a potentially more pathogenic flora than fully edentulous.\textsuperscript{16} Microbiological counting recorded in the present research revealed decreased isolation of \textit{A. actinomycetemcomitans} after 6 months in smokers, together with increased prevalence of \textit{Streptococcus spp}. This could be explained according to the fact that certain bacterial species have been proposed to be protective or beneficial to the host, including \textit{Streptococcus sanguis}. It is typically found in high numbers in periodontal sites that don’t demonstrate attachment loss, but in low numbers in sites with breakdown.\textsuperscript{17} These species probably function in preventing the colonization of pathogenic organisms, they produce \textit{H$_2$O$_2$} which is lethal for \textit{A. actinomycetemcomitans}.\textsuperscript{18} These results were supported by published data.\textsuperscript{19} where the percentage isolation of \textit{Porphyromonas gingivalis} and \textit{A. actinomycetemcomitans} were reported to be 31\% and 3\% in dental peri-implantitis patients, respectively. Yeast fungi were reported in one case at base line of smokers. After six months of implant placement yeast was found in 4 sites examined. This is in agreement with the finding of five cases positive for \textit{Candida albicans}.\textsuperscript{20} Based on these findings, the authors suggested that antimicrobial therapies for implant failures should not be implemented without a prior comprehensive microbiological analysis. Conclusions: From the results of the present study, it could be concluded that: Healthy periodontal sites harbor Putative Periodontal Pathogens. Strict oral hygiene is essential to reduce risk for biologic complications after implant placement. Smokers have higher incidence for colonization of \textit{Porphyromonas gingivalis}.

\textbf{Fig. 7:} Clinical parameters in Smokers.

\textbf{Fig. 8:} Colony Forming Units in smokers.
Fig. 9: Colony Forming Units in Non smokers.

Fig. 10: Bacterial percentage in phase one (Smoker).

Fig. 11: Bacterial percentage in phase one (Non smokers).
Fig. 12: Bacterial percentage in phase Two (Smokers).

Fig. 13: Bacterial percentage in phase Two (Non smokers).

REFERENCES


