Bacterial Plaque around Dental Implant in Smoking and Non Smoking

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Introduction

The composition of sub gingival micro flora in periodontal diseases is extremely complex, and it has been shown to consists mainly of Gram-negative anaerobic bacteria [9-11]. The specific of bacteria associated with the disease *Aggregatibacter actinomycetemcomitans* and *Porphyromonas gingivalis* have been suggested as specific risk factor in the diagnosis and treatment of periodontal disease [2]. Slots, *et al.* [9] stated that the designation *Aggregatibacter actinomycetemcomitans* and *Porphyromonas gingivalis* as periodontal pathogens presupport that destructive periodontal disease is more prevalent in periodontal sites exposed to the organisms than in those not exposed. The role of bacteria in peri-implant infection was debated already in the early era of dental implant [1]. The first indication for a specific role of bacteria in peri-implant infection originated from microscopic analysis of samples taken around implant of various designs. Implants with advanced pocket formation showed high levels of spirochetes whereas; implants with stabilized pockets not exceeding 5 mm. yielded spare predominantly Coccoid microorganisms [2].

The early colonization of peri-implant pockets by putative periodontal pathogens was studied in 20 partially edentulous individuals using anaerobic culture technique. The presence and levels of PPP in pockets and saliva was established at base line, after 6 and 12 months. Results revealed that patients with failure were associated with the presence of Porphyromonas gingivalis [12].

Prospective, split mouth, single-blind study of colonization of pristine sulci, created in42 partially edentulous patients during implant surgery was done by Quirynen., *et al.* [6] Samples were taken 1, 2, 4, 13, 26 and 78 weeks after abutment connection. Samuel T., *et al.* [7] examined subgingival plaque samples pre and post operative of placement of dental implant. It was found that for the most part, the newly created pri-implant crevices were colonized by specific bacteria within two weeks. Leonharatt., *et al.* [4], performed a study to evaluate differences of microbiota at titanium implant demonstrating clinical and radiographic evidence of loss of supporting tissue. Results showed *Porphyromonas* spp., *Porphyromonas gingivalis, Aggregatibacter actinomycetemcomitans* in 60% of failing implants, in comparison to flora resembling associated with healthy periodontium in implants surrounded with healthy tissues.

Bain and Moy, 1993 assessed the various factors that predispose implant failure in a group of 520 patients who had received 2,194 Branemark implants. Smoking was found to the most significant factor. DeBruyn and Collaert, 1994 found that smokers demonstrated a significant higher failure rate before functional loading of implants than non smokers. Schwartez-Arad., *et al.* 2002 concluded that it cannot be assumed that smoking is the only or the most significant factors in implant failure. And that further research is necessary to identify other possible factors that contribute to failure.

Accordingly this study will be performed to verify, the occurrence of putative periodontal pathogens in relation to cigarette smoking in sulci around dental implants.

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Aim of the study

- 1. Determination of incidence of periodontal pathogens: by isolation of Aggregatibacter actinomycetemcomitans and Porphyromonas gingivalis in sites subjected to surgical implant placement.
- 2. Identification of suspected pathogen by traditional method and API 20 kit (Rapid Identification).
- 3. Determination of susceptible and resistant isolates by culture sensitivity Test using Antibiotic s Disks.
- 4. Determination of possible relationship of presence of the two putative periodontal pathogens and smoking
- 5. Looking for the Gene Resistant factor by polymerase chain reaction PCR or DNA sequences
- 6. Determination of possible relationship of presence of two putative periodontal pathogen and smoking

Research Design

Subject population:

Forty patients having surgical placement of one or more dental implant in Oral and Maxillofacial surgery, College of Dentistry, RCsDP

Inclusion criteria:

- 1. Willingness and ability to sign informed consent
- 2. Partially edentulous with more than 6 remaining teeth
- 3. No active periodontitis in remaining teeth
- 4. Restoration after 6 month
- 5. Attempts will be made to recruit approximately equal numbers of males and females
- 6. Both smokers and non smokers will be included in the study

Exclusion criteria:

- 1. Systemic condition that might have influence the course of periodontal condition
- 2. Antimicrobial mouth wash 24 hours before examination
- 3. Antibiotic therapy 3 month period
- 4. No steroid or chemotherapy 3 month period

Subjects grouping:

Subjects will be divided into two main groups: smokers and non smokers, Smokers will be set in 2 groups (less than 10 cigarettes/day and more than 10 cigarettes/day)

Clinical monitoring:

The following clinical parameters will be recorded for each patient:

- 1. Modified plaque Index, Silness and Loe, 1964 [8]
- 2. Modified sulcular Bleeding Index, Muehleman, 1971 [5]
- 3. Probing Pocket depth measurement in millimeters
- 4. Clinical attachment level

Material and Methods

Media used: five different media formulated by Difco Laboratories were evaluated namely: Schaedler Agar (SA), Brain Heart Infusion

Agar (BHIA), Columbia Blood Agar (CBA), and Tryptic soy Agar(TSA), all media were enriched with 5% sheep blood, hemin (10 μ /mL), Vitamin K (5 μ /mL).

For aerobic or facultative anaerobic culture: Tryptic soy Agar supplemented with 5% defibrinated sheep blood, hemin (10 μ /mL), Cysteine hydrochloride (5 μ /mL), (Vitamin K (5 μ /mL), Pallidium chloride (0.33 mg/mL) Cadmium sulfate (20 μ /mL), Metronidazole (10 μ /mL) for *Actinomyces* spp.

For anaerobic culture: Schaedler agar with Kanamycin 100 mg/mL, Vancomycin 7.5 and sheep blood Columbia agar with 5% sheep blood, colistin 10 ng/L and Nalidixic 10 mg/L for isolation of *Porphyromonas* spp.

Material required but not provided: as standard microbiological supplies and equipments as microscope incinerator, autoclave, incubators, anaerobic chamber, standard microbiological supplies loop. Saline blanks, slides staining supplies.

Microbiologic samples: Subgingival plaque specimens from two sites per patient will be obtained, one for aerobic and one for anaerobic. Her culture media and serological and biochemical reagents.

The procedures

- 1. Bacterial samples will be taken after removal of Supragingival with light curette
- 2. Sterile paper point will be placed for 10 seconds
- 3. Place paper point in sterile tubes, the specimens for anaerobic microbiology will be placed in Stuart transport medium and send to laboratory within 1-2 hours in accordance of Jousimies., *et al.* [3], Takemoto., *et al.* 1997. The media dissolved in distilled water and spreading the samples, then the cultures media are incubated anaerobically using Gasack EZ Anaerobic Container system sachets for up to 10 days for isolation of *Actinomyces* spp at 37° C.

Identification

The cultures are identified by gram stain, coloniakl morphology, aerobic control, and specific identification by API 20 System. The isolated *Porphoromonas intermedia* and *Porphoromonas gingivalis* and *Aggregatibacter actinomycetemcomitans* strains are tested for susceptibility to Amoxicillin, metronidazole, Clindamycin, Azithromycin, tetracycline and Ciprofloxacin [1].

Data evaluation:

Clinical parameters will be computed for each subject, averaged within subjects and averaged across subjects in relation to assigned groups.

The low tested periodontal pathogens will be assessed separately for the same groups.

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