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RESEARCH ARTICLE

DETERMINATION OF METAL PARTICLES RELEASED FROM TITANIUM ENDOSSEOUS IMPLANTS BY EXFOLIATIVE CYTOLOGY: A CLINICAL EVALUATION

***Dr. Kumar Gaurav and Dr. Sheela Kumar Gujjari**

Department of Periodontology, JSS Dental College & Hospital, JSS University, Mysuru

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ABSTRACT

The revolutionary era of tooth replacement began with the invention of implants. Although success rate of 81 to 99% have been reported, there has been a failure rate of 1.5 to 6.7%. Though small these failures still pose a significant clinical, psychological and financial challenges to clinician and patients. Improper implant planning, patient compliance and hypersensitivity to implant metal are major causes for its failure as there is a void and scope of research in this area. Hence this study has been designed to determine the metal particle released from titanium end osseous implant by exfoliative cytology and histopathology

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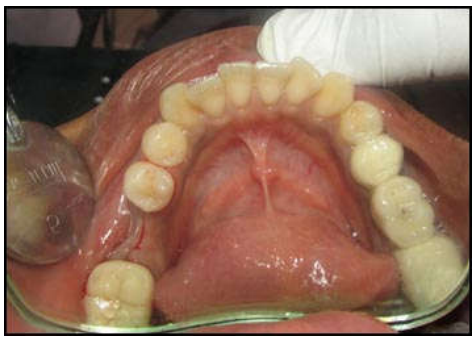
INTRODUCTION

Titanium and its alloys are widely used in end osseous implants as it is a biocompatible material with high corrosion resistance, but literature also witnesses some amount of corrosion (Dental endosseous implants, 1996; Misch, 2007). Corrosion can severely limit the fatigue life and ultimate strength of titaniumleading to implant failure. Corrosionleads to titanium release from implant surface to its surrounding tissues evoking host immune response (Zarb, 1983; Anusavice, 2003). This was hypothesized to cause type IV delayed hypersensitivity reaction causing failure of well planned implant (GujjariSheela Kumar and Musharraf Ali Nada, 2014). There are reports suggesting the presence of titanium particles in saliva and GCF and also it has been found in distant organs such as lymph nodes, liver and spleen. Olmedo *et al* demonstrated the metal particles around the dental implant was detected by exfoliative cytology of the surrounding soft tissue (Olmedo *et al.,* 2012). There is a void and scope of research to establish the nobility and biocompatibility in terms of corrosion of widely used titanium implants. Detecting corroded metal particles in surrounding miliue at early stage can prove beneficial to patients and shall aid on to greater success of implants. Hence this study was designed to determine the metal particle released from Titanium Endosseous implant by Exfoliative Cytology.

****Corresponding author: Dr. Kumar Gaurav***
Department of Periodontology, JSS Dental College & Hospital, JSS University, Mysuru.

MATERIAL AND METHODS

This was a clinical evaluation carried out over a period of twelve months among patients undergoing implant therapy at JSS Dental College and Hospital, Mysore. The sample size was estimated to be 16 assuming the release of metal particles to be 50% at 10% level of significance and 20% allowable error. A total of 16 subjects who approached for the placement of implant following the inclusion criteria and who had given written consent were selected from the outpatients presenting to Department of Periodontology and Department of Prosthodontics and Crown and Bridge JSS Dental College and Hospital, Mysore. The study duration was of 12 months. The study population comprised of 16 patients with partially edentulous area either in maxilla or mandibular with adequate bone (clinically and radio graphically) and no known systemic diseases, non smokers, with optimal oral hygieneandin an age range of 19 to 65 years. Patients with history of allergy, chronic smokers, individuals with orthodontic therapy, oral piercing, periodontal diseases and on medication containing any metals like titanium were excluded from the study. Pre surgical preperations for all subjects included in the study were carried out as per the standard protocol including blood, orthopantomograms (OPG) and intraoral periapical radiographs (IOPAR) for implant assessment and planning. All subjects underwent phase 1 periodontal therapy. Clinical parameters were evaluated and recorded on 4th week followed by surgical stent fabrication. All subjects underwent two stage implant surgical procedures.



CRESTAL INCISION

FLAP REFLECTED

INITIAL DRILL

PARALLELING PIN

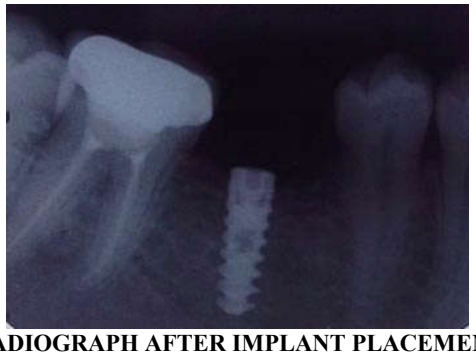
EXFOLIATIVE CYTOLOGY SAMPLE

EXFOLIATIVE CYTOLOGY - SMEAR

SMEAR FIXATION

COVER SCREW PLACEMENT

PROSTHETIC CROWN IN OCCLUSION



RADIOGRAPH AFTER IMPLANT PLACEMENT

Table 1. Description statistics of indices at different time intervals

	Baseline	3 months	6 months	12 months	F vaue	P value
Modified plaque index(mPI)	0.1094+0.20349	0.6719+0.44459	0.8219+0.40905	0.7656+0.30915	19.505	0.001
Modified bleeding index (mBI)	0.1875±0.34761	0.3906±0.28822	0.5156±0.2809	0.5156±0.2666	4.469	0.008
DIM	1.7500±0.36515	1.96062±0.2015	1.500±1.114	1.6550±1.1689	2.592	0.058
Probing depth	1.7500±0.3651	1.1250±0.3415	1.250±0.39791	1.2813±0.3966	9.911	<0.001
Peri-implant attachment(PAL)	.0000	0.781±.36372	0.6719±0.361	0.6563±0.340	28.365	<0.001
Marginal bone levels (MBL)	0.3087±0.2584	0.4737±0.1360	0.4844±0.134	0.5213±0.134	12.724	<0.001
Papillary index(PPI)	0.3750±0.5000	1.00±0.5164	1.4357±0.512	1.5625±0.512	22.551	<0.001

Statistically significant at p value < 0.05

Antibiotic and analgesic coverage along with post operative instructions were given to all the patients. The clinical parameters, radiographic parameters and exfoliative cytology findings were assessed on day of surgery followed by 3, 6 and 12 months.

Clinical parameters

- Modified plaque index (mPI), (Mombelli, 2000)
- Modified bleeding index (mBI), (Mombelli, 2000)
- Distance between the implant shoulder and the mucosal margin (DIM) in millimetres (recession scored as negative value), (Mombelli, 2000)
- Pocket probing depth (PPD) in millimetres, (Mombelli, 2000)
- Probing attachment level (PAL) in millimetres calculated by subtracting Pocket probing depth(PPD) from Distance between implant shoulder and mucosal margins(DIM) (Mombelli, 2000).

Radiographic parameters

- Peri-implant radioluscency
- Marginal bone levels (MBL) (Mengel and Flores-de-Jacoby, 2005)

The radiographic image was digitized and assessed using Adobe Photoshop measure tool. Shoulder of the implant was taken as the fixed reference point. The distance in millimetres between the shoulder of the implant and the first clear bone to implant contact, mesially and distally were noted. Corrected bone level= measured bone level x (actual implant length/ measured Changes in bone height over the entire observation period and the annual rate of change in bone level was calculated

Annual vertical bone loss= marginal bone level at baseline - marginal bone level at the end of 12 months

Cytological study

Using a micro brush (regular size, 2.0) cytological samples of the peri-implant mucosa was collected at the level of union of the sulcular epithelium and the buccal epithelium. The smear obtained was spray fixed and stained with Papanicolau method. The cover slip will be mounted on microscopic slides using Canada balsam. The slide was analyzed under transmitted light microscopy for presence of metal particles. Cytological smears were also obtained from the contralateral side of the jaw of the same individual which served as control. Metal particles if present on microscopy would be subjected to X-ray defraction (WAXS) i.e., wide angle x-ray scattering for confirmation.

- The quantitative data was presented as mean and standard deviation while qualitative data as frequency and percentage. The mean value at different time intervals with regard to each variable of interest (mPI, mBI, DIM, PPD, PAL and MBL) was compared using one way ANOVA The statistical significance was fixed at 0.05.

RESULTS

The mean age of the subjects at the time of implant insertion was 29.18 years (range= 18-48years) and 25% of the subjects were females (Table-1). The length of the implants varied between 10-11.5mm with 12.5% being 10mm and remaining 87.5% being 11.5mm in length. The diameter of the implants placed ranged from 3-3.5mm with 87.5% of implants being 3.5mm wide, 12.5% 3.0mm wide.

Exfoliative cytology examination for presence of metal particles

The exfoliative cytology specimens collected from 16 implant sites at interval of baseline, 3, 6 and 12 months showed no evidence of metal particles at 1000x magnification.

Radiographic findings

The following radiographic parameters were recorded at various time intervals during the follow-up period of 12 months

Periimplantradioluscency

No peri-implant radioluscency was seen in any of the cases during the 12 months follow-up period.

Implant success and survival rate

The success and survival rate calculated according to the Implant quality scale proposed at the International Congress of Oral Implantologists, Consensus Conference, Pisa, Italy, 2007. Was 100 % in the present study.

DISCUSSION

Currently titanium is the material of choice in implantology, whereas Williams and Williams, 2004 established that all metals whether passive shows slow removal of ions from their surface. These release of metal particle in surrounding milieu could be a possible cause of implant failure. In the present study the cytological smear samples were taken from the surrounding implant area at an interval of baseline, 3, 6 and 12 months. Pap staining and evaluation of cells under 1000x magnification showed no evidence of metal particles within the cells.

Cells indicative of delayed hypersensitivity like macrophages, plasma cells etc were not found in the samples. This finding was in contrast to the study done by Olmendo DG (Olmedo *et al.,* 2003). Who has extensively worked on assessing histopathologic evidence for titanium implant corrosion and Type IV hypersensitivity reaction in the surrounding tissues in various animal and human studies. This difference in observation can be attributed to various factors (Olmedo *et al.,* 2003). The procedure of implant placement may lead to mechanical wear of implant surface causing release of metal particle in the surrounding tissues and in the present study care was taken to minimise the malhandling of the implant to rule out this possibility.

The pH of the surrounding tissue is known to have a greater effect on the corrosion of the dental implant. Presence of bacteria and bacterial products causes drop in pH leading to corrosion of the implants (Olmedo *et al.,* 2003). Whereas in this study only one group of healthy patients were enrolled where drop in pH would have been null because of proper care and maintenance by the patients. The source of metal particles in the oral epithelial cells can be from various metallic restoration this possibility was ruled out in this study as such patients were not included. It was reported by Daniel G (Olmedo Daniel *et al.,* 2012) that saliva leaking between the metallicsuprastucture (nickel-chrome- molybdenum alloy) and the implant made of pure titanium may trigger a corrosion process as a result of differences in electrical potential (galvanic corrosion).this generates the passage of ions, such as nickel or chrome from the alloy of a crown or bridge to the periimplant tissues, with consequent bone resorption which compromises the stability of implant leading to fracture. In the present study patients with metallic prosthesis were not included. The method of tissue collection from the adjacent area of implant was exfoliative cytology using a cytobrush or wooden spatula which would have limited the tissue sample quantity for metal aggregation analysis when compared to biopsies. As present study included human subjects the biopsy procedure at regular intervals would have compromised the healing around the implant. Hence a non invasive method like exfoliative cytology was opted over biopsy. As non of the samples in the present study showed the presence of metal particles so the second objective of the study could not be met.

In the present study, all the patients were incorporated in an individually designed maintenance care program namely *'Cumulative interceptive supportive therapy'* with regular visits at intervals of 3 months. This systematic approach to the prevention and treatment of peri-implantitis was suggested by Lang *et al*., 2011. Periodontal maintenance (PM) is a critical factor in the long-term success of both periodontal and dental implant therapy. Studies have shown both modern periodontal and dental implant therapies are effective in maintaining natural teeth and replacing lost teeth, respectively. However, without a regular program of clinical reevaluation, plaque control, oral hygiene instruction, and reassessment of biomechanical factors, the benefits of treatment often are lost and inflammatory disease in the form of recurrent periodontitis or periimplantitis may result (Nicholas *et al.,* 2009). A statistically significant increase in the mean Probing pocket depth and peri-implant attachment level PPD from baseline values was observed over the 12 months follow-up period. *The annual increase in the mean probing depth was 0.4mm*.which was in accordance with the healthy successful implants. The peri-implant attachment level showed a statistically significant increase with annual loss of approximately 0.66mm. The change in the PD levels and attachment levels were seen within the first 3 months of follow-up (1.75mm and 0.78mm respectively) followed by a gradual decrease in PD and PAL in the remaining months of the study. This could be attributed to intervention with appropriate maintenance therapy. This result is in accordance with the study by Ellegard and Karoussis *et al.,* 1997. In contrary, studies by Mengel and Flores-de-Jacoby, Sbordone *et al* reported no statistically significant alterations in the PPD and PAL around implants placed in patients with a history of chronic periodontitis (Sbordone *et al.,* 1999). The mean Peri-implant marginal bone levels (MBL) levels showed significant alterations over the 12 months period compared to the baseline. *The annual mean peri-implant marginal bone loss was 0.21mm*. This could be attributed to intervention with maintenance therapy.

The mean change in papillary index from baseline to 12 month was 1.18 mm which was highly significant. The mean papillary index according to the present study at 12 month was 1.56 mm. All subjects of the present study showed complete papilla fill. This could be due to the reduction of inflammation and reinforcement of oral hygiene during the maintenance phase. The importance of a zone of keratinized gingiva / mucosa around the teeth and implants has been vigorously debated. Clinical studies by Adell *et al,* Albrektsson *et al,* indicate that absence of keratinized mucosa around the implants does not influence implant survival (Albrektason *et al.,* 1986; Adell *et al.* 1990). In the present study width of keratinized gingiva was maintained throughout the study period. In contrast, Warrer *et al* demonstrated in an experimental animal study that implants placed in areas without keratinized mucosa had higher plaque scores and higher rate of attachment loss compared with implants placed in keratinized mucosa (Warrer *et al.,* 1995). Also, in study by Ellegard *et al*, it was found that bleeding on probing and pockets ≥ 4mm tended to occur more frequently at non-keratinized sites than keratinized sites (Ellegaard *et al.,* 1997).
Out of all 16 implants placed none of the implant failed. Hence the annual survival rate in the present study as calculated according to the Implant quality scale was 100% during the period of 12 month. None of the 16 implants had PD and MBL change more than 2mm within 1 year of post loading'. Hence the annual success rate is 100% (Lang *et al.,* 2011; Bjorn Klinge *et al.,* 2005; Lindhe *et al.,* 1992; Schnitman and Shulman, 1979). The implant quality scale proposed at the International Congress of Oral Implantologists, Consensus Conference, Pisa, Italy 2007 is not only recent but also provides the management technique based on the success and survival classification. The success and survival rates were calculated according to Albrektssoncriteria. The success of implants in the present study may be credited to systemic health of patients and good oral hygiene maintenance by the patients during the study period (Albrektason *et al.,* 1986). As no metal particles were detected. In the study titanium implant could still be considered as a safe biomaterial.

Conclusion

This was a prospective study evaluated the release of metal particle from titanium end osseous implant by exfoliative cytology. The clinical and radiographic parameters were also studied to assess the outcome of the implants over a period of 12 months. Within the limits of the present study, the following conclusions can be drawn Titanium Dental implant

in systemically healthy patients have demonstrated no evidence of metal particle released in surrounding milieu. even though many implant biomaterial have been introduced, but considering all aspect titanium can be still be considered as a safe biomaterial. To the best of our knowledge, Exfoliative cytology is a simple, non-invasive and efficient technique to detect metal particle in cells exfoliated from periimplant mucosa and would seem to be a useful method to monitor dental implant corrosion. Patients were more comfortable with the procedure (exfoliative cytology). Although the present study fulfils its objectives, further studies with a larger sample size, longer follow-up and a additional control group (periimplantitis patients) is required.

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