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Comparison of Enzyme Beta Glucuronidase and Alkaline Phosphatase Levels in Peri Implant Sulcular Fluid Around Healthy and Diseased Implants – A Clinical Pilot Study

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ABSTRACT

The aim of this study is to compare the levels of the enzyme beta glucuronidase enzyme and alkaline phosphatase around healthy and diseased implants keeping the levels around healthy teeth as control. Twelve male subjects with implant prosthesis were screened postoperatively and eight was selected based on the inclusion and exclusion criteria. Selections of healthy and diseased implants were based on visual inspection of the gingiva and clinical records obtained within a period of six months prior to sampling. Plaque and gingival index levels were recorded for the healthy and diseased implants. The sulcular fluid was assayed for the levels of enzyme Beta glucuronidase and alkaline phosphatase. The result of this study clearly indicates that the enzyme beta glucuronidase and alkaline phosphatase levels surrounding the failing implants are increased when compared to successful implants. Plaque scores were similar in both the healthy and diseased. As the plaque scores do not offer much insight in this area, their levels seem to be more or less similar in health and diseased. Gingival index scores were found to be increased in diseased when compared to the healthy. From the results of this study, it can be inferred that the increased beta glucuronidase and alkaline phosphatase levels can be an important biomarker and a good predictor of implant failure. One foreseeable benefit of an oral fluid-based periodontal diagnostic would be identification of highly susceptible individuals prior to overt disease.

Keywords: Beta glucuronidase, alkaline phosphatase**,** implants, biomarker, periodontal diagnosis.

INTRODUCTION

The medical works of ancient India devote significant space to oral and periodontal problems, including descriptions of severe periodontal disease with loose teeth and purulent discharge from the gingiva, stressing tooth brushing and oral hygiene[1].

Brill confirmed the presence of GCF in humans and considered it a “transudate”. However, others demonstrated that GCF is an inflammatory exudate, not a continuous transudate. In strictly normal gingiva, little or no fluid can be collected[2,]

Teeth penetrate the integument as a structure that emerges from inside the body to outside the body. The gingival sulcular tissue is an area that provides a biologic seal, but it is also the area where the plaque bacteria challenge the host. This microbial challenge can result in a homeostasis with the host response, or it can overwhelm the host and cause tissue destruction, resulting eventually in

periodontal disease. The host, however, has exquisite defense mechanisms that involve saliva and gingival crevicular fluid (GCF), both of which have multiple capacities to interact with the bacterial challenge. These capacities range from enzymes to antibodies to polymorphonuclear leukocytes. The contribution of each of these components is unknown, although each appears to be important in the host defense to bacterial plaque. For example, decreased salivary secretion (xerostomia) results in increased gingival disease and caries. Future therapeutic approaches may be directed at stimulating components of saliva or GCF[3].

In 1806 the Italian M. Maggiolo attempted to place solid-gold roots in human jaws, and later in the nineteenth century, several other investigators used porcelain and metallic implants. In the first half of the twentieth century, several attempts were made using elaborate surgical techniques and complicated constructs of gold and other precious metals, and microscopic investigations were begun on the tissue response to various metals[1].

Together, periodic evaluation of tissue appearance, probing depth changes, and radiographic assessment are the best means of detecting changes in bone support. Clinicians should monitor the surrounding tissues for signs of periimplant disease by monitoring changes in probing depth and radiographic evidence of bone destruction, suppuration, calculus buildup, swelling, color changes, and bleeding[4].

In cases with severely reduced bone support extending into the apical half of the implant, or in cases demonstrating mobility, implant removal should be considered. After the implants are removed, the ridge defects can be reconstructed using bone graft and membrane techniques. This treatment usually enables the clinician to place new implants in a previously compromised situation[5].

Several dental health criteria have been adapted for implants. The clinical criterion most commonly reported is the survival rate, or whether the implant is still physically in the mouth or has been removed. Proponents of this method say it provides the clearest presentation of the data; crisis argue implants that should be removed because of pain, disease, or the inability to be restored still may be maintained yet wrongfully reported as successful. Reports of natural teeth used to support a prosthesis follow a similar criterion: whether the restoration is still in the mouth. Therefore survival rates rather than success rates are the most common method to report the "success" of the prosthesis, whether the prosthesis is supported by implants or natural teeth. A majority of reports that include clinical criteria include mobility, radiographic assessment, and gingival and plaque indices. Subjective criteria of discomfort and patient satisfaction also are mentioned[6].

When an implant fails before restoration, it probably did not achieve osseointegration, or the integration was weak or jeopardized by infection, movement, or impaired wound healing[6]. Late implant failures occur after delivery of the prosthesis for many reasons, including implant overload and infection[7].

Implant success is as difficult to describe as the success criteria required for a tooth. A range from health to disease exists in both conditions. The primary criteria for assessing implant quality are pain and mobility. The presence of either greatly compromises the implant; removal usually is indicated. Probing depths may be related to the presence of local disease of preexisting tissue thickness before the implant was inserted. An increasing probing depth is more diagnostic and signifies bone loss, gingival hyperplasia, or hypertrophy. Bone loss usually is evaluated best with probing rather than with radiographs. The most common cause of bone loss during the first few years of function is related to factors o stress. The bleeding index is observed easily and indicates inflammation of the gingiva. However, implant health status is not as related to sulcular inflammation as would be the case with a natural tooth[8,9].

Aim of the study

The purpose of this study is to compare the levels of the enzyme beta glucuronidase enzyme and alkaline phosphatase around healthy and diseased implants keeping the levels around healthy teeth as control.

MATERIALS AND METHODS

Eight male subjects with implant prosthesis were screened post operatively and thirty four was selected based on the following inclusion and exclusion criteria.

Inclusion criteria

1. The patients having placed the prosthesis over the implant at least or before a period of six months.
2. Patient age should be within 20-60 years.
3. The patient should not have any oral lesions.

Exclusion Criteria

1. The subjects with the history of periodontal treatment in the preceding six months.
2. Intake of non steroidal anti inflammatory drugs, immunosuppressive drugs, corticosteroids and antihypertensive drugs.
3. Antibiotic therapy and antiseptic therapy for the preceding six months.
4. Smokers.
5. Any underlying systemic conditions.

The ethical committee clearance was obtained before the start of the study. Selections of healthy and diseased implants were based on visual inspection of the gingiva and clinical records obtained within a period of six months prior to sampling. Failing implants were evidenced by mobility of the implant, the presence of fistulae or exposed implant threads or hydroxyapatite coatings[10].

Collection of peri implant sulcular fluid

Before the collection of the peri implant sulcular fluid, all supragingival plaque was removed from each sampled site. The sites chosen for sample collection were isolated with cotton roles. The fluid was collected using standardized filter paper strips held within the crevice. The strip was inserted into the sulcus or pocket until slight resistance is felt and was left in place for twenty seconds. Then it was transferred immediately into plastic vials containing 300 µL of saline with 0.1 % polysorbate 20. The fluid was later eluted from the paper strips by vortexing the sample at 3500 rpm for a period thirty minutes. The strips were then removed from the vials and the vials were sealed and frozen at -80 degree centigrade for subsequent laboratory analysis[10].

Plaque index (Loe)[11]

Implants placed in the oral cavity represent artificial surfaces colonized by bacteria from saliva and ecologic niches such as periodontal pockets, tonsils, and crypts of the tongue. Experimental and human studies have provided evidence that formation and development of a microbial film represents an important etiologic factor in the pathogenesis of peri-implant disease. Periodontal pathogens from residual pockets of remaining teeth in patients treated for periodontal disease have been documented to colonize oral implants. Mombelli and coworkers modified the original Plaque index introduced by Silness and Loe to assess biofilm formation in the marginal area around implants. It appears meaningful to monitor oral hygiene habits by quantifying plaque accumulation. The index described by Mombelli et al to assess plaque accumulation around oral implants is as follows:

Score	Criteria
0	No detection of plaque
1	Plaque only recognized by running a probe across the smooth marginal surface of the implant
2	Plaque can be seen by the naked eye
3	Abundance of soft matter

Gingival Index[11]

In addition to redness and swelling of the marginal tissues, bleeding on probing, pocket formation , and suppuration have been reported to result from peri-implant infections. Assessment of these clinical signs has been considered important in the diagnosis of periodontal diseases. Therefore, the definition of peri implant parameters based on periodontal periodontal indices such as the Gingival Index System seems indicated. Mombelli et al modified the gingival index for application around oral implants as follows :

Score	Criteria
0	No bleeding when a periodontal probe is passed along the mucosal margin adjacent to the implant
1	Isolated bleeding spots visible
2	Blood forms a confluent red line on mucosal margin
3	Heavy or profuse bleeding

Beta glucuronidase enzyme assay (Sigma Aldrich Biotech Ltd)

Beta glucuronidase was determined by release of 4-methylumbelliferone from hydrolysis of 4-methylumbelliferyl-b-D-glucuronide. 25muL of sample,10 muL of substrate and 65muL of acetate buffer were incubated for 1 hour at 37 C. (Preparation of Blanks solution: Prepared as described above, except that 25muL of sample was replaced with 25muL of saline.)

Following incubation, 2.4 m L of glycine buffer was added to each sample and blank. Five –ng/mL and 1-ng/mL standards, of 4 –methyumbelliferone were prepared while the samples and blanks were incubated. These two standards were prepared each time the assay was run to detect changes not only in the buffers used but in the flurometer. Only one standard (5ng/mL) is needed to set the flurometer maximum (4.0to 9.0) on the computerized Amino-Bowman series 2 flurometer. Fluorescence was read at an excitation wavelength of 365 nm and an emission wavelength of 450 nm[9,12].

Alkaline phosphatase assay (Sigma Aldrich Biotech Ltd)

The assay is based on the 2-stage de-phosphorylation of dioxetane substrate by the ALP enzyme. The substrate used in this study was CSPD which is the acronym for disodium 3-(-4-methoxyspiro {1,2-dioxetane-3,2'-(5'-chloro) tricycle[3.3.1.13,7] decan }-4-yl) phenyl phosphate. Upon dephosphorylation by ALP, both a hydrogen phosphate anion and a metastable chloro-aryloxide intermediate anion are formed. The electrophilic nature of the chloro-adamantyl group contributes to an electron drift which brings about a quicker fission of the dioxetane ring than that, occurring the unchlorinated AMPPD assay previously reported by Chapple et al, 1994. This rapid dissociation results in a stable chloro-adamantane group and a very unstable methyl-m-oxybenzoate anion, which rapidly decomposes and in doing so reaches ground state by emitting a photon of light of wavelength 477 nm[13].

RESULTS

Similarly higher values of gingival index are found for failure implant cases compared to successful implants. Nearly there is four-fold increase in the plaque index among failure implant cases compared to successful implant cases. Nearly two-fold increase in the values is seen among failure implant cases compared to successful implant cases in all the levels of Beta glucuronidase. The values of the Alkaline Phosphate among failure implants nearly four times higher than that of successful implants.

As far as successful implants are concerned, it is evident that plaque index is highly correlated with gingival index and gingival index does not correlate with Beta Glucuronidase of Alkaline Phosphate. Higher the value of gingival index, higher will be value of plaque index. Plaque index is significantly correlated with Alkaline Phosphate and not with Beta Glucuronidase. Beta Glucuronidase does not have correlation with any of the other three parameters.

DISCUSSION

This study was carried out on patients who have implant prosthesis in their oral cavity. Both the healthy as well as diseased implants were taken into consideration, keeping healthy teeth as control, and health and disease were differentiated as explained above. Plaque scores and gingivitis scores were also taken into consideration. Radiographs of the prosthesis along with the implant were taken.

The presence of beta glucuronidase increases with severity of the disease. The results of this study is similar to that of Bourtos et al (1994)[9].

The result of this study clearly indicates that the alkaline phosphatase levels surrounding the failing implants are increased when compared to successful implants. The results obtained here is similar to the results obtained surrounding the inflamed gingiva[12]

Plaque scores were similar in both the healthy and diseased. As the plaque scores do not offer much insight in this area, their levels seem to be more or less similar in health and diseased.

Gingivitis scores were found to be increased in diseased when compared to the healthy.

The outcome of this study clearly indicates that assessment of biochemical mediators, especially alkaline phosphatase and beta glucuronidase, investigated in this study is a good way to monitor inflammation around dental implants[14,16].

CONCLUSION

Periodontal disease is a bacteria-induced chronic inflammatory disease affecting the soft and hard supporting structures encompassing the teeth.

Beta glucuronidase and alkaline phosphatase has been highly correlated with clinical features of the disease and decreases in response to intervention therapies, and has been shown to possess predictive properties for possible future disease activity[12]. One foreseeable benefit of an oral fluid-based periodontal diagnostic would be identification of highly susceptible individuals prior to overt disease. Timely detection and diagnosis of disease may significantly affect the clinical management of periodontal patients by offering earlier, less invasive, and more cost-effective treatment therapies[1315,] The effect of antibiotics and analgesics on the enzyme levels also act as a modifying factor, which should also be considered.[17,18]

REFERENCES

1. Quirynen M. Anatomical and Inflammatory Factors Influence, Bacterial Plaque Growth and Retention in Man [DMD thesis]. Leuven: University of Leuven, 1986;173-180.
2. Prabhu MN, et al. Early diagnosis of periodontal disease based on hostresponse analysis- A review, *Indian Journal of Dental Advancements.* **2**(4), 359-361 (2010).
3. Smith QT, Geegan SJ. Repeated measurement of crevicular fluid parameters at different sites. *J Clin Periodontol;* **18**:171-176 (1991).
4. Mombelli A, Marxer M, Gabertheul T, Grunder Um Lang NP. The microbiota of osseointegrated implants in patients with a history of periodontal disease. *J Clin Periodontol;* **22**:124-130 (1995).
5. Newman M, Fleming T. Periodontal considerations of implants and implant associated microbiota. *J Dent Educ;* **12**: 737-744 (1988).
6. Priyavadhana P. et al. Wound Healing in Periodontics", *BiosciencesBiotechnology Research Asia,* **11**(2): 791-796 (2014).
7. Mombelli , Buser D, Belser UC, Lang NP. The original one-stage dental implant system and its clinical application. *Periodontol 2000*; **17**:106-118 (1998).
8. Machtei EE, Oved-Peleg E, Peled M. Comparison of clinical, radiographic and immunological parameters of teech and different dental implant platforms. *Clin Oral Implants Res*; **17**:658-665 (2006).
9. Abrahamsson I, Berglundh T, Wennstrom J, Lindhe J. The peri-implant hard and soft tissues at different implant systems. A comparative study in the dog. *Clin Oral Implants Res;* **7**:212-219 (1996).
10. Bourtos M, Bryan S, Smith T. Crevicular fluid enzymes from endosseous dental implants and natural teeth. *Int J Oral Maxillofac Implants;* **11**: 322-330 (1996).
11. Löe H. The Gingival Index, the Plaque Index and the Retention Index systems. *J Periodontol;* **38**:610-616 (1967).
12. Prabhu MN, et al. Comparison of enzyme beta glucuronidase levels around healthy and diseased implants-A clinical Study, *Indian Journal of Multidisciplinary Dentistry.* **2**(1), 93-95 (2011).
13. Prabhu MN, et al. Comparison of enzyme alkaline phosphatase levels around healthy and diseased implants-A clinical Study, *Indian Journal of Multidisciplinary Dentistry.* **1**(3), 169-171 (2011).
14. Pernetti AR, , Rosenbach, Sujatha PB. Gingival crevicular fluid levels of alkaline phosphatase in periodontal health and disease. *J Periodontol* ; **78**: 2325-2330 (2007).
15. Offenbacher S, Collins JG, Arnold RR. New clinical diagnostic strategies based on pathogenesis of disease. *J Periodontal Res;* **28**:523-535 (1993).
16. Malmström HS, Fritz ME, Timmis DP, Van dyke TE. Osseo-integrated implant

treatment of a patient with rapidly progressive periodontitis. A case report. *J Periodontal*; **61**:300-304 (1990).

17. Prabhu Manickam Natarajan. Analgesics Used In Periodontal Surgery" *International Journal of innovations in Dental Sciences,* **1**(1), 1-9 (2016).

18. Priyavadhana P, Prabhu Manickam Natarajan The role of antibiotics in treatment of chronic Periodontitis". *International Journal of Dental Sciences and Research*, **2**(1), 16-18 (2014).