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ORIGINAL ARTICLE

EVALUATION OF PRESENCE AND ACTIVITY OF THREE DIFFERENT ENZYMES IN CREVICULAR FLUID FROM DENTAL IMPLANTS

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ABSTRACT:

Background: In the literature, for monitoring the conditions of endosseous dental implants, monitoring of the clinical periodontal parameters have been widely used. For pathologies occurring in various tissues, Aspartate aminotransferase (AST) levels in the serum and body fluids have been used since decades as a diagnostic marker. Two such enzymes associated with disuse destruction due to periodontal pathologies are AST and alkaline phosphatise (AP). Increased levels of serum cathepsin K (CK) has also been found to be associated with chronic inflammatory processes. Hence; the present study was undertaken to evaluate the activity of AST in Peri-implant crevicular Fluid (PIF) obtained from healthy and pathologic tissue associated with endosseous dental implants. **Materials & methods:** The present study was conducted in the department of oral implantology and included assessment of 150 individuals that underwent prosthetic rehabilitation of edentulous areas by dental implant procedures. All the patients were divided into three study groups; group 1, 2 and 3 having patients with healthy peri-implant tissue status, patients with mucositis, and patients with peri-implantitis respectively. Recording of all the clinical parameters (probing depth, bone loss etc) was done. Collection of the PIF was done for evaluation of AST activity in the experimental groups at the time of second visit. Randomly, on the mesial or distal site, sampling of the PIF was randomly preformed from the healthy implants and implants with mucositis where as sites associated with deepest PD during the first visit, the peri-implantitis samples were obtained. Spectrophotometrical method was used for the measurement of Transaminase activity. Scandinavian method was used for the assessment of AP activity. Method described by Strbac et al was used for assessment of CK levels. SPSS software was used for the analysis of the results. **Results:** In healthy patients, the mean AST levels were found to be 0.21 U/ml. In patients with mucositis and with peri-implantitis, the mean values of AST were found to be 0.37 and 0.65 U/ml respectively. Mean PD was found to be 2.10, 2.85 and6.80 in group 1, 2 and 3 patients respectively. As far as mean BL is concerned, the value was found to be 1.56, 1.95 and 5.20 respectively in group 1, 2 and 3 respectively. The mean value of AP in group 1, group 2 and group 3 was found to be 11.20, 17.20 and 24.30 IU/mL respectively. The mean value of CK in group 1, group 2 and group 3 was found to be 1.15, 2.10 and 3.20 pmol/sample respectively. Significant difference was obtained while comparing mean AST, CK and AP level sin between peri-implantitis patients and mucositis patients. **Conclusion:** Some amount of diagnostic significance exists in context to AST, AP and CK activity in PIF of the implant patients with implant associated pathologies. **Clinical significance:** Biochemical enzymes can be used as a biomarker for predicting the inflammation around the dental implants.

Key words: Aspartate, Alakaline phosphatase, Cathespsin, Dental Implant

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INTRODUCTION

With the advancement in the frequency of use of dental implants for rehabilitation, there has been simultaneous increase in the capability of the clinicians in relation to the effective restoration of the dentition of the patients.[1] From time to time, various dental implant systems have been introduced from time to time for increasing the prognosis and outcome of dental implant procedures.[2] On the basis of chronological categories as early failures, distinguishing if the dental implants can be done within few weeks to few months of dental implants. For monitoring the conditions of endosseous dental implants, monitoring of the clinical periodontal parameters have been widely used in the literature. However, precise significance of these clinical parameters (periodontal bleeding, periodontal probing etc) in relation to the monitoring of outcome of dental implants is still unclear.[3] Gingival crevicular fluid (GCF) has been studies a number of times in the past literature for monitoring the periodontal activities.[4] Aspartate aminotransferase (AST) is the enzyme which under physiologic conditions is confined to cytoplasm but in case of cell death, is released into the extracellular environment. Two such enzymes associated with disuse destruction due to periodontal pathologies are AST and alkaline phosphatise (AP).[5] Increased levels of serum cathepsin K (CK) has been found to be associated with chronic inflammatory processes.[4] Hence; the present study was undertaken to evaluate the activity of AST, AP and CK in Peri-implant crevicular Fluid (PIF) obtained from healthy and pathologic tissue associated with endosseous dental implants.

MATERIALS & METHODS

The present study was conducted in the department of oral implantology and department of periodontics of the dental institution and included assessment of 150 individuals that underwent prosthetic rehabilitation of

edentulous areas by dental implant procedures. All the patients aged between 32 to 60 years and reported in the institution for the purpose of prosthetic rehabilitation from June 2011 to July 2014. Out of 150 individuals, 72 were males while remaining were females. Ethical approval was taken from the institutional ethical committee and written consent was obtained after explaining in written the entire research protocol. The present study included all the patients what were rehabilitated with endosseous dental implants in two-staged procedures. Patients with history of any systemic illness, any known drug allergy, who had taken any anti-inflammatory or antibiotic therapy in the past three months or who have undergone any oral dental prophylactic procedure in the past three months were excluded from the present study. Selection of only one fixture was selected for the present study in subjects in which more than one dental implant were placed. Criteria for selection in the present study were as follows:

- Minimum period of functioning of the dental implants of the subjects for the present study was 2 years.
- Peri-apical radiographs should be taken immediately after the placement of the fixtures and should be available for data assessment.
- Probing access should be present in cases in which experimental fixtures were supported by a fixed restorative material.

The entire fixtures were divided into following groups with 50 subjects in each group as shown in **Graph 1**.
Criteria for subjects with healthy dental implants included:

- Regular periodontal check should have been performed in patients at 5 months interval.
- On visual examination and assessment, no deposition of plaque at supra-gingival position should be detected on the areas of restored dental implants.
- On visual examination, supra-gingival plaque should not be present in more than twenty percent of the teeth in the oral cavity.
- Presence of 0 score of modified sulcus bleeding index (mSI).[6]
- Absence of peri-implant mucositis on visual detection
- Around all the sites of the fixtures, Probing depth (PD) should not be more than 3 mm.
- When post-surgical radiographs were taken immediately and subsequently, bone loss (BL) should not exceed 2 mm on the fixture's either side.

Criteria for subjects with Mucositis dental implants included:

- Occasional or absence of periodic maintenance of periodontal tissues of the subjects after prosthesis placement
- Positive detection of redness and swelling of the mucosa of the peri-implant tissues
- On visual examination, presence of supra-gingival plaque on the restored dental implants
- mSI score of 2 or 3 on the fixture
- Detection of PD upto 3 mm around the fixture's sites
- Maximum upto 2mm BL on the either sides of the fixture based on the criteria described above

Criteria for subjects with Peri-Implantitis Implants dental implants included:

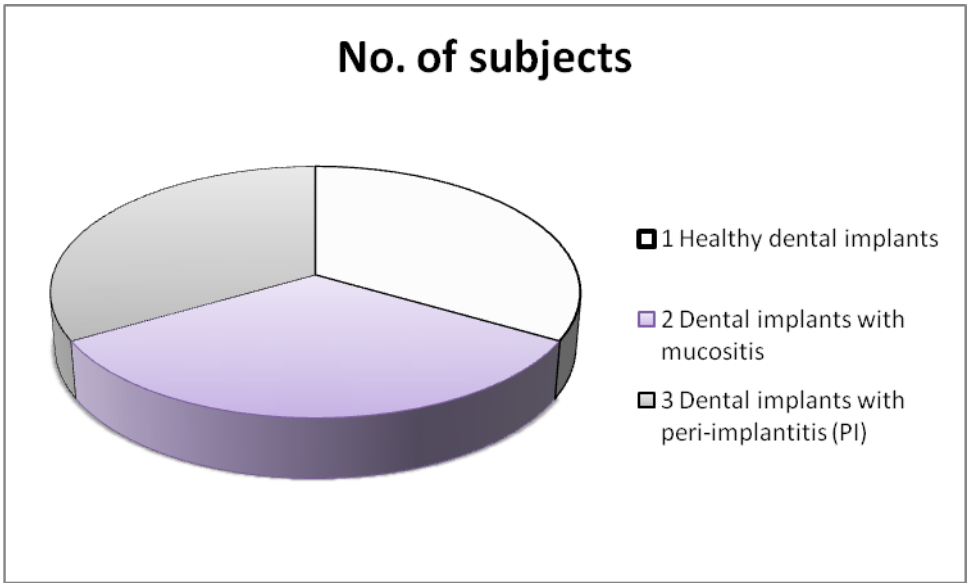
- Absence supporting treatment after the prosthesis placement
- Positive detection of supra-gingival plaque on the resorted dental implants
- Presence of 2 to 3 mm of mSI score
- Presence of PD of equal to more than 6 mm in minimum of one site around the fixture
- Presence of BL to a minimum of half of the length of the fixture
- Recording of the mobility of the fixture and suppuration was also done.

Recording of all the clinical parameters was done with standard well calibrated probe with standard 20 gram of probing force. Consideration of only the worst clinical parameter was done which were detected in one of the four sites (mesial, distal, buccal and lingual). Collection of the PIF was done for evaluation of AST activity in the experimental groups at the time of second visit. Randomly, on the mesial or distal site, sampling of the PIF was randomly preformed from the healthy implants and implants with mucositis where as sites associated with deepest PD during the first visit, the peri-implantitis samples were obtained. Removal of all the supra-gingival plaque was done prior to the PIF collection followed by isolation of the sampling sites with cotton pellets. Gently air streaming was done followed by drying for 50 seconds. Sterile 40 number endodontic paper pints were used for the collection of PIF from the base of the sulcus. These paper points containing the specimens were sent to the laboratory for assessment. Measuring of the weight of the paper pints before and after the sampling procedure was done with an analytical balance. The difference in the weight of the paper points was considered as the values of adsorbed PIF volume. Expression of the AST activity was taken from the value of the PIF volume. Spectrophotometrical method was used for the measurement of Transaminase activity.[6, 7] Scandinavian method was used for the assessment of AP activity.[8] Method described by Strbac et al was used for assessment of CK levels.[9] Expression of the results was done as AST Units/ml in PCF. SPSS software was used for the analysis of the results. One way ANOVA, chi-square test and Mann-Witney U test were used for the assessment of the level of significance. P-value of less than 0.05 was taken as significant. For determination of linear association between the AST values and clinical parameters, Spearman's rank correlation coefficient was used. Value of AST of more than or equal to 0.4 U/ml was taken as threshold for positive test.

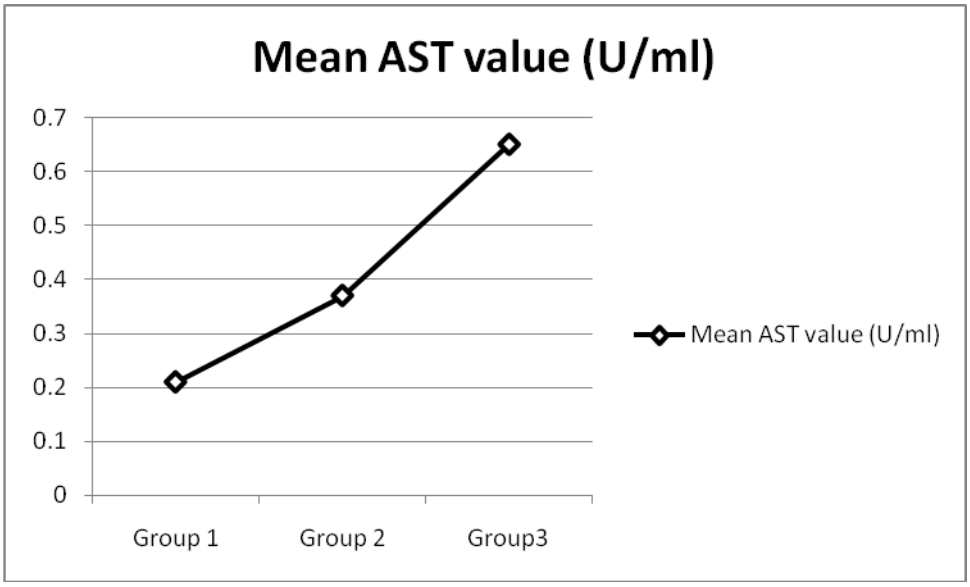
RESULTS

Graph 1 shows the division of the patients in various groups. Mean values of AST in PIF of patients from various groups is highlighted in **Graph 2**. In healthy patients, the mean AST levels were found to be 0.21 U/ml. In patients with mucositis and with peri-implantitis, the mean values of AST were found to be 0.37 and 0.65 U/ml respectively. The mean value of AP in group 1, group 2 and group 3 was found to be 11.20, 17.20 and 24.30 IU/mL respectively (**Graph 3**). The mean value of CK in group 1, group 2 and group 3 was found to be 1.15, 2.10 and 3.20 pmol/sample respectively (**Graph 4**). **Graph 5** shows the value of AST and other clinical parameters in patients of different groups. Mean PD was found to be 2.10, 2.85 and6.80 in group 1, 2 and 3 patients respectively. As far as mean BL is concerned, the value was found to be 1.56, 1.95 and 5.20 respectively in group 1, 2 and 3 respectively. **Table 1** shows the p-values for comparison of various clinical parameters and AST values in between various study groups. Significant difference was obtained while comparing mean AST, AP and CK level sin between peri-implantitis patients and mucositis patients. While comparing the various periodontal parameters in between study groups, significant differences were obtained.

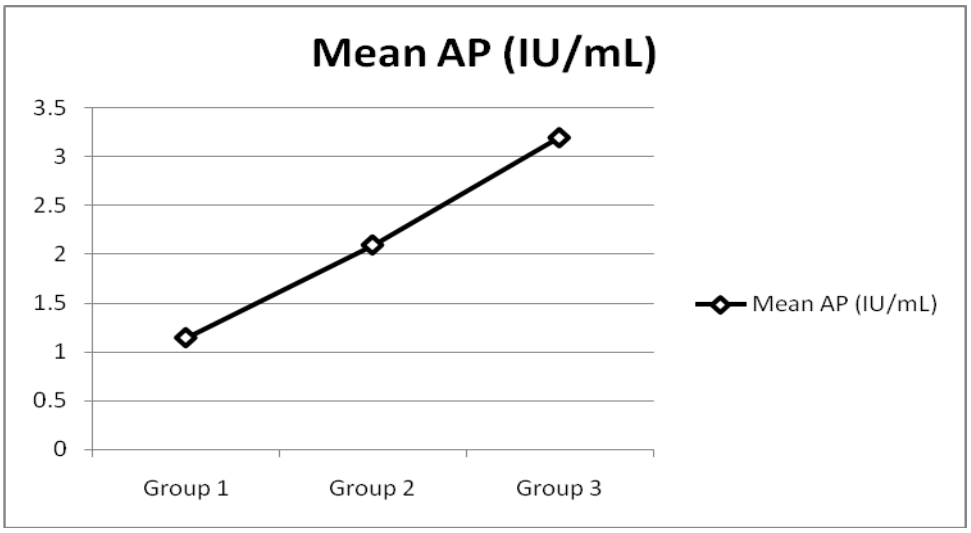
Graph 1: Distribution of subjects into various groups



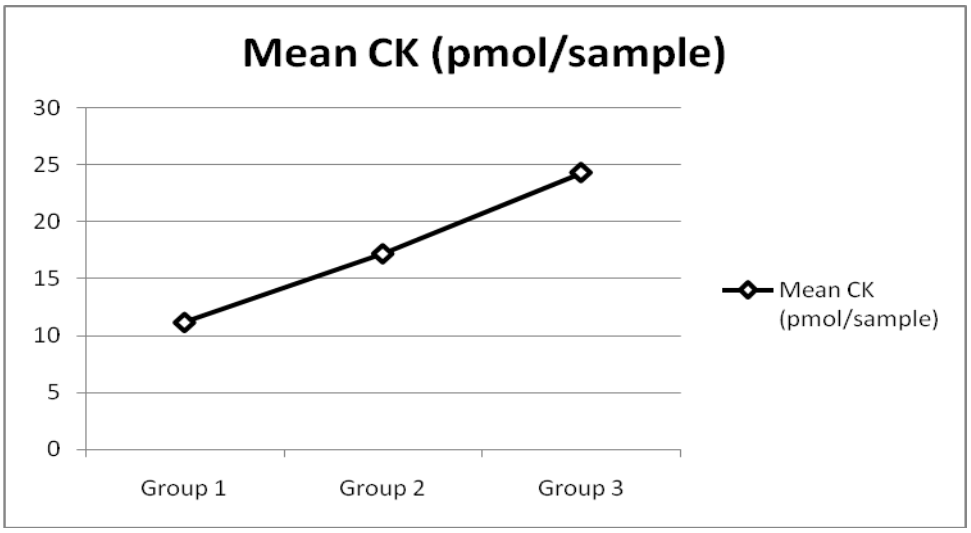
Graph 2: Mean values of AST in PIF of patients from various groups



Graph 3: Mean values of ALP in PIF of patients from various groups



Graph 4: Mean values of ALP in PIF of patients from various groups



Graph 5: Value of AST and other clinical parameters in patients of different groups

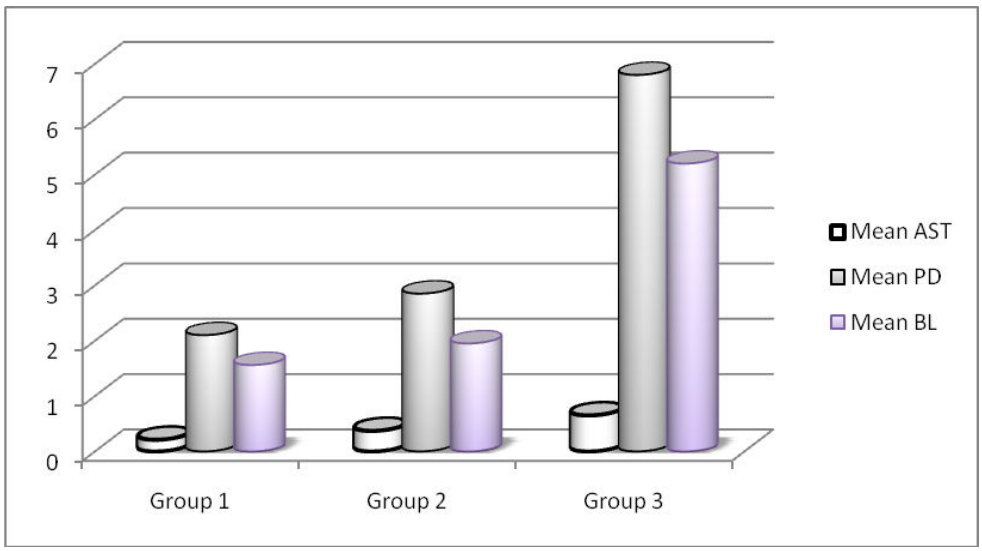


Table 1: p-value for the difference of AST levels and clinical parameters in between the various study groups

Clinical parameter	Group 1 Vs Group 2	Group 2 Vs Group 3	Group 1 Vs Group 3	Group 1 Vs Group 2 Vs Group 3
Mean AST	0.51	0.01*	0.03*	0.03*
Mean AP	0.02*	0.01*	0.03*	0.01*
Mean CK	0.03*	0.02*	0.04*	0.04*
Mean PD	0.01*	0.04*	0.01*	0.01*
Mean BL	0.02*	0.01*	0.04*	0.021*
Mean mSI	0.02*	0.03*	0.03*	0.02*

*: Significant

DISCUSSION

In the modern day dentistry, endosseous dental implants have become a very important constituent of the replacement therapy for rehabilitating missing teeth. Despite of the high success rate of the dental implant therapy, failure rates of dental implants have been reported in a number of previous studies. Improved surgical techniques of dental implants have also improved the success rate of dental implants.[10, 11] Controlling the onset and progression of inflammation and pathologies in relation to the peri-implant tissues have also resulted in improvement of the long-term survival rates of the patients who have undergone dental implant therapy.[12, 13] Diseases process of the tissue in the periphery of the dental implants has also proved to be responsible for the failure of dental implants which have been further attributed to dental plaque. Several investigators from time to time have assessed various clinico-radiographic parameters in dental implant patients. No current diseases status is exhibited by the radiographic details of the patients with dental implants showing osseous resorption.[14, 15] AST is found to be an important constituent of GCF in periodontal inflamed tissues. It is an important intracellular enzyme released into the extracellular environment after cell death and has been used widely in the diagnosis of various hepatic and cardiac pathologies.[16]

In the present study, we observed that in all the cases of healthy implants, dental implants with mucositis and dental implants with peri-implantitis, AST activity was demonstrated on regular basis. Also higher AST activity was observed in cases of peri-implantitis in comparison with the subjects of the other two groups. We also observed significant association of AST, AP and CK activities with clinic-radiographic parameters in cases of healthy dental implants. Our results are in correlation with the results of previous studies which also observed significantly higher values of AST, AP and CK around dental implant cases with increased probing parameters.[17, 18] Rühling et al analyzed the AST levels in the crevicular fluid (CF) of dental implants which exhibited peri-implantitis and evaluated the correlation of AST levels and progressive loss of attachment. They assessed 20 patients who received 42 endosseous cylindric titanium implants and evaluated the radiographic features in relation to the pre-existing bone loss, clinical measurements and AST analysis in CF. The values were measured twice in the span of six months. They analyzed a total of 168 sites in 7 patients in which 13 sites showed attachment loss of equal to or greater than 1.0 mm. They observed low positive and high negative predictive values along with a sensitivity of 15 percent. From the results, they concluded that AST has a very limited value as a diagnostic maker in assessment of peri-implant diseases.[19] Paknejad et al investigated the presence of AST and alkaline phosphatase (ALP) levels in crevicular fluid in dental implants subjects with and without clinic-radiographic features of peri-implantitis. They analyzed 17 dental implant cases which presented with signs and symptoms of peri-implantitis in a total of 12 patients and compared the results with those of 13 subjects who received a total of 17 dental implants and showed absence of peri-implant pathology. They used filter paper strips for collecting the peri-implant crevicular fluid from the base of the sulcus and observed a significant difference between the activities of AST and ALP in between the two study groups. Also a significant activity of AST was found to be associated with the amount of Bleeding on probing. From the results, they concluded that for establishing the role of peri-implant crevicular fluid as the diagnostic fluid, future longitudinal studies are required.[20] Fiorellini et al evaluated the association of AST levels with routine periodontal clinical parameters around the peri-implant surface. They assessed a total of 20 healthy subjects who received 59 dental implants and recorded their clinical periodontal parameters. However, they didn't observe any significant difference between clinical periodontal indices and increased AST levels on individual basis.[21] Sánchez-Pérez A et al evaluated the presence of AST levels in the peri-implant crevicular fluid in patients with and without peri-implantitis. They evaluated the AST levels in 60 successful implant cases that were inserted in 25 patients. Twice collection of the samples was done for the measuring the levels of AST both the times. Clinical periodontal parameters were recorded and evaluated for the presence of mucositis. They observed a significant difference between the AST levels when compared at different time intervals. From the results, they concluded that in patients with mucositis, AST is a reliable predictor.[22] Paolantonio et al investigated the activity of AST in the peri-implant crevicular fluid (PCF) among healthy and diseases dental implant patients to study their diagnostic role. They assessed 81 fixtures form 81 healthy patients and divided them into three study groups. First group comprised of healthy patients while

the other two groups included dental implant cases with mucositis and peri-implantitis respectively. All the groups consisted of 27 patients in each group. Collection of PCF was done with the help of endodontic paper pints and measurement of the AST activity was done with spectrophotometrical analysis. They observed that in healthy implant cases and implant cases with mucositis, the mean value of AST was found to be 0.26 U/ml and 0.38 U/ml respectively. They didn't observe any significant difference between the healthy implant cases and implant cases with mucositis in relation to the AST activity. From the results, they concluded that further investigation of the AST levels in implant pathologies could open up a new field and role of AST as a diagnostic marker.[23] Strbac et al evaluated the correlating of concentration of CK secreted into the crevicular fluid in the peri-implant areas and concluded that higher amount of association exists in between CK and peri-implant inflammation.[9] Ishikawa and Cimasoni also observed a positive correlation of AP levels in gingival crevicular fluid and amount of inflammation in the periodontal areas. [24]

CONCLUSION

Some amount of diagnostic significance exists in context to AST, AP and CK activity in PIF of the implant patients with implant associated pathologies. However, future studies are required for establishing the diagnostic role of AST in peri-implant pathologies.

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