

# Correlation of Peri-implant Health and Aspartate Aminotransferase Levels: A Cross-Sectional Clinical Study

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*At present, there are no diagnostic tools that permit early detection of peri-implantitis. The purpose of this cross-sectional study was to evaluate the correlation of aspartate aminotransferase (AST) levels with traditional periodontal clinical parameters around dental implants, since AST has been associated with destruction of cardiac, hepatic, and periodontal tissues. Twenty healthy volunteers with 59 implants were recruited from the Harvard School of Dental Medicine clinics. Clinical parameters evaluated included: AST level, probing depth (mm), Gingival Index (0, 1, 2, or 3), and bleeding on probing (0 or 1). Utilizing the site or implant as the unit of measure, the authors found a statistically significant association of increased AST activity with positive bleeding on probing, increased probing depth, and increased Gingival Index. No statistical correlations were found between clinical indices and increased AST levels when the results were examined on an individual patient basis. This cross-sectional study was able to demonstrate a statistical correlation between diseased clinical periodontal parameters and elevated AST levels. (INT J ORAL MAXILLOFAC IMPLANTS 2000;15:500–504)*

**Key words:** aspartate aminotransferase, dental implants, diagnosis, gingival crevicular fluid, peri-implantitis

Endosseous dental implantation to provide support for replacement of missing teeth has become an important component of modern dentistry. Although osseointegration can be predictably achieved, failures do sometimes occur. The short-term success of dental implants has been made possible as a result of improved surgical techniques and materials.<sup>1–4</sup> Long-term survival has been related to patient-dependent factors, including ability and willingness to control etiologic factors responsible for the onset and progression of peri-implant tissue inflammation.<sup>5,6</sup>

Overall, longitudinal clinical trials have shown a low incidence of implant failure.<sup>7–10</sup> Implant failures have been in part the result of a disease process in the soft and hard tissues termed *peri-implantitis*. Generally, this process has been assumed to be related to bacterial plaque with or without overload.<sup>5,6</sup> The criteria for evaluating the success or failure of implants have been developed by several investigators.<sup>8,11–14</sup> Assessments have included radiographs and dental indices, such as bleeding on probing, pocket depth, and mobility. Currently, radiographic examinations alone have been utilized as the means to evaluate success with respect to peri-implant bone levels. As a diagnostic tool, radiographs depict only a past pattern of osseous resorption; they do not reveal current disease status. Once this anatomic loss of bone has occurred, the disease has progressed to an advanced stage and regeneration may be difficult or impossible to accomplish. Therefore, it is essential that a diagnostic tool for early detection be developed to evaluate a change in peri-implant status prior to irreversible bone loss.

An area of investigation for the early detection of periodontitis has been gingival crevicular fluid (GCF) analysis. Gingival crevicular fluid found at the gingival sulcus or periodontal pocket is a serum-like

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fluid exudate from capillaries. Several diagnostic tests based on metabolic markers have been developed.<sup>15-19</sup> These include markers of proteolytic enzymes, tissue damage, and osseous breakdown. Some of these same markers have been identified in peri-implant crevicular fluid and related to implant disease status.<sup>20-22</sup>

In inflamed periodontal tissues, GCF has been found to be made up of enzymes, such as aspartate aminotransferase (AST).<sup>23</sup> Aspartate aminotransferase is an intracellular enzyme released from injured or dead cells and has been used in medicine to diagnose cardiac and hepatic disease.<sup>24-27</sup> Aspartate aminotransferase activity has been shown to be related to gingival health in several animal and human models. Animal studies demonstrated that an elevated AST level was detected in experimentally induced periodontitis.<sup>23,28</sup> Cross-sectional human studies indicate that a correlation exists between AST levels and periodontal disease severity.<sup>29,30</sup> Longitudinal investigations have shown a correlation between AST levels and gingival inflammation, suggesting that this enzyme may be used to assess site-specific risk of future attachment loss.<sup>31-34</sup>

Since traditional measures have limitations as prognostic indicators for implant success, the purpose of the present cross-sectional study was to evaluate whether AST levels around implants are associated with clinical parameters, including peri-implant probing depth, Gingival Index, and bleeding on probing.

## MATERIALS AND METHODS

The study protocol was approved by the Harvard Medical School Committee on Human Studies. Each patient who entered the trial signed a consent form and met specific inclusion criteria. Healthy volunteers were recruited from the Harvard School of Dental Medicine clinics for this cross-sectional investigation. Inclusion criteria were: (1) presence of at least 1 endosseous dental implant restored with appropriate prosthesis, (2) no history of antibiotic treatment prior to the study for 3 months, (3) no history of medical conditions that required antibiotic prophylaxis, (4) no smoking habit, and (5) negative history of chronic corticosteroid use.

A complete oral examination was given prior to performance of the AST assay and measurement of clinical parameters. First, AST levels were measured as previously described at the mesiobuccal, distobuccal, and direct lingual/palatal of the implants and scored as 0, 800, or 1,200  $\mu$ IU.<sup>35</sup> The test involves collection of crevicular fluid for 30 seconds with filter paper strips. The presence of AST activity is indicated

by a colorimetric reaction. The test trays contain the dried substrate and indicator reagents necessary for the reactions. The dry ingredients were reconstituted by adding 3 drops of buffer solution. After the test samples were collected, the reaction was started by adding 1 drop of starter solution to each well, except for the 2 wells designated as control wells. To assist the trained test reader, each tray of microwells included control wells of 800 and 1,200  $\mu$ IU for comparison. Prior to study initiation, the AST reader was trained against known controls prepared by the manufacturer. Next, clinical parameters of peri-implant probing depth (mm), Gingival Index (GI) (0, 1, 2, or 3),<sup>36</sup> and bleeding on probing (BOP) (0 or 1) were recorded by a single examiner at the same sites analyzed for AST activity. Criteria for implant health and disease were established. Healthy implants were defined as having probing pocket depths less than 4 mm, a GI of 0, no BOP, and an AST score of 0. Diseased implants were defined as having probing pocket depths  $\geq$  4 mm, a GI  $\geq$  1, presence of BOP, and presence of an 800 or 1,200  $\mu$ IU AST score.

Probing calibration was performed to assess examiner measurement error. To calculate measurement error, the study examiner's results were compared to those of a previously calibrated individual to confirm adequate interexaminer reliability.<sup>37</sup> A paired *t* test revealed that the standard deviation for probing depth was 0.57 mm, with no significant differences when compared to the calibrated examiner ( $P > .05$ ). In addition, percent agreement was also calculated. Percent agreement within 1 mm was 98%. When the threshold was extended to 2 mm, percent agreement was 100%. The analysis of the data consisted of descriptive and bivariate statistics utilizing the site, implant, and patient as the units of measure. A mean and standard deviation were calculated for the clinical parameters and demographic information. For the statistical analysis, AST scores of 0, 800, and 1,200 were assigned 0, 1, and 2, respectively. To assess the correlation between AST scores and clinical parameters, Spearman's rank correlation test was used at the patient and implant level of analysis. The Wilcoxon rank sum test was used to estimate the correlation of clinical parameters to AST level on a site-specific basis.

## RESULTS

Twenty patients (13 men and 7 women, 17 partially edentulous and 3 completely edentulous) with 59 implants were examined in this cross-sectional study (total of 177 sites examined). There was a total of 45 Straumann implants (Institut Straumann

**Table 1 Spearman's Correlation Utilizing Implants and Patients as Units of Measure**

Clinical parameter	P values	
	Implant	Patient
Probing pocket depth	.0016	NS
Bleeding on probing	.0324	NS
Gingival Index	.0115	NS

NS = not statistically significant ( $P > .05$ ).

AG, Waldenburg, Switzerland) and 14 Nobel Biocare implants (Nobel Biocare, Göteborg, Sweden). Comparison of the 2 implant systems in regards to AST activity was not performed due to the limited number of Nobel Biocare implants assessed. The mean time in function was  $14.3 \pm 8.7$  months. Fifty implants had been restored with fixed prostheses, and 9 were restored with removable dentures. The mean and standard deviations for pocket depth, BOP, and GI were  $4.15 \pm 1.08$  mm,  $0.35 \pm 0.39$ , and  $1.46 \pm 0.41$ , respectively.

The significance of correlations between AST and the clinical parameters was dependent on the unit of measure. When the site was used as the unit of measure, increased AST activity was found to be significantly associated with "diseased" clinical parameters by the Wilcoxon rank sum test. The levels of significance were .0017, .0021, and .0011 for pocket depth, BOP, and GI, respectively. The Z scores for pocket depth, BOP, and GI were 3.0, 3.1, and 3.2, respectively.

When the implant was utilized as the unit of measure, significant correlations between the increased AST and the number of diseased implants were observed (Table 1). Implants with elevated AST levels were correlated with higher "diseased" clinical parameters. Spearman's correlation coefficients were 0.43, 0.30, and 0.35 for pocket depth, BOP, and GI, respectively, and were statistically significant ( $P \leq .05$ ). No statistical correlations were found between clinical indices and AST levels when the results were examined at the patient level (Table 1).

## DISCUSSION

The present criteria for evaluating the disease status around dental implants have been based on clinical and radiographic changes. Both criteria are often indicators of extensive pathologic changes and may not be reflective of current disease status. Radiographic bone loss is visualized only after extensive demineralization, and clinical parameters, including the gingival and plaque indices, are often subjective

in nature. Measurements such as probing pocket depth and probing attachment have associated examiner error and can be complicated by anatomic factors. The tissues surrounding dental implants have been found to be structurally different from the natural dentition. Around implants, the gingival tissues form a tight adhesion, similar to junctional epithelium around teeth. However, since no cementum is present, the periodontal probe can easily pass apically to the epithelial junction.<sup>38,39</sup> As a result, the measurements for pocket depth may differ from those of the natural dentition, especially when inflammation is present. An often-used clinical parameter has been BOP. In healthy gingival tissue surrounding the implants, there is usually no detectable bleeding. However, due to differences in the attachment apparatus between teeth and dental implants, BOP may not be an accurate indicator of peri-implant inflammation. Bleeding on probing has been reported to be a frequent finding around healthy dental implants.<sup>40</sup>

The relationship between bacterial populations in peri-implant disease progression has also been investigated as a prognostic indicator.<sup>41,42</sup> These investigations indicate trends similar to those found around the natural dentition. A shift from a gram-positive to a gram-negative population occurs with progression of disease. Other investigators have demonstrated that shallow pockets harbor a non-pathologic bacterial population, whereas deeper pockets ( $> 5$  mm) are populated by a pathogenic microflora. However, the identification of specific bacterial species has not given clinicians the ability to determine the presence of active disease or the risk of future disease progression.

As a result, limitations associated with the use of traditional clinical parameters necessitate the development of objective biochemical markers to assess peri-implant health. The early recognition of peri-implantitis would permit the clinician to intervene more rapidly, increasing the likelihood of treatment success. Recent investigations have attempted to characterize inflammatory mediators that may precede anatomic changes around dental implants. Interleukin-1 $\beta$  has been found to be elevated at diseased compared to healthy sites.<sup>20</sup> Elevated prostaglandin E<sub>2</sub> and C-telopeptide pyridinoline cross-links levels have been associated with risk of peri-implant disease progression.<sup>21,22</sup> An early and objective means to monitor peri-implant health may be diagnostic AST. This enzyme has been associated with destruction of cardiac, hepatic, and periodontal tissues and measures the presence of active tissue breakdown.<sup>23-25</sup> In healthy sites, AST has not been detected outside of cells. When cell death occurs, an

elevated level can be found in the associated serum or fluid. In dentistry, AST activity has been demonstrated to be present in gingival crevicular fluid and has been shown to be an indicator of active periodontal tissue breakdown. The relationship of AST levels to health, gingivitis, and periodontitis has been evaluated in both animal and human studies.<sup>29-33</sup> These studies, conducted in both cross-sectional and longitudinal designs, have correlated clinical parameters, including probing pocket depth and probing attachment levels, to differing thresholds of enzyme activity. In a multicenter study, the risk of periodontal disease progression over 1 year was associated with the presence or absence of baseline AST.<sup>34</sup>

The present cross-sectional study investigated the relationship of AST activity and clinical parameters around dental implants. Elevated AST levels were statistically associated with positive BOP, pocket depth  $\geq 4$  mm, and GI  $\geq 1$ , utilizing the site or implant as the unit of measure. These findings were similar to those of a previous study in which diseased and healthy sites of the natural dentition were compared with the AST activity.<sup>30</sup> This investigation indicated that AST levels were found to be elevated at active disease sites, as compared to healthy sites within the same individuals. These results supported the concept that site-specific elevated AST levels were associated with disease. The results around dental implants in the present study allow for potential use of AST to monitor disease status at the site level.

When the data were analyzed for significance at the patient level, no statistical correlations were found between increased clinical indices and AST levels. The lack of a relationship between AST activity and any clinical indices is within expectations. The data utilized to evaluate individual patients were the means of AST activity and the clinical parameters. The mean of each variable suffered the loss of site information, which resulted in a dilution of the relationship between AST and clinical indices. Patients with low AST levels should not be interpreted as having all healthy implants, since a diseased site may be present. This observation was also consistent with the disease process of dental implants, which occurs at specific sites. Therefore, utilization of this assay in clinical practice could include monitoring of specific implants or implant sites within the mouth where diagnostic uncertainty exists.

The results of this investigation should be interpreted with caution due to the cross-sectional design. Although elevated AST was correlated to increases in the clinical parameters, it cannot be certain as to which elevation initiated the process. For example, bleeding upon probing may have resulted in an 800 or 1,200  $\mu$ IU AST. In addition, the

enhanced predictability of treatment decisions and implant prognosis based upon AST status cannot be determined from a cross-sectional study. Therefore, to better characterize AST activity, future study considerations should include a longitudinal study design, increased sample size, and the inclusion of additional clinical parameters.

## CONCLUSION

This study examined the relationship of AST and clinical parameters. The results indicated a statistical correlation between the clinical parameters of disease and health to AST levels at the site and implant level. The importance of these findings may be that in the absence of traditional parameters, such as bleeding on probing or gingival erythema, AST may indicate risk or lack of risk for disease progression at specific implants or implant sites. An alternative hypothesis is that the presence of elevated AST levels at specific implant sites, in conjunction with traditional signs of disease, may identify sites at an increased risk of peri-implant breakdown. It appears that the AST test may be useful as an adjunct to traditional clinical parameters.

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