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Original

Use of aspartate aminotransferase in diagnosing periodontal disease: a comparative study of clinical and microbiological parameters

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Abstract: The objective of this study was to assess the association between the levels of enzyme aspartate aminotransferase (AST) in gingival crevicular fluid (GCF) with the BANA hydrolysis microbiological test (Perioscan™) and clinical periodontal diagnostic measurements, such as bleeding on probing, plaque index, gingival index, probing depth, and attachment level in patients with chronic periodontitis using an enzymatic test (PocketWatch™). One hundred and forty-seven sites were evaluated in 22 patients with a probing depth of ≥ 5 mm at selected sites. AST and BANA enzymatic tests were carried out, and clinical parameters recorded. Pearson's chi-square and Fisher's exact tests were used for statistical analysis. There was no statistical correlation between AST levels and any of the analyzed parameters. The lack of any association between the factors studied does not indicate, however, that the latter cannot be used in diagnosing the actual periodontal condition of patients and/or sites. However, more research should be carried out to evaluate the true relationship between AST and periodontal disease. (J. Oral Sci. 45, 33-38, 2003)

Key words: aspartate aminotransferase; enzymatic tests; periodontal diagnosis; gingival crevicular fluid; periodontal disease.

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Introduction

The correct diagnosis of periodontal conditions is the key to a treatment plan, prognosis and maintenance of periodontal health. Knowledge of disease progression and pathogenesis has influenced the study of methods for periodontal diagnosis. Traditional procedures, such as probing and radiographic assessment, provide information of what has already occurred, and not what is occurring or will occur with disease progression (1,2).

Determining the activity of periodontal disease has a direct impact on treatment (3). Loesche (4) proposed that the need for treatment would be established if there are large quantities of microorganisms present. An active site is characterized by continued loss of bone support, connective tissue, or clinical attachment level.

On the other hand, host response factors, such as gingival crevicular fluid components, have been extensively studied lately, as well as serum and cell components (5). There are more than 50 indicators of inflammatory and immune response identified in gingival crevicular fluid (GCF) (1,2).

Adolph & Lorenz (6) and Wolf & Williams (7) have reported the presence of high levels of aspartate aminotransferase (AST) in serum as a strong indicator of myocardial infarction. The enzyme AST is found in all cells, and its levels vary in different cell types. Cells release AST into the extracellular space during periods of tissue necrosis or trauma, resulting in a detectable enzyme quantity in the peripheral circulation. Higher levels of AST are found in the GCF of diseased sites than in healthy sites, and have been reported as a possible marker of periodontally active sites (8-24). Significant AST levels have also been found in human gingival epithelial cells, human gingival fibroblasts and human periodontal ligament fibroblasts

(14).

It is difficult to draw any conclusions about the relationship between specific pathogens and the destructive process in specific sites at the time of sample collection (25). The monitoring of factors associated with host response and periodontal tissue destruction has been suggested for assessing tissue destruction activity (3). An association between the presence of certain specific pathogens and disease markers in the fluid suggests the possibility of using a combination of bacterium and host response markers for assessing the risk of active periodontal disease (21,22).

The aim of this study was to analyze the association between the presence of the enzyme AST measured according to the PocketWatch™ test with microbiological parameters of the BANA hydrolysis test and clinical parameters of bleeding on probing, probing depth, attachment level, plaque index and gingival index in sites with periodontal disease.

Materials and Methods

Twenty-two patients with chronic periodontal disease were selected from the Periodontal Clinic of Araraquara Dental School to participate in this study. Their ages ranged from 28 to 63 years (mean ± SD, 39 ± 8.3) and there were 10 males and 12 females, with a total of 147 sites. The study was approved by the Ethics Committee of the Araraquara Dental School, and all subjects volunteered to participate and signed an informed consent form. After anamnesis, clinical examination was conducted by a single previously trained examiner using a Williams sterile periodontal probe and an oral mirror (Hu-Friedy). Patients who had sites with a probing depth of ≥ 5 mm in single rooted teeth, and no endodontic lesion, occlusion-related dysfunction or cavity or restoration problem were included in the sample and underwent testing.

Day 7: Enzymatic Test (Pocketwatch™, Steri-Oss®, San Diego, CA, USA)

Approximately 1 week after site selection, enzymatic testing was conducted. Initially, a test tray was prepared by adding 3 drops of reconstitution buffer (Bottle A) to each test well. Selected site areas were air-jet dried and isolated with a cotton swab. A sampling paper strip was delicately placed into the gingival pocket and left for 30s for fluid absorption. The strip was then placed in the corresponding plastic well containing the buffer solution. The reaction was then started by adding one drop of starter solution (Bottle B) to each test well. This procedure was repeated for each selected site of each patient. Strips containing blood or saliva were discarded.

Day 9: Microbiological (BANA) Test

The microbiological assessment was carried out 2 days later using the BANA test (Perioscan™, Oral-B® Laboratories, Belmont, CA, USA), presented in card form, to diagnose the presence of *Porphyromonas gingivalis*, *Treponema denticola* and *Bacteroides forsythus* in the subgingival bacterial plaque. These periodontopathogens produce a trypsin-like protease that hydrolyzes the N-benzoyl-DL-arginine-2β-naphthylamide (BANA) substrate, when incubated, resulting in a variable colorimetric reaction.

The clinical specimen earmarked for microbiological examination was collected from the same dental surface where the PocketWatch™ test was carried out. Thus, after the supragingival plaque was removed and discarded with gauze and sterile curettes, samples of the subgingival bacterial plaque were collected. A BANA hydrolysis reaction was carried out according to the methodology recommended by Löesche et al. (26). Results were read by two independently trained examiners and were considered positive if a blue color was present, and negative in the absence of color.

One week later, the following clinical parameters were analyzed: plaque index (PI) (27); gingival index (GI) (28); probing depth (PD); attachment level (AL), and bleeding on probing (BP). Probing depth, attachment level, and bleeding on probing were done with the aid of a controlled-force electronic probe (Florida Probe® Corporation, Gainesville, FL, USA) and a crystal acetate plate with previously marked grooves for each selected site. All clinical parameters and patient data were recorded on individual forms previously prepared for the research study.

Pearson's chi-square test and Fisher's exact association test were used for statistical analysis ($P < 0.05$).

Results

Of 147 sites, 125 (85%) were AST positive and 124 (84%) were BANA positive. Of those BANA-positive sites, 105 (84.7%) were also AST positive and only 19 (15.3%) were AST negative. However, this same percentage profile was also found among BANA-negative sites, as can be seen in Fig. 1. Using Pearson's chi-square and Fisher's exact association tests we concluded that there was no significant association between the BANA and AST results.

Of sites positive for bleeding on probing, 113 (86.3%) were also AST positive and only 18 (13.7%) were AST negative. On the other hand, among sites negative for bleeding on probing, 12 (75%) were AST positive and only 4 (25%) were AST negative (Fig. 1). Despite this difference, these data were also not statistically different.

AST positive and negative percentage profiles (Table 1) were very similar for plaque indices 1 through 3, and for plaque index 0 these values were slightly different (Fig. 1). Despite this difference, the percentage of profiles was not statistically different.

AST positive and negative percentage profiles (Table 1) were very similar for all gingival indices from 1 through 3. Thus, we concluded that there was no association between AST and gingival index variables (Fig. 1).

Probing depth and attachment level variables were jointly compared to the AST results. A comparison between the AST+ and AST- groups was made using Hotelling's T^2 multivariate test, which resulted in a value of P = 0.7326, indicating that there was no significant difference between probing depth and attachment level as a function of the AST being classified as either positive or negative.

The results of the percentage of BANA and AST-positive sites, according to plaque and gingival indices were analyzed. For the plaque index values, a correlation analysis and a quadratic regression fit method were utilized. For the gingival index results, this same type of analysis was not possible due to the low number of points. AST+ and BANA+ values were very similar for both PI 1 and GI.

Discussion

The enzyme AST is present in the blood, but its levels

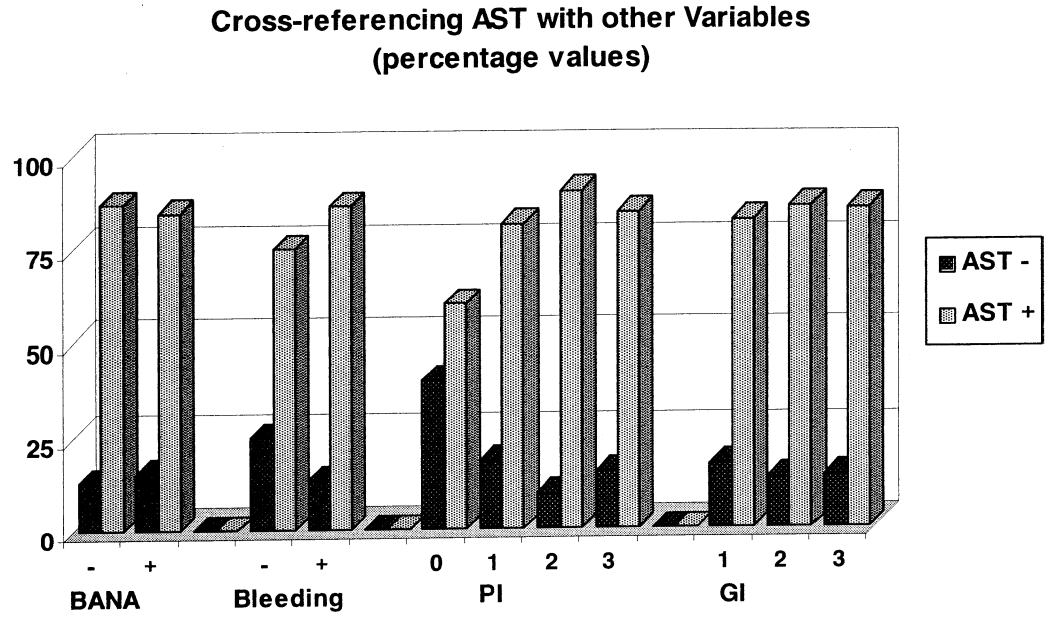

Fig. 1 Cross-referencing AST with other variables (percentage values). PI: plaque index, GI: gingival index.

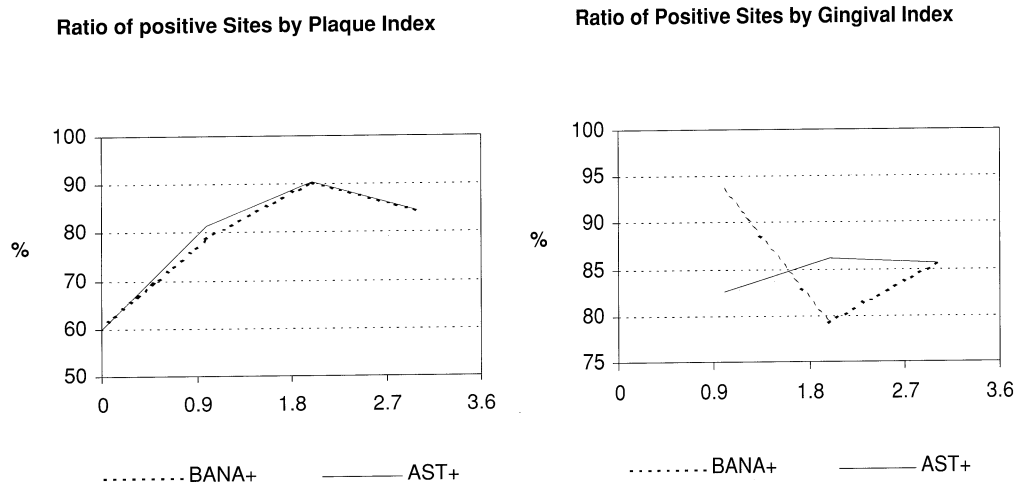

Fig. 2 Ratio of BANA and AST positive sites by PI and GI.

are lower than those necessary for the test to be positive, except in acute or severe cases of liver, muscle or heart disease. Since none of the patients reported or presented these problems, this indicates that the AST originated from the fluid and was due to changes occurring in periodontal tissue. However, we must consider the results of this study with caution. Cross-sectional studies, such as this one, have a limited value for detecting periodontal disease and comparing clinical indices of its presence or absence.

In this study, when the presence of AST in fluid was assessed in relation to BANA hydrolysis, a correlation between the two tests and the presence of periodontal disease was found (85% of sites with a periodontal pocket of ≥ 5 mm were AST+ and/or BANA+). Although the percentage of AST+ and BANA+ sites had high and similar values, 85% and 84% respectively, there was no statistical correlation between them. Sites with an AST+ result had similar values for BANA positive and BANA negative sites, and the same occurred with AST- sites. The data from this study are similar to those of Smith et al. (21), who did not find a positive association between AST levels and BANA, although AST levels showed a significant correlation in sites with periodontal disease in patients with chronic periodontitis.

This lack of correlation between the two examinations could be associated to the fact that the BANA test only detects the presence of three periodontopathogenic bacteria, and therefore does not cover other possible pathogens causing periodontal damage, such as *Actinobacillus actinomycetemcomitans*. Another possible factor could be the quantity of pathogens detected through BANA, which might not be sufficient to cause a host response detectable by means of the PocketWatch™ test.

Table 1 Percentage of AST and BANA-positive sites, by the Plaque Index and Gingival Index

Plaque Index	BANA +	AST +
0	60.0	60.0
1	78.9	81.6
2	90.4	90.4
3	84.6	84.6
Gingival Index	**BANA +**	**AST +**
1	93.5	82.6
2	79.3	86.2
3	85.7	85.7

Analyzing AST+ and BANA+ sites with plaque and gingival indices, it was possible to observe very similar values between them, showing a relationship between positive sites and these two clinical parameters. Although this association did not generally occur, when only positive sites were analyzed, both tests correlated to one another through the assessed parameters. In fact, this trend for a significant correlation between these parameters in AST positive and BANA positive sites suggests that their activity may be indicative of changes that have occurred and/or are occurring in periodontal tissue (21).

In the present study, no association was found between high AST levels and bleeding on probing. According to Chambers et al. (8), the lack of any correlation between these parameters is beneficial, because a test for disease activity should detect the product of periodontal destruction rather than that of gingival inflammation. Haffajee et al. (25) reported that bleeding on probing is associated with a high ratio of false positive interpretations. The presence of bleeding on probing proved reliable for assessing gingival status, however its absence did not prove reliable for monitoring gingival status in inflammatory processes. In addition, the capability of a cross-sectional study for detecting markers of active periodontal destruction is limited and depends directly on the frequency and duration of destructive episodes (10).

Correlating AST levels and plaque and gingival indices demonstrated that the occurrence of AST+ sites was associated with sites with some inflammation of gingival tissue. In addition, upon analyzing the percentage values of AST+ sites with these two parameters, we noted a high incidence of gingival inflammation with higher AST+ scores, and that this incidence increased with greater inflammation. In regression analysis of these AST+ sites with plaque and gingival indices, there was a significant correlation with the plaque index and also a trend to close values for gingival index, indicating a possible relationship between diseased sites and an inflammatory condition.

Wong et al. (22), Shimada et al. (20) and Kamma et al. (24), analyzing the association of plaque and gingival indices with AST in sites with a probing depth of ≥ 4 mm, did not find any significant difference between positive and negative sites, particularly in relation to the gingival index, and such results are consistent with our study. This may be indicative that increased AST levels in fluid can occur prior to clinical manifestations of inflammation assessed

by the measurements of such indices.

Probing depth was a clinical parameter used both for patient selection and for a clinical correlation with AST levels, because in sites with a probing depth of ≥ 5 mm the percentage of AST+ sites would be greater (21,25). Although a high ratio of AST+ sites was found (85% were AST+) in pockets with a probing depth of ≥ 5 mm, there was no statistically significant difference between probing depth as a function of the AST site being classified as either positive or negative.

Persson et al. (18) reported a higher level of enzyme activity in sites with proven attachment loss in relation to those where no changes had occurred, although fluctuation of values had been observed. These results were also consistent with, but did not confirm, the studies by Chambers et al. (9) and Imrey et al. (10), in which increased AST levels occurred during periods of attachment loss. Nevertheless, Smith et al. (21) report having found no significant correlation between AST positive sites and probing depth, probably due to the sensitivity (60%) and specificity (57%) of AST values for differentiating sites with periodontitis and gingivitis. Similarly, studies by Wong et al. (22) and Shimada et al. (20) likewise did not find any significant differences in relation to probing depth and attachment level between AST+ and AST- sites, but there was a correlation between healthy and diseased sites. The findings of our study were similar to these results.

According to Ivic-Kardum et al. (11), AST levels in gingival crevicular fluid play a key role in providing earlier data for assessing periodontal condition, both before and after therapy is initiated, with no need to wait for the slow changes detected by the clinical indices presently used.

In the present study, the sites with changes in AST levels did not correlate with PI and GI, possibly due to hygiene habits. Although no suggestions were made concerning hygiene habits, we noted that some patients had improved their hygienic practices after the beginning of the study. This could cause a decrease in these indices at the time of final analysis. In addition, these indexes are directly linked to the resistance of each organism and to the local conditions of the oral cavity, which cause the detection of each parameter to vary considerably from person to person. Since the detection of AST is not linked to these factors, it can be reliable for assessing actual periodontal condition. However, we would like to emphasize the importance of associating a test based on changes that have occurred in the host with the clinical parameters of periodontal disease so that a more accurate diagnosis can be made of the condition of any given site or patient.

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