Effect of Non-surgical Therapy on GCF Aspartate Aminotransferase levels in Chronic Periodontitis patients

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ABSTRACT

During inflammation and cell death, Aspartate Aminotransferase (AST) gets liberated in the extracellular fluid such as Gingival Crevicular Fluid (GCF). Its concentration is increased in periodontitis; and after nonsurgical therapy, when the inflammation is reduced, AST levels will be decreased. So a study was planned to analyze the level of AST in GCF at baseline and 3 months after non-surgical therapy in patients with chronic periodontitis. Statistical correlations were calculated to determine the relationship between AST and periodontal parameters like probing pocket depth (PPD), clinical attachment level (CAL), gingival index (GI) and plaque index (PI). There was a statistically significant difference in AST levels between baseline and 3 months after therapy. Improvement in clinical parameters was observed and there was a corresponding reduction in AST levels. It was thus concluded that AST level may be a useful adjunct as a biomarker in the assessment of periodontal disease as evident by its reduction in GCF levels.

Keywords: Biomarker, Aspartate Aminotransferase, Gingival Crevicular Fluid

INTRODUCTION

The advance diagnostic measures have shown that pathogenesis of periodontal disease is episodic and does not occur in a continuous linear manner.1 There are various risk factors which are responsible for periodontal destruction. Risk factors may be environmental, behavioural or biological which when present, increase the likelihood that an individual will get the disease. The microbial tooth deposits having pathogenic bacteria, systemic diseases such as diabetes, and local risk factors such as tobacco smoking are the important associated factors.

To overcome the microbial insult, host immune response is activated which is a critical determinant of periodontal disease pathogenesis. The marginal gingival and sulcular area act as battle field where this interaction between bacteria and host takes place. The sulcus is bathed with a fluid, the gingival crevicular fluid (GCF). Its presence has drawn the attention of various investigators since 19th century. Waerhaug (1952)2, Brill and Krasse (1958)3 are the earliest pioneer workers who analyzed the volume, composition and role of GCF in defence mechanism.4

Various components of GCF that have been studied including tissue degradation products e.g. hydroxyproline and glycosaminoglycans, mediators of inflammation and bone resorption e.g. interleukines (IL) and prostaglandin (PG), collagenase and proteases, enzymes that help in the physiology of cell and are released only upon cell death e.g. lactate dehydrogenase and aspartate aminotransferase (AST) also known as SGOT (serum glutamate oxaloacitic transaminase).5,6,7 These may act as biomarkers.

Biomarker is a signal that serves as a guide or indicator of the state of a living organism. In more sophisticated context, hormonal or enzymatic changes in response to toxic substances serve as biomarkers.8 AST is particularly important in the transport of reducing equivalents across the mitochondrial membrane via the malate aspartate shuttle and is a sensitive indicator of necrosis in a number of tissues.9 AST levels in serum, cerebrospinal fluid and joint fluid have been used for several decades in medicine as a diagnostic aid for assessing the cell death and tissue destruction.10 AST levels in blood serum have served for many years as the basis of a test for tissue

degradation in disease such as myocardial infarction, hepatic disorders and renal pathology etc. In GCF it has been reported as a possible marker for distinguishing between active and inactive disease sites. AST has been thought to be a useful indicator of periodontal disease activity. 1, 11

During inflammation and cell death this enzyme is not utilized and gets liberated in the extracellular fluid such as GCF where it can be assessed. So, it is hypothesized that during gingival inflammation its concentration will be increased and after nonsurgical therapy when the microbial load is reduced AST levels in gingivitis and periodontitis may also be decreased. So its level in GCF can be used as a diagnostic biological marker to predict the activity and progression of periodontal disease and the earliest interception for its treatment.

MATERIALS AND METHOD

Selection of subjects, test sites and experimental design

A prospective, interventional, comparative, bio-chemical study was planned, aimed at comparing subjects with pre-treatment and post-treatment evaluations of Aspartate Aminotransferase (AST). An ethical clearance was obtained from Ethical Committee on Human and/or Animal subjects'Research, Kothiwal Dental College and Research Centre (KDCRC), Moradabad.

A total number of 20 patients diagnosed as chronic generalized periodontitis were selected from the out-patient department of Periodontics, KDCRC Moradabad. Subjects having good general health, minimum of 20 natural teeth, excluding 3rd molars, definite clinical evidence of chronic periodontitis, 5-8 mm probing pocket depth, mean GI $\geq$ 2 with definite loss of attachment were included for evaluation. Sites selected for evaluation of AST in GCF were having probing pocket depth of $\geq$ 5mm on at least one location on a minimum of 6 teeth in the mouth. 12

Exclusion criteria included :

1)Periodontal therapy other than standard prophylaxis during the previous 6 months.

2)Use of antibiotics within the previous 3 months.

3)Systemic diseases (cardiovascular disease, diabetes, blood disorders, hepatitis, renal disorders) that could influence the course of periodontal disease.

4)Pregnant women or lactating mothers.

5)Inability of the persons to cooperate because of their physical or mental status or daily routine.

6)Subjects with full crown, orthodontic bands or denture clasps on teeth.

7)Teeth diagnosed as of very poor prognosis.

Periodontal status was assessed by using Probing pocket depth (PPD), Clinical attachment level (CAL), Gingival index (Loe and Silness 1963), and Plaque index (Silness and Loe 1963). Probing pocket depth (PPD) was measured with UNC-15 (University of North Carolina) probe.

SITE SELECTION

The selection of test site was made one day before the collection of crevicular fluid. One test site was selected from each patient having pocket depth of 5-8 mm and clinical evidence of attachment loss. The clinical parameters were recorded on first day and gingival crevicular fluid (GCF) was collected on the following day. At baseline this was done to eliminate the mechanical effect of procedures of recording of clinical parameters. Oral hygiene instructions were given to the patient and scaling and root planing (SRP) was done. The clinical parameters and GCF- AST quantification was repeated 3 months post-operatively and recorded.

COLLECTION OF GCF

The gingival margin was dried with air and cotton swabs. Supragingival plaque was removed with a curette/scaler and GCF sample was obtained from mesiobuccal, distobuccal and/or mid buccal site. A standard volume of 1.0 micro liter of crevicular fluid was collected in a Hirschman volumetric micropipette positioned extrasulcularly. These microcapillary pipettes were calibrated from 0 to 5microlitre with a calibration mark after each microlitre, and obtained from "Sigma chemical company" (St. Louis, U.S.A.). If plaque or debris clogged the micropipette or blood contaminated the GCF, the GCF collection was repeated. Samples thus collected were immediately sent to the laboratory for the analysis of Aspartate Aminotransferase enzyme. The biochemical analysis was done for estimation of AST enzyme concentration

at baseline and three months after SRP.

ESTIMATION OF AST LEVEL IN GCF

The Erba SGOT (AST) kit was used for quantitative estimation of AST activity. This kit is based on the reference method of the International Federation of Clinical Chemistry (IFCC).7 AST activities are measured photometrically by measuring transamination of aspartic acid and oxaloacetic acid. AST activity was measured on semi automated autoanalyser.

STATISTICAL ANALYSIS

Comparison between the diseased and healthy site for all the measures of periodontal parameters and AST levels were analyzed by applying student paired "t" test. Karl Pearson's correlation coefficient ("r") was calculated among different parameters of periodontal disease and AST concentration.

RESULTS

Test of significance among various parameters;-

Mean PI at baseline was 1.70 ± 0.4022 which reduced to 0.96 ± 0.4388 after 3 months of SRP. Mean percentage reduction was 43.38% which was statistically very significant ($t = 3.74$, $p < 0.01$).

Mean GI at baseline was 2.14 ± 0.2497 which reduced to 1.10 ± 0.3183 after 3 months of SRP. Mean percentage reduction was 48.54% which was statistically very significant ($t = 12.65$, $p < 0.01$).

Mean PPD was 3.76 ± 1.0338 at base line which reduced to 2.75 ± 0.7988 after 3 months of SRP. Mean percentage reduction was 28.26% which was statistically very significant ($t = 7.33$, $p < 0.01$).

Mean CAL at base line was 4.21 ± 0.9222 which reduced to 3.23 ± 0.7385 after 3 months of SRP. Mean percentage reduction was 24.47% which was statistically very significant ($t = 6.80$, $p < 0.01$).

Mean AST at baseline was 3276.91 ± 1350.25 which reduced to 1658.22 ± 539.67 after 3 months of SRP. Mean percentage reduction was 49.40% which was statistically very significant ($t = 6.00$, $p < 0.01$).

Coefficient correlation between different parameters and AST levels at Base line;-

PI and AST were found to have statistically insignificant correlation ($r = 0.02$, $t = 0.07$, $p > 0.1$).

GI and AST have shown statistically very significant correlation ($r = 0.91$, $t = 9.28$, $p < 0.01$).

PPD and AST Correlation was statistically significant at 95% level ($r = 0.53$, $t = 2.65$, $p < 0.05$).

CAL and AST was statistically insignificant correlation was found ($r = 0.33$, $t = 1.48$, $p > 0.1$).

Coefficient correlation between different parameters and AST levels after 3 months of SRP;-

PI and AST correlation was statistically insignificant ($r = 0.01$, $t = 0.02$, $p > 0.1$).

Between GI and AST an insignificant correlation was found ($r = 0.31$, $t = 1.36$, $p > 0.1$).

PPD and AST correlation was weakly statistically significant. ($r = 0.38$, $t = 1.75$, $p < 0.1$).

Between CAL and AST statistically very significant correlation was observed ($r = 0.83$, $t = 6.32$, $p < 0.01$).

TABLE 1: Test of significance (Paired't' test) among various parameters

PERIODONTITIS	Parameter	Mean ±SD		% Reduction	't' value	'p' value
		Baseline	After 3 months			
	PI	1.70±0.4022	0.96±0.4388	43.38%	3.74	p*<0.01
	GI	2.14±0.2497	1.10±0.3183	48.54%	12.65	p*<0.01
	PPD	3.76±1.0338	2.75±0.7988	28.26%	7.33	p*<0.01
	CAL	4.21±0.9222	3.23±0.7385	24.47%	6.80	p*<0.01
	AST	3275.91±1350.25	1658.22±539.67	49.40%	6.00	p*<0.01

Graph No. 1: Curve showing comparison of AST levels at baseline and after 3 months

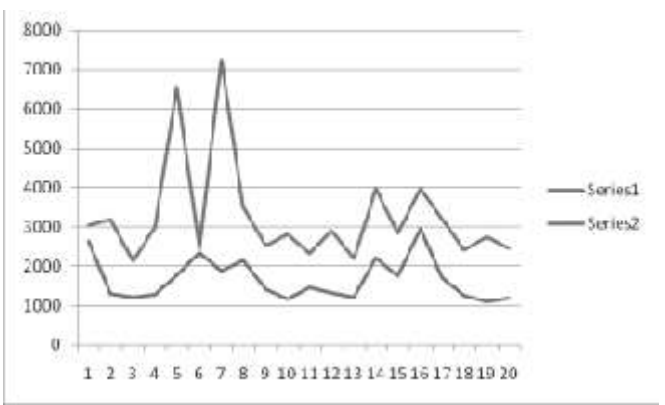

TABLE 2: Coefficient correlation between different parameters at base line (Karl Pearson's correlation coefficient test)

S. No.	Parameter	'r' value	$'r'_{tab}$ value	$'t'_{cal}$ value	'p' value
1.	PI and AST	0.07	0.07	t_{cal}=1.73	p^{*}<0.1
2.	GI and AST	0.91	0.23	t_{cal}=2.88	p^{**}<0.01
3.	PPD and AST	0.55	2.69	t_{cal}=2.13 t_{tab}=1.73	p^{**}<0.05 p^{**}<0.01
4.	CAL and AST	0.33	1.48	t_{cal}=1.73	p^{**}<0.1

TABLE 3: Coefficient correlation between different parameters after 3 months (Karl Person's correlation coefficient test)

S. No.	Parameters	'r' value	$'r'_{tab}$ value	$'t'_{cal}$ value	'p' value
1.	PI and AST	0.11	0.32	t_{cal}=1.73	p^{*}>0.1
2.	GI and AST	0.31	1.36	t_{cal}=1.73	p^{*}>0.1
3.	PPD and AST	0.58	1.79	t_{cal}=1.73 t_{tab}=2.1	p^{**}<0.1 p^{***}<0.05
4.	CAL and AST	0.63	6.32	t_{cal}=2.86	p^{***}<0.01

p*>0.1-Statistically insignificant, p**<0.1-Statistically weakly significant, p***>0.05-Statistically insignificant

DISCUSSION

AST levels ranged from 2127.9 to7251.6 µIU/L at baseline. Kolte Rajashri et al. (2003)7 have reported 1200 µIU/L to 4400 µIU/L at diseased sites. Golub et al. (1976)13 , Cohen et al. (1991)14, 15, Chamber et al. (1991)16 and Persson GR and Page RC (1992)17 have also observed in their studies a higher levels of AST at diseased sites. Some sites have shown an abnormally high AST levels which may be due to active periodontal destruction at those sites. Perinetti et al. (2003) and Shimada Koichi (2000) in their longitudinal studies have observed higher levels with disease active sites 18, 9. AST levels were found to be reduced (Table 1) very significantly (P<0.01) after treatment when compared by applying paired 't' test. This reflects that the AST release is associated with inflammation / necrosis. Persson GR et al. (1990)19, Mc Culloch CAG (1994) 20, Chambers D.A. et al. (1984)21 have also quoted the association between enzyme level and gingival inflammation in their studies. This could be because of marked improvement in gingival status after SRP. The residual inflammation in deep pockets may be responsible for lesser reduction of AST levels.

To measure the effect of SRP, the paired 't' test was applied to find the reduction in the periodontal parameter before and after treatment and a statistically very significant reduction (p<0.01) was found suggesting that SRP and motivation for oral hygiene measures helped in improving the periodontal health of subjects (Table-1) . SRP is considered as a gold standard therapy in the treatment of periodontal diseases. This is established in our study also and is in agreement with several studies like those of Li R (1992)22, Page Roy C (1992)23, Mangnesson Ingvar et al. (1996)24, Shimada K et al. (1999)1, Shimada K et al. (2000)9, Tsalikis L et al. (2001).25

On correlating AST levels with clinical parameters by Karl Pearson's coefficient correlation test, there was very significant correlation between AST level and GI (p<0.01). (Table-2) before treatment (at baseline), suggesting that AST level is correlated with severity of gingival inflammation. This observation is in agreement with the findings of the studies of Persson GR et al (1990)19, Kolte Rajashri (2003).7 Magnsson Ingvar et al. (1996) have also found that AST levels consistently increasing with increase in GI.24

A positive correlation was present after treatment between GI and AST (Table 3) but it was statistically insignificant (p>0.1). This might be due to presence of inflammation in deep pockets. SRP reduced the inflammation but might be unable to completely eliminate it in deep pockets. Presence of inflammation is believed to be related to the penetration of antigenic substances via gingival sulcus and junctional epithelium. 7 Even in clinically healthy gingiva with GI scores '0' small foci of inflammatory cells are found in the connective tissue near the base of the sulcus. According to study performed by Page and Schroder (1975), histologically a classical vasculitis of vessels subjacent to JE and gingival sulcus, extracellular presence of serum protein and alteration of most coronal portion of JE could be seen.7 Since, deep periodontal pocket having larger surface area are exposed to external environment, a higher concentration of AST is expected. This finding also

supports that only SRP may not be a sufficient treatment modality for complete debridement of tooth surface and inflamed periodontal tissues; An additional surgical approach may be required for elimination of pocket.

A positive correlation was found between PI and AST at baseline and after 3 months of SRP, but was statistically insignificant ($p > 0.1$) (Table 2, 3). This can be explained on the basis of the pathogenicity of plaque supporting the specific plaque hypothesis (Loesche W. J. et al. 1976).25 Specific pathogenic microorganisms in the plaque causes tissue destruction which is responsible for the release of AST in the GCF. A study performed by Kuru B. and Yilmaz S. et al. (1999)26 concludes that P. gingivalis, Actinobacillus actinomycetemcomitans and Prevotella intermedia were detected in AST positives (AST > 600µIU) while Aggregatibacter actinomycetemcomitans species were found in AST negative sites (AST < 600 µIU). An another study of Kuru B. et al.. in (1999)26 shows that C. rectus, E. corrodens, F. nucleatum, Capnocytophaga species, P. intermedia and P. gingivalis were significantly higher in positive than negative sites. Additionally, PI measures the amount and extent of plaque on the tooth surface supragingivally only. This may be the reason for insignificant correlation between PI and AST. In their studies Silva Emilio Barbosae et al. (2003)27 and Magnusson Ingvar et al. (1996)24 have also found statistically insignificant correlation between AST levels and PI.

The present study also shows a wide fluctuations and variation in GCF-AST levels and overlap of enzyme levels when correlated with PPD among different patients. Page and De Rouen (1992)10, Imery et al. (1991)28 and Persson GR (1995)29 and Page RC 199223 observed fluctuation probably resulted from the known episodic nature of periodontal destruction. There might be active destruction going on at sites showing higher levels of AST activity. Deep pockets provide an excellent habitat for microorganism where sufficient nutrition through GCF and protected environment is available to bacteria away from superficial surfaces of gingiva where environmental resistance is more compared to deeper site. Karl Pearson correlation coefficient test was applied to find the correlation between PPD and AST levels at pre-and post treatment (Table 2,3). A positive and statistically significant association ($p < 0.05$) was found at pretreatment sites. This finding suggest that increased periodontal destruction resulted by microbial activity leads to increased AST concentration. Study performed by Persson et al. (1995)29 also supports this statement. Post treatment PPD values were found to be weakly correlated ($p < 0.1$, $p > 0.05$) with AST values. After SRP when microbial load was reduced, periodontal destruction was also reduced which resulted in the decreased AST concentrations in GCF but reduction was not much significant. This might be due to residual inflammation and ulcerations in the tissues even after SRP in the deep pockets. SRP is not sufficient to remove the inner lining epithelium of the pocket. AJ Smith et al. (1998) have also found no statistically significant correlation between PPD and AST levels.30

Between CAL and AST at baseline, a positive correlation was found though it was not statistically significant, but a statistically very significant correlation was found at post treatment stage between CAL and AST ($p < 0.01$). This finding suggests that resolution of inflammation at diseased site along with clinical attachment gain is strongly associated with decreased AST levels. Chambers DA et al. (1991)16, Persson GR and Page RC (1992).17 In their study have also found a positive correlation between AST and CAL. The weak correlation at the baseline may be due to presence of more inactive sites at the time of GCF collection.

However, the detection of the disease at the earliest stage is not possible with the clinical parameters. It requires a very sensitive and specific test. In this aspect AST has got very important role to detect the, quiescent, active and vulnerable sites for periodontal destruction.

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