Original Article

Association of adipocyte fatty acid-binding protein and tumor necrosis factor alpha with periodontal health and disease: A cross-sectional investigation

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ABSTRACT

Background: Adipocyte fatty acid binding protein (A-FABP) is a novel biomarker of inflammation for various chronic systemic diseases. Since periodontitis is a chronic inflammatory disease, this study explores the association of A-FABP with periodontal disease parameters and tumor necrosis factor-alpha (TNF-α) levels in gingival crevicular fluid in periodontal health and disease.

Materials and Methods: This original research article describes a cross-sectional study conducted at the Department of Periodontics, M. R. Ambedkar Dental College and Hospital, Bangalore, India. This cross-sectional investigation was conducted on sixty subjects which were divided into three groups of twenty subjects each – healthy, gingivitis, and chronic periodontitis. Clinical parameters – plaque index, bleeding index, probing depth, and clinical attachment loss were recorded. Gingival crevicular fluid samples were analyzed for A-FABP and TNF-α levels using ELISA. One-way analysis of variance was used to find the significance of study parameters on a continuous scale between three groups. Pearson's correlation has been used to find the relationship between Gingival crevicular fluid concentration of markers and periodontal parameters. Multiple linear regression analysis was applied to the study. The statistical significance was considered at $P < 0.05$.

Results: Mean concentration of A-FABP (6.43 ± 2.51) and TNF-α (3454.82 ± 1566.44) was highest in the periodontitis group, and the difference among the groups was statistically significant ($P < 0.05$). A positive correlation was observed between clinical attachment loss and the two markers among all groups. The correlation between A-FABP and TNF-α in periodontitis groups was positive and statistically significant ($P < 0.05$). Multiple linear regression model was statistically significant ($P < 0.05$) indicating that there is a significant relationship between the set of predictors and the clinical attachment loss.

Conclusion: A-FABP and TNF-α levels in GCF were significantly elevated in the presence of inflammation. A-FABP has a probable stimulatory effect on TNF-α; however, its role needs to be explored. A-FABP could serve as a novel inflammatory biomarker of periodontitis and the scope of using A-FABP inhibition as a treatment modality could be investigated with interventional studies.

Key Words: Adipocytes, chronic periodontitis, fatty acid-binding proteins, gingival crevicular fluid, tumor necrosis factor-alpha

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INTRODUCTION

Chronic periodontitis affects nearly 89.6% of adults >35 years in India.[1] It is defined as "an infectious disease resulting in inflammation within the supporting tissues of the teeth, progressive attachment loss, and bone loss."[2] Pathogenic bacteria in oral cavity lead to chronic inflammation, tissue destruction, and simultaneous healing, but the hallmark of periodontitis is alveolar bone loss and tissue destruction from an imbalance between the healing and destruction. Periodontal tissue destruction is mediated by neutrophils, macrophages, and T and B lymphocytes. These cells produce inflammatory mediators such as cytokines, arachidonic acid metabolites, and proteolytic enzymes, which collectively lead to bone resorption by the activation of several distinct host degradative pathways.[3]

Adipokines are bioactive molecules produced by adipose tissue and studied extensively as biomarkers of inflammation.[4] Among 600 odd adipokines, a novel biomarker called adipocyte fatty acid-binding protein (A-FABP) also known as fatty acid-binding protein-4 (FABP4) or aP2 has gained immense interest in recent years in the research on inflammatory diseases such as atherosclerosis[5] and insulin resistance.[6] It is a peptide produced by adipocytes, macrophages, and endothelial cells and is suggested as the third adipokine after adiponectin and leptin. Currently, it is used as an established inflammatory marker in diabetes mellitus, endothelial dysfunction, atherosclerosis, and obesity-related disorders. It plays a vital role in the crossroads of inflammation and metabolic responses.[7] Administration of FABP4 inhibitor has shown to be beneficial in insulin resistance, diabetes mellitus, fatty liver disease, and atherosclerosis in experimental models.[8] Periodontitis is also an inflammatory disease, and there is a possibility to find an association between A-FABP and periodontal inflammation. The only study in dental literature on A-FABP showed its serum levels decreased after nonsurgical periodontal therapy was performed.[9]

A-FABP plays a role in cell signaling cascades, which in turn leads to the production of pro-inflammatory cytokines such as interleukin (IL)-1, IL-6, and tumor necrosis factor-alpha (TNF-α).[10] TNF-α also a member of adipokine family is a well-established pro-inflammatory marker for periodontitis. Several roles of TNF-α in periodontitis have been investigated including stimulation of osteoclast formation,[11] regulation of matrix metalloproteinases, enzymes that degrade connective tissue and enhancing host response to periodontal pathogens by stimulating signal transduction pathways.[12,13] Its use as a potential biomarker for the diagnosis of periodontal diseases is strongly supported in a systematic review by Madureira *et al.* in 2018.[14] A-FABP is known to elevate the levels of TNF-α through a positive feedback loop involving c-Jun NH2-terminal kinases (c-JNK).[10]

Although our understanding of the inflammatory mediators continues to evolve, their specific roles in periodontal pathogenesis are yet to be determined. To date, there has been no attempt to estimate the GCF levels of A-FABP in periodontal health and disease, although it is extensively researched for its role in systemic disorders. Hence, this investigation was designed to evaluate the association between A-FABP and TNF-α in periodontal health and disease.

MATERIALS AND METHODS

Study population

This cross-sectional investigation was conducted at the Department of Periodontics, M. R. Ambedkar dental college and hospital, Bangalore, India. This study was approved by the human subjects ethics board of M. R. Ambedkar dental college and hospital and was conducted in accordance with the Helsinki Declaration of 1975, as revised in 2013. The nature and purpose of the study were explained to the subjects, and written consent was taken. The subjects belonging to both genders and in the age range of 18–65 years were allocated to three groups, namely, healthy, gingivitis, and periodontitis based on the following exclusion and inclusion criteria.

A diagnosis of periodontal disease or health was made in accordance with the clinical criteria for periodontal disease classification agreed upon by consensus at the world workshop in periodontics in 1999,[15] as follows

Healthy subjects
Subjects who are systemically healthy PD< 3mm, absence of clinical attachment loss, and bleeding on probing (BOP) ≤5% of sites examined.

Gingivitis subjects
Subjects with inflammatory changes in gingival tissue with probing depth (PD) ≤3 mm and presence of BOP ≥50% of sites examined.

Periodontitis subjects

Subjects with inflammatory changes in gingival tissue, eight or more teeth with probing depth (PD) ≥5 mm, clinical attachment loss (CAL) ≥3 mm, and presence of BOP.

Exclusion criteria were history of underlying systemic disease, obese and overweight individuals, history of periodontal treatment in the past 6 months, under antibiotic treatment during the past 6 months, history of tobacco use in any form, pregnant females and lactating mothers, patients on steroid therapy, immune-modulatory drugs, oral contraceptives, or any medication affecting periodontal health.

The recruitment of patients is represented in a flow chart [Figure 1]. Out of the 200 patients screened, 77 agreed to participate, and 60 patients fulfilled the inclusion criteria.

Recording of periodontal parameters

After recording demographic factors, all patients underwent a full-mouth periodontal examination that included the plaque index (PI) by Silness and Loe,[16] bleeding index (BI) by Ainamo and Bay,[17] Probing depth (PD),[18] and clinical attachment loss (CAL).[18] PD and CAL were recorded at six sites per tooth using the UNC-15 graduated probe (University of North Carolina, Hu-Friedy, Chicago, IL, USA). All parameters

Figure 1: Flow chart depicting the selection and recruitment of subjects.

were recorded by one examiner (first author). This examiner was trained in a calibration process to reduce intraexaminer error. The examiner recorded all the periodontal parameters on two occasions, 24 h apart, and the correlation coefficient was calculated to be in acceptable range (0.721–0.773).

Collection of gingival crevicular fluid

The subjects were asked to rinse their mouth vigorously with water to cleanse the teeth of loosely adherent debris. Samples of GCF were obtained from predetermined sites the following day after examination to avoid contamination of the sample with blood after screening using color-coded, calibrated, volumetric, microcapillary pipettes with 5 µL range (Sigma chemical company, St Louis, MO, USA). The site with deepest probing depth was selected and isolated with cotton rolls. Volumetric micropipettes were placed extra crevicular at the entrance of the gingival crevice, and the 5 µL GCF samples were collected from each patient. The pipettes contaminated with blood or saliva were discarded, and the pipette with collected fluid was wrapped in a sterile aluminum foil to prevent oxidation. Samples were placed in plastic vials and immediately stored at −70°C until analyzed for A-FABP and TNF-α with commercially available ELISA kits.

Method of adipocyte fatty-acid binding protein and tumor necrosis factor-alpha estimation

The levels of A-FABP (Bioassay technology laboratory, Shanghai, China) and TNF-α (Ray Biotech, Inc., Norcross, GA, USA) were estimated using commercially available ELISA kits following the manufacturer's instructions. The GCF samples were diluted with phosphate-buffered saline to achieve the required volume. The dilution factor was noted. They were placed in duplicate in two wells. Buffered saline was used as a blank in one well, and standards were produced according to the manufacturer's guidelines. The optical density of each sample was recorded using an ELISA reader and converted into concentrations using the standard curve and its function. Each of these values was then multiplied by the dilution factor to give the concentration in the samples. Two samples were obtained for each sample, and the average of these values was taken as the final measured concentration.

Statistical analysis

A priori power analysis was carried out to calculate the optimal sample size. A sample size of 18 in
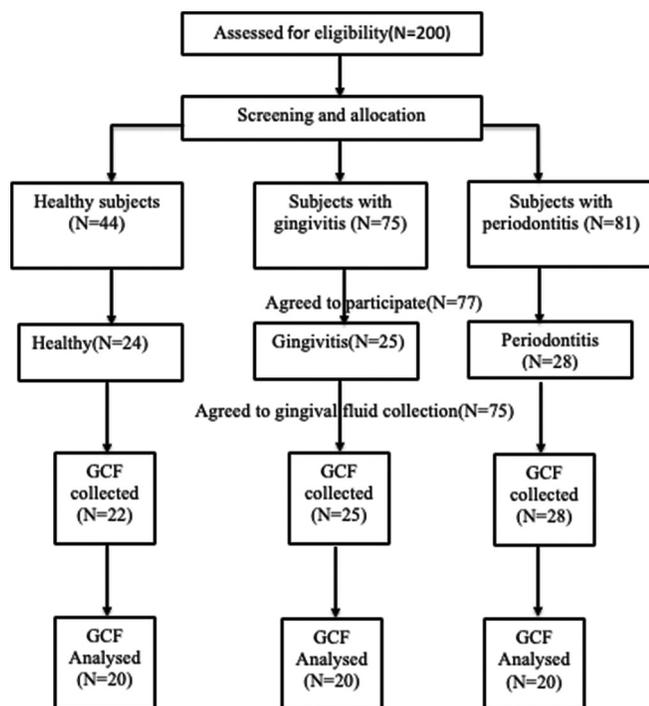
each group was calculated to be required to detect a significant difference of 0.5 pg/μL of TNF-α with 80% statistical power and a 5% level of significance. A descriptive statistical analysis was carried out in the present study. Results on continuous measurements are presented as mean ± standard deviation, and results on categorical measurements are presented in number. Significance is assessed at 5% level of significance. One-way analysis of variance was used to find the significance of study parameters on a continuous scale between three groups. Pearson's correlation has been used to find the relationship between GCF concentration of markers and periodontal parameters. Multiple linear regression analysis was applied to the study. The statistical significance was considered at $P < 0.05$.

RESULTS

This was an observational study involving three groups. Table 1 shows demographic distribution among the three groups. The difference in the mean age and gender distribution among the three groups was not statistically significant ($P = 0.07$).

Clinical parameters such as PI, BI, probing depth (PD), and clinical attachment loss (CAL) were recorded for all three groups and compared [Table 2]. The mean PI was 0.30 ± 0.22 in the healthy group, 1.17 ± 0.16 in the gingivitis group, and 2.01 ± 0.43 in the periodontitis group. The difference in their

means was statistically significant ($P < 0.001$). The mean BI was 4.10 ± 0.85, 79.09 ± 6.74, and 90.47 ± 6.27 in healthy, gingivitis, and periodontitis groups, respectively. The difference in mean BI scores was statistically significant ($P < 0.0001$). The PD scores were 2.26 ± 0.38, 2.74 ± 0.42, and 5.00 ± 0.70 in healthy, gingivitis, and periodontitis groups, respectively. Mean CAL was 0.03 ± 0.10 in the healthy group, 0.38 ± 0.47 in the gingivitis group, and 3.38 ± 0.76 in the periodontitis group, respectively. The difference in means of PD and CAL among the three groups was statistically significant ($P < 0.001$). All the periodontal parameters such as PI, BI, PD, and CAL were highest among the group periodontitis group and least among the healthy subjects.

The mean concentration of A-FABP and TNF-α in GCF was recorded among all three groups [Table 2]. Mean concentration of A-FABP was 2.22 ± 0.56 in the healthy group, 2.66 ± 0.63 in the gingivitis group, and 6.43 ± 2.51 in the periodontitis group. It was highest in the periodontitis group ($P < 0.001$). The mean concentration of TNF-α was least in the healthy group (949.84 ± 519.91) and highest among the periodontitis group (3454.82 ± 1566.44). The gingivitis group had a mean concentration of 2303.32 ± 1033.98. The difference between the three groups was statistically significant ($P < 0.001$).

Pearson's correlation was performed between the clinical parameters and the GCF concentration of A-FABP and TNF-α among the three groups [Table 3]. In all three groups, the clinical parameters showed a positive correlation with the concentration of A-FABP and TNF-α. In all three groups, the correlation between CAL and both markers was statistically significant ($P < 0.05$). Probing depth was positively and significantly correlated with the markers in all groups ($P < 0.05$). All other parameters showed a statistically significant correlation with A-FABP in gingivitis and periodontitis groups.

Table 1: Comparison of demographic data (age and gender) among the three groups

Parameter	Healthy	Gingivitis	Periodontitis
Age (years)	27.45±9.09	30.5±9.26	34.35±9.48
Gender (%)			
Male	45	60	50
Female	55	40	50

Values are given as percentage or mean±SD, samples were age and gender matched (*P*=0.07). SD: Standard deviation

Table 2: Comparison of means of clinical parameters and gingival crevicular fluid parameters among the three groups

Parameter	Healthy (*n*=20)	Gingivitis (*n*=20)	Periodontitis (*n*=20)	ANOVA, *P*
PI	0.30±0.22	1.17±0.16	2.01±0.43	<0.001*
BI (%)	4.10±0.85	79.09±6.74	90.47±6.27	<0.001*
PD (mm)	2.26±0.38	2.74±0.43	5.006±0.70	<0.001*
CAL (mm)	0.03±0.10	0.38±0.47	3.38±0.76	<0.001*
A-FABP (ng/mL)	2.229±0.561	2.665±0.636	6.430±2.515	<0.0001*
TNF-α (pg/mL)	949.84±519.91	2303.32±1033.98	3454.82±1566.44	<0.0001*

*Statistically significant, values are mean±SD. PI: Plaque index; BI: Bleeding index; PD: Probing depth; CAL: Clinical attachment loss; A-FABP: Adipocyte fatty acid binding protein; TNF-α: Tumor necrosis factor alpha; SD: Standard deviation; ANOVA: Analysis of variance

Gingivitis and periodontitis subjects showed a positive correlation between the concentration of A-FABP and TNF-α in GCF. In the periodontitis group, the correlation between them was statistically significant ($P = 0.0001$).

Multiple linear regression analysis was done in gingivitis and periodontitis group [Tables 4 and 5]. In the gingivitis group, BI was taken as the dependent variable. GCF concentration of A-FABP, TNF-α, Age, PI, PD, and CAL was independent variables. All periodontal parameters have a positive correlation on BI. With an adjusted $R^2 = 0.592$, the analysis suggests that 59% of bleeding can be attributed to the predictors. The F test score was 5.602 and statistically significant ($P = 0.004$).

Multiple linear regression analysis in the periodontitis group [Table 5] was performed using CAL as the dependent variable and GCF concentration of A-FABP, TNF-α, Age, BI, PI, and PD as the independent variables. Age, BI, PD, and the markers showed a positive correlation, although not significant. The predictors have a significant effect on clinical attachment loss, as interpreted by the elasticity. The adjusted $R^2 = 0.546$ suggests that, after adjusting, 55% of clinical attachment loss is explained by the predictors. The F-test score was 4.808, and the overall model was statistically significant at $P = 0.008$ indicating that there is a significant relationship between the set of predictors and the clinical attachment loss.

DISCUSSION

Periodontitis is a complex disease, in which disease expression involves intricate interaction of biofilm with host immune-inflammatory response and subsequent alteration in bone and connective tissue homeostasis. Although primarily protective, a hyperresponsive inflammatory response could result

Table 3: Correlation between adipocyte fatty acid-binding protein and tumor necrosis factor alpha in gingival crevicular fluid and clinical parameters in the three groups

Correlation	Healthy		Gingivitis		Periodontitis	
	r	*P*	*r*	*P*	*r*	*P*
A-FABP and PI	0.165	0.24	0.446	0.02*	0.799	0.00001*
A-FABP and BI	0.194	0.20	0.439	0.02*	0.417	0.03*
A-FABP and PD	0.376	0.05*	0.401	0.03*	0.847	0.000001*
A-FABP and CAL	0.409	0.03*	0.631	0.001*	0.721	0.0001*
TNF-α and PI	0.058	0.40	0.422	0.03*	0.542	0.006*
TNF-α and BI	0.147	0.26	0.307	0.09	0.354	0.06
TNF-α and PD	0.359	0.05*	0.010	0.48	0.531	0.007*
TNF-α and CAL	0.441	0.02*	0.662	0.007*	0.678	0.0005*
A-FABP and TNF-α	0.091	0.35	0.346	0.06	0.731	0.0001*

*Statistically significant. PI: Plaque index; BI: Bleeding index; PD: Probing depth; CAL: Clinical attachment loss; A-FABP: Adipocyte fatty acid binding protein; TNF-α: Tumor necrosis factor alpha

Table 4: Multiple regression analysis in the gingivitis group (with bleeding index as predictor)

Variable	Coefficients	SE	*t* statistics	*P*	Lower 95%	Upper 95%	Regression statistics
Intercept	42.648	11.499	3.708	0.002*	17.805	67.491	Multiple *R*: 0.849
Age	0.255	0.204	1.248	0.233	−0.186	0.697	*R*²: 0.721
PI	4.266	7.465	0.571	0.577	−11.861	20.395	Adjusted *R*²: 0.592
PD	9.823	2.693	3.647	0.002*	4.004	15.641	SE: 4.306
CAL	8.370	3.520	2.377	0.033*	0.764	15.976	*F*-test: 5.602
A-FABP	−1.920	2.277	−0.843	0.414	−6.840	2.998	*P*: 0.004*
TNF-α	−0.000	0.001	−0.645	0.529	−0.003	0.002	

*Statistically significant. PI: Plaque index; PD: Probing depth; CAL: Clinical attachment loss, A-FABP: Adipocyte fatty acid-binding protein; TNF-α: Tumor necrosis factor alpha; SE: Standard error

Table 5: Multiple regression analysis in the periodontitis group (with clinical attachment loss as predictor)

Variables	Coefficients	SE	*t* statistics	*P*	Lower 95%	Upper 95%	Regression statistics
Intercept	−4.325	4.372	−0.989	0.340	−13.770	5.120	Multiple *R*: 0.830
Age	0.007	0.022	0.336	0.741	−0.041	0.056	*R*²: 0.689
PI	−0.407	0.696	−0.584	0.568	−1.912	1.097	Adjusted *R*²: 0.546
BI	0.039	0.032	1.198	0.251	−0.031	0.110	SE: 0.659
PD	0.760	0.471	1.612	0.130	−0.258	1.779	*F*-test: 4.808
A-FABP	0.016	0.178	0.094	0.925	−0.369	0.403	*P*: 0.008*
TNF-α	0.0002	0.0001	1.455	0.169	−0.0001	0.0005	

*Statistically significant. PI: Plaque index; BI: Bleeding index; PD: Probing depth; A-FABP: Adipocyte fatty acid-binding protein; TNF-α: Tumor necrosis factor alpha; SE: Standard error

in enhanced tissue destruction.[19] The release of virulence factors of periodontal pathogens such as lipopolysaccharides (LPS) into the circulation triggers inflammatory process which causes the secretion of pro-inflammatory cytokines IL-1, TNF-α, and IL-6, from inflammatory cells that are primarily responsible for periodontal tissue destruction. The macrophages at the site produce these mediators including A-FABP through the toll-like receptor pathways[20] which in turn signals the release of TNF-α from various cells that orchestrate events, leading to inflammation, connective tissue degradation, and osteoclastic bone resorption. Periodontal tissue destruction is attributed mostly to host-derived mechanisms than to proteolytic enzymes of microbial origin.

The systemic challenge of bacterial toxins such as LPS derived from periodontal lesions represents an essential link between periodontitis, metabolic dysregulation seen in diabetes and cardiovascular diseases. The relationship of periodontal disease with diabetes and diabetes with coronary heart disease is well described in the literature.[21,22] Periodontal infection is thought to have a role in atherosclerotic disease progression, and periodontal pathogens have been identified in atherosclerotic plaques.[23] Therefore, periodontitis is closely associated with systemic conditions and the underlying core process remains to be inflammation. Thus, it is safe to assume that the inflammatory biomarkers elevated in any one of the above systemic conditions might also be elevated in periodontitis.

The adipose tissue traditionally considered as an energy storage tissue is presently recognized as the largest endocrine organ in the body, secreting a large number of bioactive proteins known as adipokines. Dysregulated production of these adipokines is a crucial mediator that links obesity, diabetes, and vascular complications.[24] TNF-α is a well-established pro-inflammatory adipokine secreted by endothelial cells, macrophages, and adipocytes. It is known to cause chronic inflammatory state and insulin resistance among obese individuals.[25] Elevated levels of TNF-α are a well-known risk factor for destructive periodontal disease[13] by stimulating osteoclast formation.[11]

A-FABP is essentially an adipokine but is also produced in significant amounts by the macrophages at the site of inflammation. Prospective studies on Chinese cohorts demonstrate that serum A-FABP is an independent predictor for Type 2 diabetes, metabolic syndrome, and atherosclerosis.[5,6,26] The plasma level of A-FABP decreases in obese patients who have lost weight.[27] It modulates cell signaling in macrophages during inflammatory response by a positive feedback loop.[10] Notably, treatment of macrophages with recombinant A-FABP significantly increased inflammatory responses including chemokine signaling and TNF-α- nuclear factor-kappa b (NF-kB) signaling pathways.[28] A-FABP is a novel biomarker that has been extensively studied in the field of immunology, cardiovascular diseases, and metabolic diseases but sparingly in the field of periodontics. In the light of current understanding of A-FABP and its role in the production of pro-inflammatory markers, this investigation was designed to explore the association between A-FABP and TNF-α in chronic periodontitis.

This investigation was conducted on healthy, gingivitis, and periodontitis patients. The difference in the mean age and gender among the three groups was statistically insignificant, thereby eliminating age and gender-related bias. Periodontitis was prevalent in older subjects in our study similar to the findings in studies done by Löe H *et al*.[29] and Brown *et al*.[30] Patients were selected according to guidelines to rule out confounding effects. Smokers, patients on any immune-modulatory or anti-inflammatory drugs, patients with a history of systemic diseases, obese/overweight, and pregnant females were excluded from the study. The concentrations of TNF-α and A-FABP were measured in GCF and correlated to clinical parameters. In a few samples, markers were not detected, which could be due to disease activity at the time of collection, technical problems (e.g., procedural errors such as vigorous plate washing and dried wells). The results of the present study show that the mean concentration of TNF-α was significantly higher in the periodontitis group than the other two groups. Evaluation of the association between TNF-α and gingival inflammation as measured by PI and BI was done along with its association with periodontal disease severity using PD and CAL using linear correlation analysis. Results revealed a positive correlation between TNF-α and all the clinical parameters among all three groups. These results corroborate with results from a study by Gokul,[31] which also showed elevated TNF alpha concentration in the GCF among periodontitis and gingivitis patients in comparison with periodontally healthy controls. Similarly, Fujita *et al*.[32] concluded a significant increase in TNF alpha in disease site in chronic periodontitis. However, Cetinkaya *et al*.[33]

reported a contrasting finding to our study, stating a significant increase in TNF alpha in GCF of healthy individuals compared to chronic periodontitis. These variations could be attributed to several factors including smaller sample sizes and methods of sample collection. However, a recent systematic review by Madureira *et al*.[14] concluded an association of chronic periodontitis with increased levels of TNF-α in GCF, supporting the use of TNF-α as a potential biomarker for diagnosis of periodontal disease which is consistent with the findings from our study.

The mean GCF levels of A-FABP were found to be significantly elevated in the periodontitis group as compared to healthy and gingivitis groups. There is no published literature on the A-FABP in GCF. Serum A-FABP is an established biomarker, known to be elevated in various chronic inflammatory diseases such as vascular inflammation,[34] Type 2 diabetes mellitus[6] and cardiovascular diseases such as atherosclerosis[5] and endothelial dysfunction[35] and all the above diseases are known to be associated with periodontitis.[36,37] Therefore, this newly defined biomarker could be used in the research on periodontitis and its association with systemic diseases.

Correlation between the various clinical parameters and the GCF concentration of A-FABP was evaluated using Pearson's correlation. All the clinical parameters showed a positive correlation with GCF levels of A-FABP in all three groups. A significant positive correlation was seen in the gingivitis and periodontitis group between the clinical parameters and A-FABP concentration in the GCF indicating that increase in the clinical parameters resulted in an increase in the levels of A-FABP in GCF, suggesting that it is a biomarker positively associated with the inflammation seen in periodontal diseases. There are no other published reports to compare our study results. It can be inferred from our study that patients with high levels of A-FABP in GCF are likely to have periodontitis although the cross-sectional study design limits our understanding as to exclude that periodontal tissue destruction might be the actual cause of elevated levels of A-FABP in the periodontitis group.

The current understanding of A-FABP's role in inflammation is not only limited to being a lipid chaperone but also as an inducer of inflammatory response through activation of inhibitor of nuclear kappa b kinase (IKK), NF-KB, and C-JNK-AP-1 pathways, leading to increase in cytokines such as IL-1, 6, and TNF alpha.[38] The results of our study support this fact by showing a positive correlation between A-FABP and TNF-α among all groups indicating their association in healthy, gingivitis, and periodontitis subjects.

Based on the results from the present study, it can be deduced that the concentration of A-FABP in GCF is higher in periodontitis because of elevated levels of inflammatory changes seen in the tissues and the reduced levels in gingivitis could be attributed to reduced inflammatory burden seen in gingivitis as compared to periodontitis. Our findings are indirectly in accordance with the results of a study by Li *et al*.[9] where they observed a reduction in the serum concentration of A-FABP after scaling and root planing in periodontitis subjects indicating its probable role in the inflammatory pathways, resulting in periodontal destruction. However, they estimated the serum changes in the levels of A-FABP before and after the intervention. The serum is not an ideal source to analyze the inflammatory status of the gingival tissue specifically but represents inflammatory changes occurring anywhere in the body. We have estimated the GCF levels of A-FABP because of the unique and exclusive ability of GCF to reflect the periodontal tissue status through the presence or absence of biomarkers in health and disease. Multiple regression analyses indicated that the joint effects of variables on the predictor CAL were statistically significant as seen by the results of the *F*-test, and the overall study model was statistically significant in the periodontitis group.

To the best of our knowledge, our study is the first to estimate the GCF levels of A-FABP in periodontal health and disease. The results should be cautiously interpreted in the light of its limitations. The results are suggestive of pro-inflammatory role of both A-FABP and TNF-α. Their exact interaction with each other in the cascade of the inflammatory process is of limited understanding. In lieu of our study findings, it is plausible that TNF-α levels are elevated as a result of increased A-FABP levels. Regardless of the limitations of the study design, which is being a cross-sectional one with small sample size, A-FABP could be considered as a potential biomarker for periodontal disease. Its GCF levels could be used to distinguish healthy and diseased states. Whether the elevated levels of AFABP are a result of periodontitis alone has to be established. Studies with interventional

study design and larger sample sizes are needed to address the lacunae of knowledge regarding its role in periodontal disease and to validate its use as a novel inflammatory biomarker of periodontal disease progression. The study groups can be expanded to involve periodontitis subjects with systemic diseases as well. A-FABP levels in saliva can be investigated in future studies and used for comparative investigations. Evidence suggests that inhibition of A-FABP has a beneficial effect on diabetes and atherosclerosis,[8] and hence, further investigation is warranted in this direction to evaluate the effect of A-FABP inhibition in subjects with periodontitis and a probable treatment modality could be established.

CONCLUSION

A-FABP and TNF-α levels in GCF were significantly elevated in the presence of inflammation as compared to healthy states. A-FABP has a probable stimulatory effect on TNF-α; however, the exact role needs to be explored further. A-FABP could serve as a novel inflammatory biomarker of periodontitis and the scope of using AFABP inhibition as treatment modality could be investigated in future with interventional studies.

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Conflicts of interest
The authors of this manuscript declare that they have no conflicts of interest, real or perceived, financial or non-financial in this article.

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