Correlation of Serum Fetuin A and Matrix Metalloproteinase-7 Levels in Periodontitis and the Outcome of Initial Periodontal Therapy on Their Levels - A Preliminary Report

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ABSTRACT

BACKGROUND

Fetuin A and MMP7 have been implicated in pathogenesis of the periodontal disease. To date, there is limited evidence that exists on its role in periodontal inflammation. The present study is the first of its kind which is aimed to correlate their concentrations in serum of patients with different periodontal status and explore the impact of periodontal therapy on their levels.

METHODS

One hundred and twenty, sex matched subjects (60 males and 60 females) belonging to common age group (30-39) were recruited and divided into four groups (n=40) based on clinical and radiographic parameters [PH-Group I;CG- Group-II; CP- Group IIIa; PTCP- Group IIIb (3 months post nonsurgical periodontal therapy)]. Serum samples were collected from each patient, Fetuin A and MMP 7 levels were analysed by enzyme-linked immunosorbent assay. Statistical analysis was performed by parametric and non-parametric tests.

RESULTS

A significant down regulation of Fetuin A levels (258.7 ng/ml) and concomitant upregulation of MMP-7 levels (256.7 pg/ml) were noted as the periodontal disease progressed ($p<0.01$). Further, periodontal therapy demonstrated a significant increase in serum Fetuin A and decrease in MMP-7 (37.5 pg/ml) levels ($p<0.01$). This indicates a negative correlation of Fetuin A and positive correlation of MMP-7 with the extent of periodontal inflammation and establishes the potency of periodontal therapy in stabilizing their levels.

CONCLUSIONS

Taken together, our data suggests that higher circulatory Fetuin A level has a protective role and helps in maintaining periodontal tissue homeostasis. The reduction of Fetuin A in CP highlights its predictive importance as an anti-inflammatory biomarker. Concurrently, it could pose as one of the possible linking factors between inflammatory conditions and vascular calcifications.

KEY WORDS

Fetuin A, MMP-7, Chronic Periodontitis, Serum, Non-Surgical Periodontal Therapy, Anti-Inflammatory Biomarker, Vascular Calcifications

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BACKGROUND

Inflammation plays a pivotal role in both the development of atherosclerosis and periodontal disease.[1] Chronic periodontitis is characterized by infection and inflammation of the periodontal tissue, leading to destruction of bone surrounding the teeth.[2] There is a growing evidence on the association between periodontal inflammation and vascular calcification. However, the underlying biological mechanisms that explain if and how periodontitis and other inflammatory diseases enhances the formation of calcifying atheromas are not well defined.[1,3] Further, there is little evidence that calcifying atheromas, which is a leading cause of cardiovascular disease could contribute to this link.

Calcifying atheromas may be initiated by several mechanisms including loss of inhibition of calcification, induction of bone formation and cell death, which can nucleate initial calcification processes.[4,5] Notably, several proteins may be involved in suppressing calcification in atheromas; these proteins include osteopontin, matrix Gla protein, osteocalcin and the alpha-2-Heremans Schmid glycoprotein (the human homologue of Fetuin A).[6,7]

α - 2 Heremans-Schmid glycoprotein (AHSG), also termed human Fetuin A, is a major serum glycoprotein that was initially identified in 1944.[8] AHSG/Fetuin A is a 46 kDa serum glycoprotein that is commonly synthesized by hepatocytes.[9] Until recently the exact function of this abundant serum protein remained unclear. Currently, there is little knowledge regarding its multifunctional roles. It has been shown that it helps remodel skeletal bones by capturing Ca2+ ions in the circulation, and depositing them in the extracellular region of bone marrow.[7] It also plays a role in bone metabolism and it has various functions such as ectopic calcification inhibition.[10] Fetuin A is considered to be a negative acute phase reactant protein and acts as a protective agent for vascular calcification by inhibiting calcium salt precipitation, preventing the mineralization by acting as "crystal poisoning" and also by interfering with the differentiation of the cells with mineralizing phenotype.[11,12] Hence, high levels of Fetuin A in serum decreases vascular calcification and any decrease in Fetuin A levels is considered as an independent risk factor for cardiovascular disease.[13]

MMPs are an important family of zinc-dependent endopeptidases that are involved in the destruction of the extracellular matrix in periodontal diseases.[14-16] As Fetuin A can interact with MMPs, we considered that the binding of MMPs to Fetuin A may facilitate decrease in its levels in periodontitis and could act as a risk factor for cardiovascular disease.[17] In view of this relationship, we considered that matrix metalloproteinases (MMPs) which are up-regulated in periodontitis could reduce the levels of Fetuin A in serum, as a consequence of their ability to degrade this protein.[17]

Till date, there are no studies in the literature examining the serum levels of MMP7 and Fetuin A in vivo. Hence, the present study which is first of its kind is aimed to compare and correlate serum levels of MMP7 and Fetuin A in periodontal health and disease. Secondly, to explore the impact of non-surgical periodontal therapy on their levels which could further throw light on its participation in the progression of periodontitis.

METHODS

Study Population and Study Design

This is a cross sectional human clinical study aimed to determine and correlate the levels between MMP 7 and Fetuin-A levels in the bold of patients with periodontitis. The study group cohort included adult subjects who were aged (30-39 years) and sex matched. A total of one hundred and twenty systemically healthy individuals attending the clinical appointments visiting the outpatient section of the Department of Periodontology, Krishnadevaraya College of Dental Sciences and Hospital, Bengaluru were selected and recruited for the study when they satisfied the inclusion and exclusion criteria. The inclusion criteria were: patients aged 30-39 years who were systemically healthy, co-operative patients who were able to attend follow up, patients who had not received any periodontal treatment within the past six months of baseline examination and dentition with at least 20 functional teeth. The exclusion criteria were: presence of any known systemic disease (history of diabetes mellitus, liver diseases, cardiovascular disease, kidney diseases, rheumatoid arthritis, bone diseases, pulmonary disease, viral and fungal infections), patients with calcification disorders (Chronic kidney disease with vascular calcification, hyper-para-thyroidism, excessive intake of vitamin D, valvular calcifications and aortic stenosis), pregnancy and lactation, obese patients, history of aggressive periodontitis and antibiotic and/or anti-inflammatory drug usage within the last 6 months before the start of the study. The eligible subjects, who volunteered, were informed of the nature, potential risks and benefits of their participation. The clearance for the study was obtained from the institution ethical committee of Krishnadevaraya College of Dental Sciences and Hospital, affiliated to the Rajiv Gandhi University of Health Sciences, Bangalore, India. All ethical and design assessment were carried out in line with the Helsinki Declaration (updated in 2002). The flow chart in Figure 1 depicts the screening and selection of patients. Out of a total of 141 patients who were screened, 120 patients were selected who met the inclusion and exclusion criteria and agreed to participate. Each participant provided a written informed consent before taking part in the study.

Clinical Examination

The participants underwent a clinical assessment. Based on the Plaque index[18], BOP[19], Gingival Index (Modified Gingival Index (mGI),[20] Ramfjord Periodontal Index (PDI),[21] full mouth probing pocket depth and relative attachment loss (using acrylic stent as a reference) were recorded at six sites per tooth and subjects were categorized into three groups (Armittage 1999).[22] Measurements were performed by a single calibrated examiner. This examiner received training in calibration process to reduce intra examiner calibration error. Ten participants were randomly chosen and the researcher recorded all clinical measurements on two distinct occasions with 48 h apart, and the correlation coefficient was calculated. Calibration was deemed acceptable when measurements were greater than 80% at the millimetre level.[23] According to their periodontal conditions, the participants were consecutively allocated to one of three groups. Group 1 consisted of 40 periodontally healthy subjects who had a mean GI < 0.5 and no

interproximal site with relative attachment loss. Group 2 included 40 patients with gingivitis who had a mean GI ≥ 0.5; no relative attachment loss and PPD > 3 mm. Group 3 comprised 40 patients with periodontitis who had interproximal relative attachment loss of ≥ 5 mm and PPD >5 mm in at least 30% of teeth present. All clinical parameters were recorded at baseline and 3 months after initial therapy. PPD and RAL were measured using a UNC-15 periodontal probe (Hu- Friedy Manufacturing Co., Chicago, IL, USA).

Interventions

Subjects with chronic periodontitis (Group IIIa) received non-surgical periodontal therapy which was performed by a single clinician.

Collection of Samples

The serum samples were collected from all the participants at baseline who acted as pre-treatment controls. After local antisepsis, 3 mL of peripheral venous blood was collected from the ante cubital vein. The blood samples were immediately centrifuged at 2000 g for 10 minutes and the serum was obtained. All samples were stored at −80°C until required for analysis. Later serum was collected, twelve weeks (3 months) following Scaling and Root Planning (SRP). All the participants were under a stringent oral hygiene programme.

Statistical Analysis

The sample size calculations were performed using primary outcome variables (serum Fetuin A levels). Based on the previous mean Fetuin A levels in serum it was predicted that the sample population of 40 participants (120 in total) would be sufficient to provide 80% statistical power and 5% level of significance.[24] The data was subjected to Shapiro-Wilks test to check for the normal distribution of the data, it was inferred to be non-parametric. To test the hypothesis of equality of means for the three groups with respect to serum of Fetuin A and MMP-7 concentrations, Kruskall- Wallis analysis test was carried out at 5% level of significance**.** When there were significant differences (P <0.001), post-hoc two-group comparisons were assessed with Bonferroni-corrected Mann-Whitney U tests, and P values <0.001 were considered to be statistically significant. Finally, a Spearman rank correlation analysis was conducted to identify the relationship of correlation between serum Fetuin A and MMP 7 with clinical periodontal parameters. P value of <0.001 was considered significant. Statistical analysis was carried out using SPSS statistical software (ver. 19.0; SPSS Inc., Chicago, IL, USA).

RESULTS

Clinical Findings

The characteristics of the subject analysed in this study are shown in Table 1. There were no significant differences in age or gender distribution among the study groups (p > 0.05) and has no impact on the results. The BOP, GI, PI, PD, RAL were significantly higher in group IIIa compared to group I and group II subjects (p <0.001). Following periodontal treatment, the clinical measure presented a marked decline (p< 0.001).

Biochemical Findings

All the serum samples were detected positive for Fetuin A and MMP-7. Fetuin A and MMP7 levels in the serum did not show any statistically significant difference in the age and sex of the study population. The concentration of Fetuin A in the serum was higher in periodontally healthy subjects than in periodontally diseased individuals. On the contrary MMP-7 in the serum was higher in subjects with periodontal disease compared to healthy individuals. The mean values of MGI, PI, BOP%, PD and RAL obtained depict that the MMP-7 concentrations in the serum rise with increasing inflammation, and decrease after NSPT whereas the serum concentration of Fetuin A decreases with the increase in inflammation (Table 2).

Correlations

Correlation between the serum concentrations of Fetuin A and MMP-7 as well as clinical parameters were analysed using the Spearman's rank correlation. As shown in Table 3 the serum concentrations of Fetuin A had a significant negative correlation with serum concentration of MMP 7. Fetuin A in serum was negatively correlated with clinical parameters (p<0.001). On the other hand, there was a positive correlation between total amount of serum MMP-7 levels and clinical parameters in all the tested groups. The finding of spearman correlation for group III showed, a notably negative link of Fetuin A whereas MMP-7 showed a positive link between the number of sites with probing pocket depth >6 mm or 4-5 mm (p<0.005)

	Group				
Variables	**I (n=40) H**	**II (n=40) G**	**IIIa (n=40) CP**	**IIIb (n=40) PTCP**	**p Value**
Age(years)	33.7	34.9	34.6		0.4+
PI	0.48±0.15‡	1.62±0.17‡	2.38±0.36‡	0.76±0.13‡	<0.001*
MGI	0.59±0.23₹	2.48±0.37₹	2.39±0.37₹₸	0.75±0.12₸	<0.001*
PPD			6.45±1.2	4±0.9	<0.001*
RAL			10.3±1.2	7.8±1.05	<0.001*
BOP%	14.6±2.34đ	66.2±4.77đ	83.9±3.5đ	24.7±7.2đ	<0.001†

Table 1. Showing the Clinical Parameters of the Sample Areas in Study Groups (Mean ± SD)

\+ No significant difference among groups (p> 0.05)
* Significant difference among groups by Kruskal-Wallis test, P< 0.001
†Significant difference in BOP% among groups (One-way ANOVA, P<0.001).
‡Significant difference in pairwise comparison of PI (Mann- Whitney U test, P< 0.001)
₹₸Significant difference in pairwise comparison of MGI among PH, CG and CP and between CP and PTCP (Mann Whitney U test, P< 0.001)
đ Significant difference in pairwise comparison of BOP% (Scheffe post hoc test, P < 0.001)

Variables		Mean	SD	Z	p-Value
Serum fetuin A (ng/ml)	CP	258.73	3.07	-3.920	<0.001*
	PTCP	285.44	5.57		
Serum MMP7 (pg/ml)	CP	256.73	48.38	-3.888	<0.001*
	PTCP	156.81	33.22		

Table 2. Intra-Group Comparison of Serum Fetuin A and Serum MMP-7 between CP and PTCP Using Paired t Test

* p<0.001 statistically significant

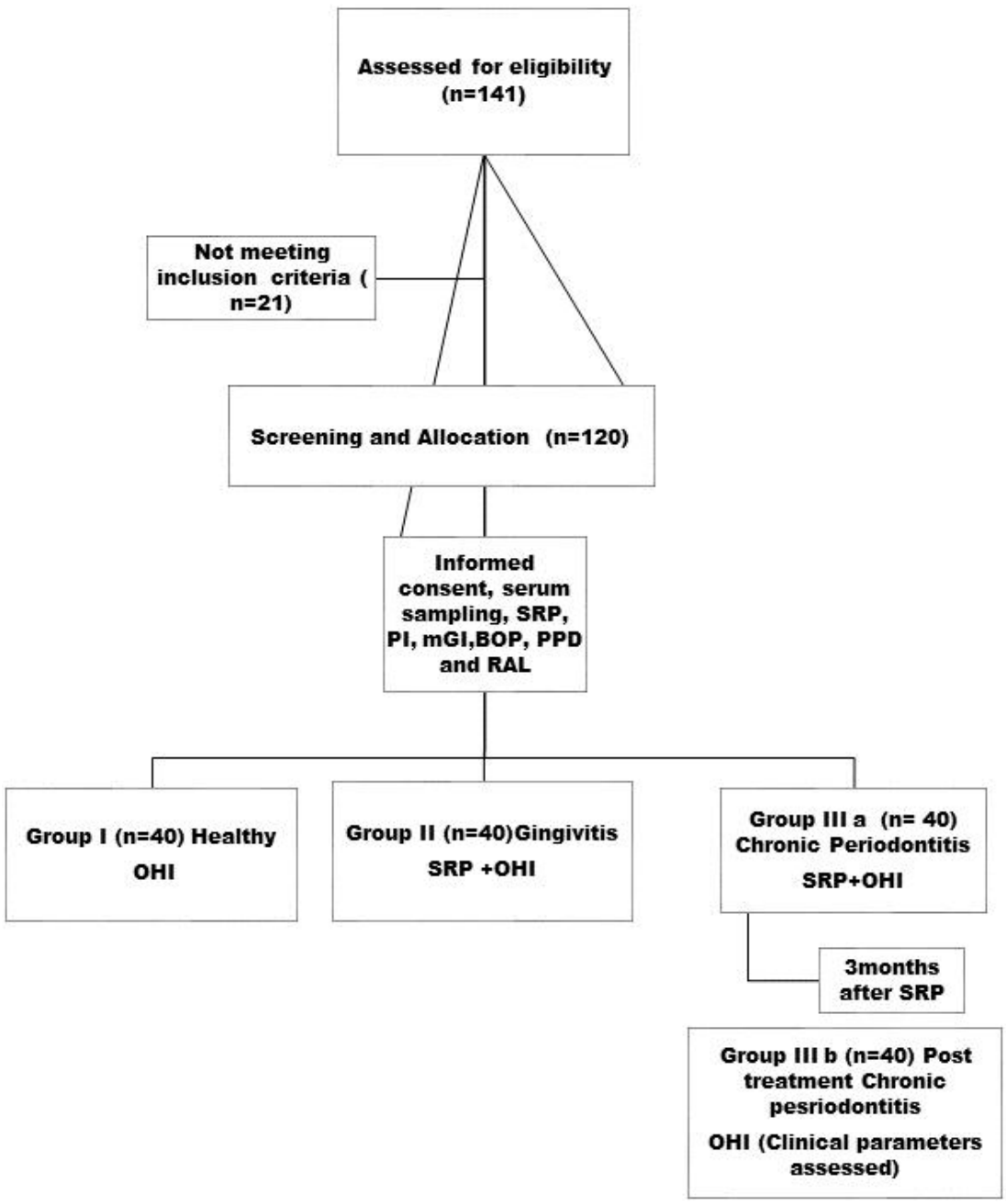

Figure 1. Study Design. BOP, Bleeding on Probing; OHI, Oral Hygiene Instructions; mGI, Modified Gingival Index; PI, Plaque Index; PPD, Periodontal Probing Depth; RAL, Relative Attachment Level; SRP, Scaling and Root Planning

			PI	BOP	mGI	Fetuin A	MMP7	PD	RAL
Group I	Fetuin-A	P	0.001*	0.681	0.001*	.	0.332		
	MMP-7	P	.617	0.186	0.491	0.332	.		
Group II	Fetuin-A	P	0.551	0.301	0.504	.	0.001*		
	MMP-7	P	0.303	0.762	0.284	0.001*	.		
Group III a	Fetuin-A	p	0.045	0.023	0.001*	.	0.010*	0.227	0.935
	MMP-7	p	0.079	0.001*	.308	0.010*	.	0.103	0.745
Group III b	Fetuin-A	P	0.001*	0.090	0.009	.	0.001*	0.784	0.373
	MMP-7	P	0.001*	0.768	0.212	0.001*	.	0.946	0.638

Table 3. Results of Spearman's Rank Correlation (r) Test to Compare Serum Fetuin A and MMP-7 Levels with Clinical Parameters among the Groups

r- Spearman's rank correlation, p- P- value, N- Sample size
*Correlation is significant if the p value is less than 0.05

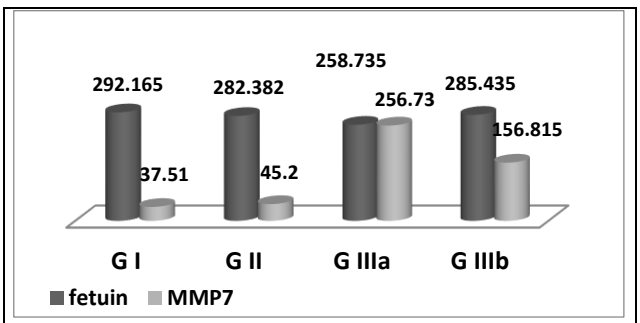

Figure 2. Comparison of Serum Fetuin A and MMP-7 Levels between Study Groups

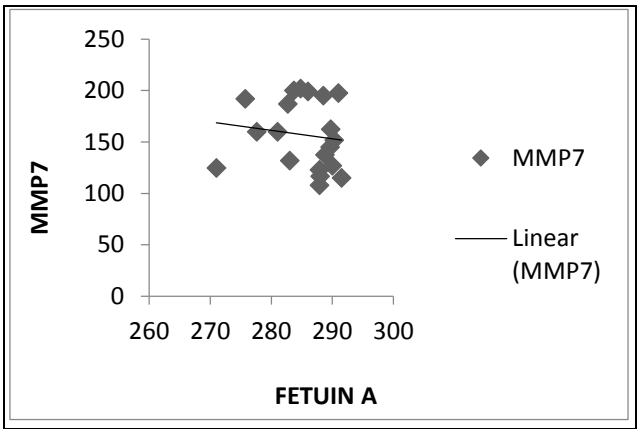

Figure 3. Intragroup Comparison of Serum Fetuin A and MMP-7

DISCUSSION

Fetuin A is often considered as a mediator that links chronic inflammation to vascular calcifications.[25] Currently, there is emerging evidence on its anti-inflammatory attributes and it being a predictive biomarker of systemic calcification. Chronic periodontitis being an inflammatory disease and even though several enzymes are increased in CP patients, none of these enzymes to date is known to potentially inhibit the function of Fetuin A. Recently, it has been demonstrated in the invitro studies that MMP-7 being a signature inflammatory mediator and biomarker in chronic periodontitis patients could reduce the levels of Fetuin A in serum as a consequence of their ability to degrade this protein.[17] Therefore, we aimed to explore for the first time the relationship between serum Fetuin A with MMP-7 and the impact of non-surgical periodontal therapy on their levels in chronic periodontitis patients. This would add new information to our knowledge that may fill some gaps on our limited understanding of the role of Fetuin A involvement in the development of CVD in CP patients.

We validated our methodology by recruiting systemically healthy subjects to eliminate the effect of systemic diseases on Fetuin A and MMP-7 levels. Further, the demographic data such as age was standardized between 30-39 yrs so as to reduce the effect of chronic nature of periodontal disease progression. Approximately equal number of male and female patients was considered in all the three groups to reduce the impact of extraneous variables in our study. Fetuin A levels in serum were examined in the current study in relation to another inflammatory biomarker in the same samples which could provide more valuable information on their association with each other as well as the progression of periodontal disease. Fetuin A molecule was adequately recovered and quantified to be 87%.

The present study indicates that there is an inverse relationship and a reverse trend between Fetuin A and MMP-7 as the periodontal disease progressed and highlights the predictive significance of Fetuin A as a negative APP in CP. The obtained lower levels of Fetuin A which was observed with the severity of periodontal disease could be attributed to its anti-inflammatory action, first it suppresses the release of proinflammatory cytokines such as TNF from macrophages (macrophage deactivation).[26] Secondly it inhibits the production of the high mobility group box 1 protein (HMGB1),which is a secondary mediator of inflammation that is secreted by activated macrophages and monocytes.[27,28] Third, it acts as an endogenous ligand for innate immune toll like receptor 4 (TLR4) thus, capable of attenuating infection and injury elicited inflammatory responses.[29,30] Fourth, it inhibits hepatocyte binding factor to hepatic receptors on the junctional epithelium thus, preventing its migration and pocket formation.[31] Fifth, it enhances cellular uptake of cationic inhibitors of proinflammatory cytokines (TNF) and thereby, prevents the over production of proinflammatory cytokines.[32] It downregulates inflammation by inhibiting some pathological pathways, such as those related to TGF-β and insulin receptor tyrosine kinases.[32] All this direct to a reasonable notion that Fetuin A is a key player in downregulating inflammation due to its anti-inflammatory properties and thus helps in maintaining periodontal health.

The results of our study on serum Fetuin A concentration are in agreement with the previous study done by Turer et al (2016) who showed that the total amount of serum levels of Fetuin A were decreased in gingivitis and CP groups compared to control.[24] Several lines of evidence in the previous studies suggest that Fetuin A levels are down regulated in various inflammatory conditions.[25,32] Our data reveal that there is a moderate negative association of Fetuin A levels and MMP-7 in CP patients. This association is in accordance several other studies indicating reduced levels of Fetuin A with inflammatory cytokines.[24,33,34,35] Also, previous in vitro study by Schure et al has shown that complete digestion of human Fetuin occurs within 12 hours when it is incubated with a 10-fold molar excess of MMP-7.[17] Therefore, MMP-7 is considered to be relevant for the study of pathophysiological degradation of Fetuin A. Likewise, our study also showed a rise in MMP7 levels and a concurrent decrease in Fetuin A levels.

Our study for the very first time evaluated the impact of non-surgical periodontal therapy on the levels of serum Fetuin A and MMP-7. On the one hand, the results showed an increase in Fetuin A concentration post treatment as compared to CP. On the other hand, there was a decrease in MMP7 levels following phase I therapy in CP. This implicates that periodontal treatment was effective in improving the periodontal parameters and increasing the Fetuin A levels after 3 months. Since, our study is first to evaluate the role of initial periodontal therapy on Fetuin A and MMP7 levels a direct comparison with other studies could not be carried out. However, a similar comparison with other systemic markers is carried out. Studies by Mattila et al.(2002), D'Aiuto et al.(2005,2013), Vidal et al.(2009, 2013), Bokhari et al. (2012) have also shown that non-surgical periodontal treatment of chronic periodontitis results in significant reductions in systemic markers of inflammation a similar trend seen in our study.[36-38]

Further, it has been observed that in chronic inflammatory diseases such as pancreatitis and rheumatoid arthritis, there was lowering of serum Fetuin A levels by 20-30%, the observations collectively suggesting that Fetuin can be classified as a negative APP during infection or other inflammatory process.[25] Periodontitis and RA are chronic inflammatory conditions with similar pathological features. Also, it is important in this context to recall that many calcification disorders, including arteriosclerosis and calciphylaxis, have an inflammatory component and that

human Fetuin A is a negative acute phase protein that is downregulated after infection or trauma.[32] Since, Fetuin A is a potent inhibitor of systemic calcification it is considered as a potential cardio protective protein. There are many proposed mechanisms by which Fetuin A inhibits calcification. First, it acts as a direct crystal inhibitor (poison) for calcium phosphate induction of mineralisation.[10,39] Second, Feutin A results in inhibition of differentiation of cells that regulate the process of mineralisation e.g. Osteoblasts. Thirdly, it could act as a decoy receptor for osteoinductive cytokines, thereby mopping up available proteins that would otherwise stimulate osteodifferentation.[17] Finally, antiapoptotic activity of Fetuin A has been observed in smooth muscle cells and dampening of the cell-specific responses is said to alleviate the detrimental consequences of local inflammation, cell death and cartilage degradation.[40] The proven protective function of Fetuin A in many animal models of inflammation, the inhibition of proinflammatory compounds and the inhibition of crystal induced neutrophil activation collectively suggests that it can be considered as an endogenous inhibitor of pathological mineralization or calcification in soft tissues. Therefore, any situation that lowers Fetuin A serum concentration will increase the risk of systemic calcification.

It could be noticed from our study that the degradation of Fetuin A, possibly related to MMP7 released into the bloodstream in patients with periodontitis could explain, at least in part, the association between untreated periodontal diseases and cardiovascular diseases in general and vascular calcification in particular as its cardio protective function is lost.

Despite the validation of our materials and methodology there does exist a few limitations in this study. First of all, large variability in the types of Fetuin A and the detection techniques needs to be addressed, as it prevents a very meaningful comparison with other studies. Secondly, we could have correlated serum levels with GCF levels as periodontitis is a site-specific disease. Thirdly, due to small sample size we failed to correlate Fetuin A levels at different stages of PD. Finally, considering that this is a typical cross-sectional epidemiological study and not a prospective investigation on this relationship, no final conclusion with respect to causality and temporality of the explored connection can be drawn. Hence, future studies are required to assess the clinical significance of these findings to elucidate the precise mechanism of vascular calcifications in patients with CP including the role of decreased Fetuin A levels and to analyse the potential of using Fetuin A as a therapeutic agent to reduce ectopic calcification.

CONCLUSIONS

There is an inverse relationship between serum Fetuin A and MMP-7 levels with the severity of periodontal inflammation. The initial periodontal therapy helped in moderation of Fetuin-A levels. Decreased Fetuin-A levels can lead to progression of periodontal inflammation owing to the loss of its anti-inflammatory protective role. Concurrently, it could pose as one of the possible linking factors between inflammatory conditions and vascular calcifications.

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