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Evaluation of Periodontal Clinical and Biochemical (GCF-Sialic Acid and Chondroitin Sulfate) Parameters in Various Grades of Fluorosis in Patients with Periodontitis

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Abstract

Aim: The study aimed to evaluate the clinical parameters and gingival crevicular fluid (GCF) levels of SA (sialic acid) and CS (chondroitin sulfate of glycosaminoglycans) in dental fluorosed patients with and without periodontitis.

Materials and Methods: A total of 40 patients were selected and clinical parameters were assessed. The GCF-SA and CS levels were estimated. The selected patients were permanent residents of endemic fluorosis belt in and around Davangere.

Results: The overall group wise estimation of GCF SA showed significantly higher SA levels in the FD group ($p < 0.005$), whereas the GCF CS levels showed no significant difference between FH and FD group ($p = 0.886$).

Conclusion: The grade wise dental fluorosis assessment of periodontal clinical and biochemical parameters (GCF- SA and CS) revealed similar features between fluorosed healthy and diseased (periodontitis) patients. However, overall comparison between the fluorosed healthy and diseased patients showed significant changes in diseased group.

Clinical Relevance: Dental fluorosis is known to bring about changes in both during health and periodontal disease status. The estimation of biochemical parameters like GCF SA and CS support the routine periodontal clinical changes.

Keywords: *Dental Fluorosis; Periodontium; Health; Periodontitis; Sialic Acid; Chondroitin Sulfate*

Introduction

Dental fluorosis is a condition showing direct evidence of toxic effect of fluoride such as cemental necrosis, osteosclerosis and calcification of ligament in human periodontal region as a consequence of the life time exposure to high-fluoride water levels. Fluoride also had a toxic effect on the alveolar bone of permanent teeth that eventually lead to osteonecrosis and recession of the alveolar crest. The adverse effects of fluoride leading to fluorosis requires to be eliminated by taking precaution and considering the potential risks.

The literature on fluoride and dental caries is well discussed in contrast to periodontal tissues. However, a recent review by Vandana KL has presented an epidemiological association between fluorosis and periodontal disease, the influence of dental fluorosis on periodontal structures along with the comparison of influence of periodontal treatment on fluorosed and non-fluorosed teeth [1]. Various hard tissue and soft tissue changes caused by fluorosis are presented. The possible consequences of periodontitis need to be recognized earlier to prevent the disease and its progression.

Vandana KL and MS Reddy reported a higher occurrence of periodontitis in dental fluorosed subjects and increased severity of periodontitis as the degree of dental fluorosis increased [1]. AK Susheela has reported the sialic acid (SA) and glycosaminoglycans (GAG) ratio as a biochemical indicator of bone destruction in skeletal fluorosis patients [2].

The Sialic acid, a biomarker with its biological activity as a defense molecule against oxidative stress would serve as an important marker in GCF to ascertain the host immune response while the GAG, an important structure entity of periodontal bone provides a marker for bone destruction. In a given clinical situation the estimation of biomarkers more than one based on host immunity and tissue breakdown products complements the clinical diagnosis and prognosis.

SA and chondroitin sulfate (CS) are two biomarkers that are known to be associated with periodontitis [3] as well as fluorosis [4]. The Medline search using the words dental fluorosis, sialic acid, chondroitin sulfate, GCF revealed no studies.

Thus, the comparative assessment of clinical parameters and GCF levels of Sialic Acid and Chondroitin sulfate [GAG (glycosaminoglycans)] levels in dental fluorosis subjects with and without periodontitis were undertaken.

Materials and Methods

The patients for this study who gave their consent to participate were selected. The study was approved by Institutional Review Board (IRB no: IEC/1802/2016-1207) in accordance with RGUHS. The study duration was of nine to twelve months.

Both the sexes in the age group of 25 - 50 years were included according to the inclusion and exclusion criteria and divided to two groups of fluorosed healthy (FH) and fluorosed with periodontitis (FD).

Systemically healthy subjects with dental fluorosis were selected based on the inclusion criteria; Subjects lived in the endemic water fluoridated (1.5 - 3 ppm) area for 5 - 10 years in and around Davangere [2] with mottled tooth enamel i.e. dental fluorosis stains. Systemically healthy periodontally diseased individuals had to have at least three teeth with probing pocket depth (PPD) > 3 mm, in at least two quadrants and radiographic evidence of bone loss [5]. Subjects with any metabolic bone diseases, (Pagets disease, hyperparathyroidism, hypophosphatasia) infectious diseases, (tuberculosis, hepatitis, HIV) autoimmune diseases, (rheumatoid arthritis) and diabetes, subjects undergoing orthodontic or any antibiotic therapy, subjects with other intrinsic dental stains, tetracycline stains or any other dental developmental anomalies such as enamel hypoplasia, amelogenesis imperfecta and dentinogenesis imperfecta etc. also pregnant or lactating patients, smokers and alcoholics were excluded from the study.

The dental fluorosis was assessed using Jackson's fluorosis index (JFI) [6]. The clinical parameters recorded were plaque index (PI) [7], gingival bleeding index (GBI) [8], PPD and CAL in FD group were recorded as per community periodontal index (CPI) [9] criteria and CPI-PPD and CPI-CAL scores were designated and mean values of each grade was calculated for every subject.

In the selected 40 dental fluorosed subjects with and without periodontitis, human gingival crevicular fluid (GCF) was collected for estimation of sialic acid (SA) and chondroitin sulphate (CS) of glycosaminoglycans (GAG) by colorimetric analysis of skoza and mohos and ELISA (Enzyme Linked Immune Sorbent Assay) kit Kinesis (USA) from Krishgen, Mumbai, India) respectively [10]. GCF samples were collected from the three teeth using paper points and were pooled. After 30 seconds, the resultant GCF samples were transferred to PBS solution and stored at -80°C until analysed [11].

Collected data was subjected to statistical spss analysis using one way anova and unpaired t test, to evaluate the results and correlation of clinical and biochemical parameters.

Results

The current study included 40 subjects which was distributed into two groups of twenty subjects each.

The demographic information is presented in table 1.

The clinical parameters such as PI, GBI, PPD and CAL between FH and FD groups are presented in table 1- 6 and graph 1-5.

Patient		FH (Mean)	FD (Mean)	P Value
Age		41.8	45.4	0.12 NS
Gender	M	1.5	1.5	0.824 NS
	F	1.5	1.5	
Pi		2.58	4.14	0.000 S
GBI		2.28	3.87	0.000 S
CPI-PPD			3.23	
CPI-CAL			0.78	
SA		496.65	686.33	0.005 S
CS		47.05	46.52	0. 686 NS

Table : 1: *Demographic table of full mouth fluorosis.*
FH: Fluorosed Healthy; FD: Fluorosed Diseased; M: Male; F: Female; PI: Plaque Index; GBI: Gingival Bleeding Index; CPI: Community Periodontal Index; PPD: Probing Pocket Depth; CAL: Clinical Attachment Loss; SA: Sialic Acid; CS: Chondroitin Sulphate.

JFI score	A (%)	B	C	D	E	F
Total (100%) 1167 Teeth	3.34	12.42	15.08	9.94	23.39	35.81

Table 2: *Percentage of occurrence of different JFI grades (FH +FP).*
JFI: Jackson's Fluorosis Index; FH: Fluorosed Healthy; FP: Fluorosed Periodontitis.

Flourosis Healthy						Fluorosis Diseased				
JFI	No	%	Mean	STD deviation	SIG P value	N	%	Mean	Std. Deviation	SIG P value
A	7	8.4	2.43	.79		5	6.17	4.20	.84	
B	14	16.87	2.43	.85		15	18.52	4.20	.77	
C	14	16.87	2.71	.61	0.749 NS	12	14.81	4.00	.85	0. .908 NS
D	11	13.25	2.81	.40		13	16.05	4.31	.75	
E	17	20.48	2.53	.87		17	20.99	4.18	.81	
F	20	24.1	2.55	.67		19	23.46	4.00	.943	

Table 3A: *Grade wise expression of plaque index between FH and FD groups.*
JFI: Jacksons Fluorosis Index; NS: No Significant Difference, when p > 0.1.

	Groups	Mean	Std Dev	Sig Sig. (2-Tailed)
PI	FH	2.58	0.72	0.000 S
	FD	4.14	0.82	

Table 3B: *Overall comparison of plaque index between FH and FD groups.*

PI: Plaque Index; FH: Flourosis Healthy; FD: Fluorosis Diseased; S: Highly Significant Difference, when P < 0.05.

Fluorosis Healthy						Fluorosis Diseased				
JFI	No	%	Mean	Std Deviation	Sig P Value	N	%	Mean	Std. Deviation	Sig P Value
A	7	8.4	2.29	0.76		5	6.17	4.20	0.84	
B	14	16.87	2.07	0.83		17	18.52	3.53	0.80	
C	14	16.87	2.43	0.65	0.742 NS	15	14.81	3.93	0.88	0.314 NS
D	11	13.25	2.36	0.67		13	16.05	4.00	0.71	
E	17	20.48	2.12	0.99		17	20.99	4.12	0.70	
F	20	24.1	2.40	0.75		19	23.46	3.74	0.99	

Table 4A: *Grade wise expression of gingival bleeding index between FH and FD groups.*

JFI: Jacksons Fluorosis Index; NS: No Significant Difference, when p > 0.1; STD: Standard.

	Groups	Mean	Std Dev	Sig Sig. (2-Tailed)
GBI	FH	2.28	0.79	0.000 S
	FD	3.87	0.84	

Table 4B: *Overall comparison of gingival bleeding index between FH and FD groups.*

GBI: Gingival Bleeding Index; FH: Flourosis Healthy; FD: Fluorosis Diseased; S: Highly Significant Difference, when P < 0.05; Std: Standard; Dev: Deviation; Sig: Significance.

PPD CPI Score						Cal CPI Score				
JFI	No	%	Mean	Std Deviation	Sig P Value	N	%	Mean	Std. Deviation	Sig P Value
A	6	10.34	3.17	0.26		6	10.34	0.50	0.84	
B	11	18.97	3.52	0.53		11	18.97	0.93	0.73	
C	8	13.79	2.88	1.22	0.349 NS	8	13.79	0.86	0.90	0.367 NS
D	6	10.34	3.29	0.40		6	10.34	0.92	0.80	
E	11	18.97	3.24	0.36		11	18.97	0.45	0.52	
F	16	27.59	3.31	0.43		16	27.59	1.05	0.78	

Table 5: *JFI grade wise CPI scores of PPD and CAL in fluorosis diseased group.*

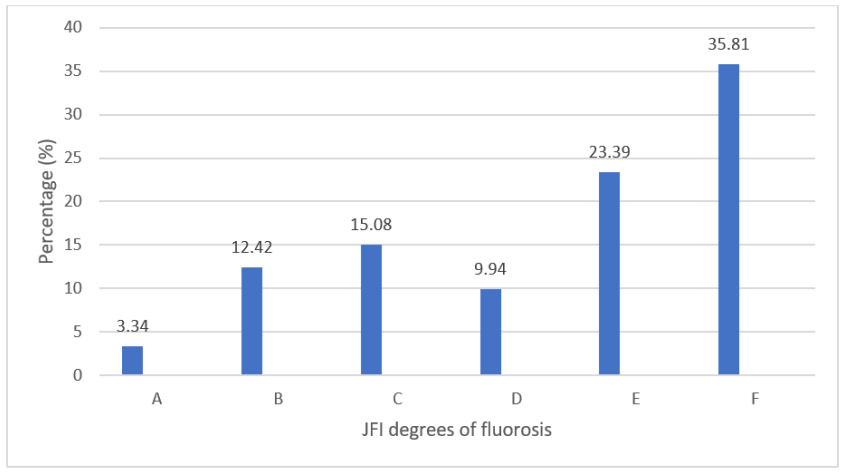
JFI: Jacksons Fluorosis Index; CPI: Community Periodontal Index; PPD: Probong Pocket Depth; CAL: Clinical Attachment Loss; NS: No Significant Difference, when p > 0.1; SIG: Significant; STD: Standard.

SA (Ngl/µl)						Range Min Max		CS (Ngl/µl)					Range Min max	
JFI	No	%	Mean	Std Deviation	Sig P Value			N	%	Mean	Std. Deviation	Sig P Value		
B	2	10	486.00	38.18		459	513	2	10	74.35	12.52		66	83
C	2	10	501.00	12.73	0.965 NS	492	510	2	10	52.10	10.04	0.121 NS	45	59
D	1	5	501.00	.		501	501	1	5	63.40	.		63	63
E	6	30	494.50	19.39		462	510	6	30	38.60	19.16		18	67
F	9	45	499.00	27.94		459	534	9	45	43.69	15.55		12	61

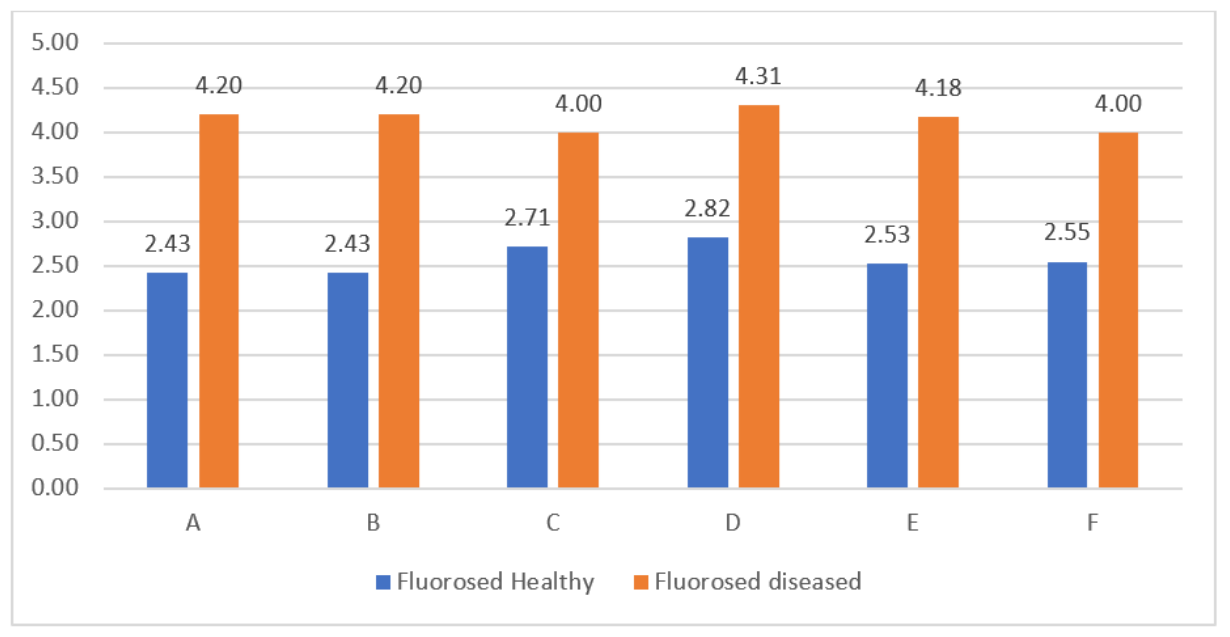
Table 6A: *Biochemical parameters of JFI grade wise in fluorosis healthy group.*
JFI: Jacksons Fluorosis Index; SA: Sialic Acid; CS: Chondroitin Sulphate; NS: No Significant Difference, when p> 0.1; S: Significant; STD: Standard; MIN: Minimum; MAX: Maximum.

SA (Ngl/µl)						Range Min max		CS (Ngl/µl)					Range Min max	
JFI	No	%	Mean	Std Deviation	Sig P Value				%	q	Std. Deviation	Sig P Value		
B	3	15	759.00	111.73		672	885	3	15	52.33	0.96		51.30	53.20
C	3	15	713.00	40.51	0.013 S	672	753	3	15	69.43	22.34	0.184 NS	46.00	90.50
D	3	15	516.00	107.79		393	594	3	15	36.37	10.96		25.10	47.00
E	5	25	743.40	67.70		663	825	5	25	42.70	20.68		23.50	74.80
F	6	30	705.00	78.78		633	858	6	30	45.62	15.98		32.30	75.50

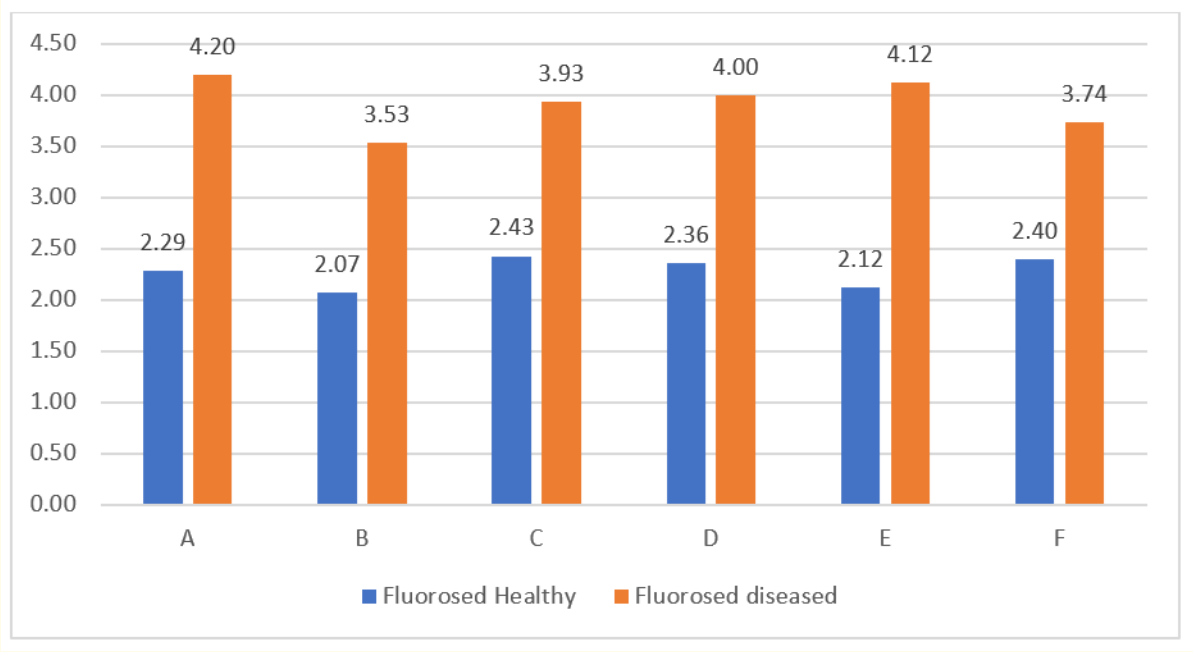
Table 6B: *Biochemical parameters of JFI grade wise in fluorosis diseased group. S: Highly Significant Difference when p < 0.05 and; NS: No Significant Difference, when p > 0.1; SA: Sialic Acid; CS: Chondroitin Sulphate; MIN: Minimum; MAX: Maximum.*



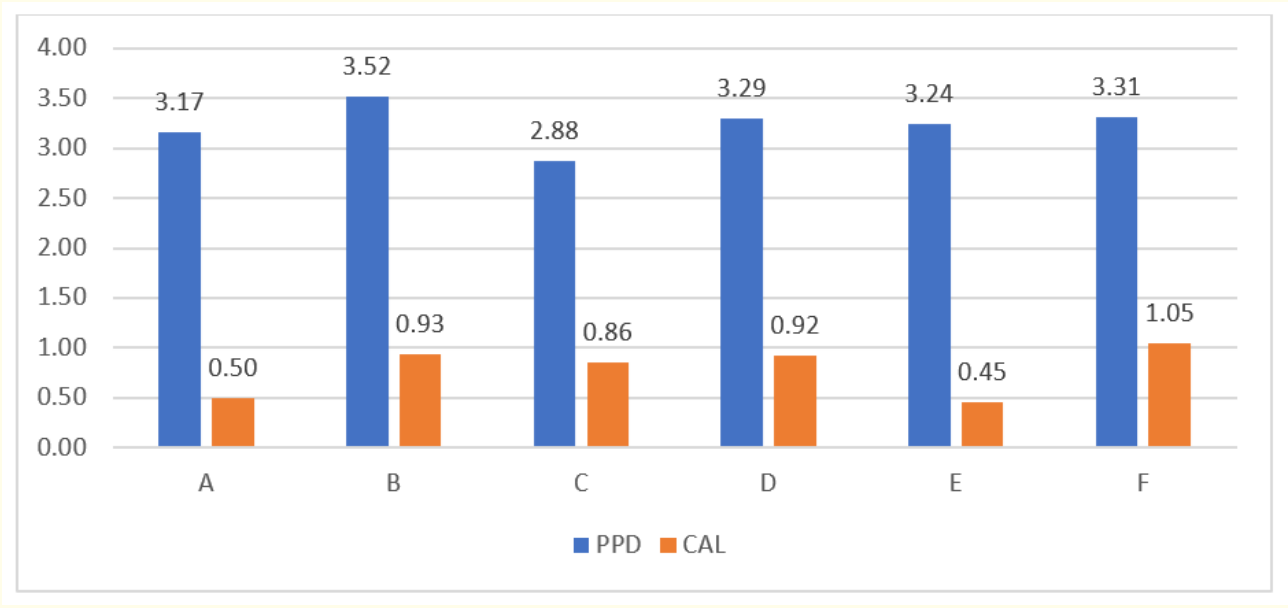
Graph 1: *Percentage of occurrence of various JFI grades in FH and FD group.*



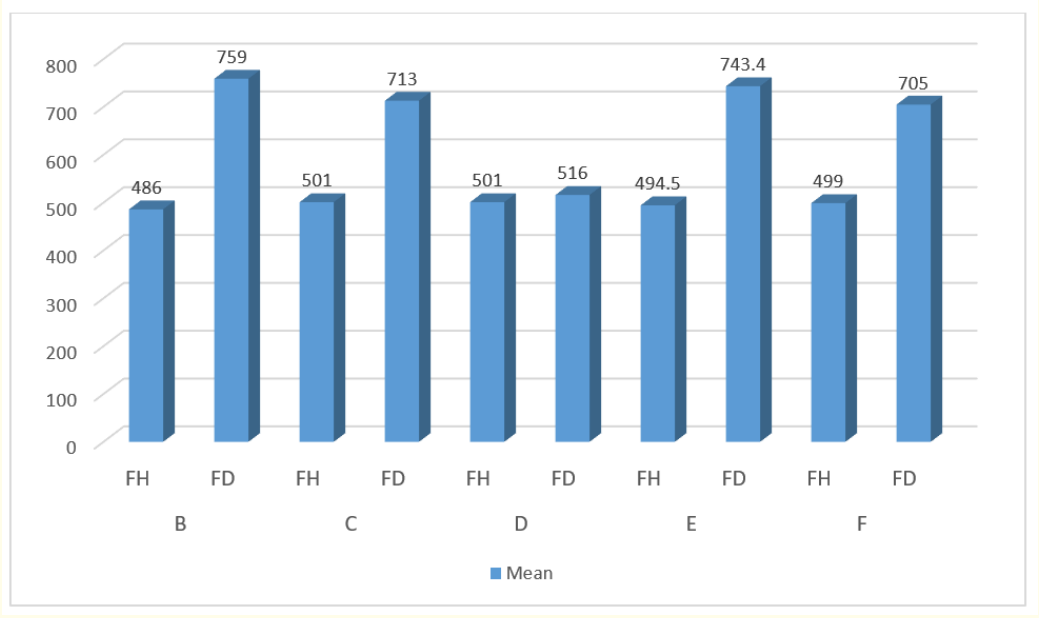
Graph 2: *Grade wise expression of plaque index between FH and FD groups.*



Graph 3: *Grade wise expression of gingival bleeding index between FH and FD groups.*



Graph 4: *CPI score of PPD-CAL of various grades of JFI in fluorosed diseased group.*



Graph 5: *GCF SA levels between FH and FD groups.*

The percentage of occurrence of different grades of Jackson's fluorosis index is presented in table 2 and shows higher incidence of grade 'F' (35.81%) and least incidence of grade 'A' (3.34%).

No significant difference were noted in the grade wise expression of plaque index between FH and FD groups (p = 0.749 and 0.908 respectively) (Table 3A).

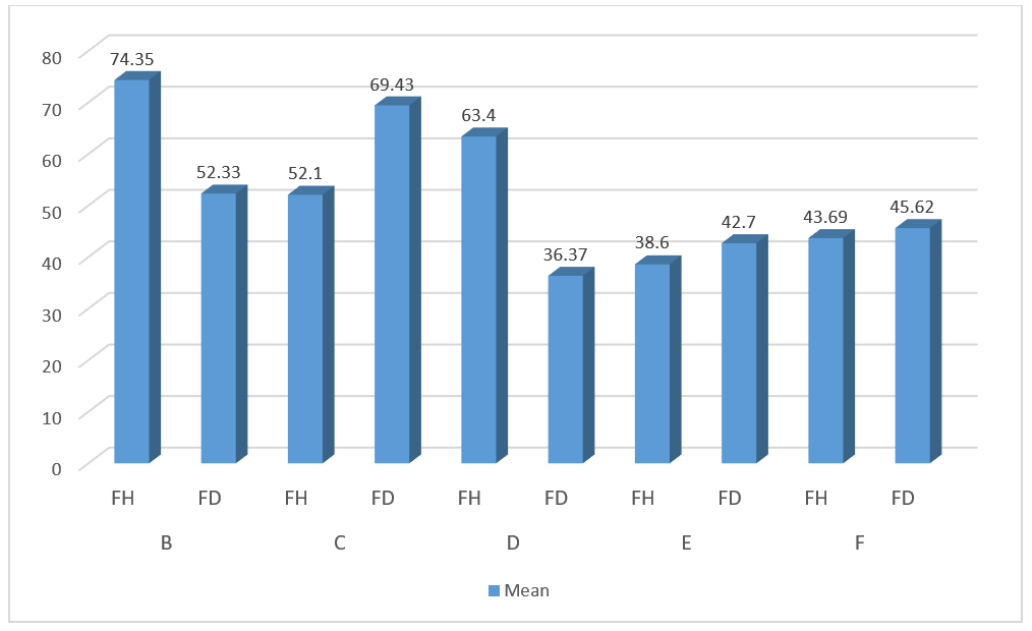
The overall comparison of plaque index between FH and FD groups showed significant difference between them (p = 0.000) (Table 3B).

In FH and FD groups, the grade wise expression of gingival bleeding index showed no significant difference (p = 0.742 and 0.314 respectively) (Table 4A), however the overall comparison between the two groups revealed significant difference (p = 0.000) (Table 4B).

In FD group, the JFI grade wise estimation showed maximum PPD score in JFI B grade, however the PPD scores were not significant in different grades (p = 0.349). The CAL was found to be maximum in JFI B grade but there was no significant difference among the JFI grades (p = 0.367) (Table 5).

In FH group, the JFI grade wise estimation showed maximum GCF SA levels in JFI C grade (501 ± 12.73 ng/µl) however the GCF SA levels were not significant in different grades. The range of GCF SA was (459-534 ng/µl). The GCF CS level was maximum in the JFI B grade and there was no significant difference amongst the JFI grades. The range was 12 - 83 ng/µl (Table 6A) (Graph 5 and 6).

In the FD group, the JFI grade wise estimation showed maximum GCF SA levels in JFI B grade (759 ± 111 ng/µl) which had the significant difference amongst different JFI grades. The ranges observed was 393-885 ng/µl. The JFI grade wise estimation showed maximum GCF CS levels JFI B grade (69.43 ± 22.34), however the values were not significant amongst different grades (Table 6B) (Graph 5 and 6).



Graph 6: *GCF CS levels between FH and FD groups.*

The overall group wise estimation of GCF SA showed significantly higher SA levels in the FD group ($p < 0.005$), whereas the GCF CS levels showed no significant difference between FH and FD group ($p = 0.886$) (Table 7).

	Grps	Mean	Std. Deviation	Sig P Value
SA	FH	496.65	23.06	0.005 S
	FD	686.33	104.20	
CS	FH	47.05	18.30	0. 686
	FD	46.52	17.90	NS

Table 7: *Overall biochemical parameters estimation in FH and FD groups.*
SA: Sialic Acid; CS: Chondroitin Sulphate; FH: Flourosis Healthy; FD: Fluorosis Diseased; S: Highly Significant Difference, when $p < 0.05$; NS: No Significant Difference, when $p > 0.1$.

Discussion

The use of gingival crevicular fluid (GCF) analysis has recently risen to the forefront in biochemical techniques used in periodontal diagnosis [12]. Fluoride also has a toxic effect on the alveolar bone of permanent teeth that eventually lead to osteonecrosis and recession of the alveolar crest [13]. In the endemic areas of fluorosis, the toxic effect of fluoride on cementum and bone may lead on to destruction of periodontal structures through a non-inflammatory process, as well enhance the regular plaque induced inflammatory through oxidative burst. Based on the above reports the occurrence of periodontitis is significantly observed and well-reported.

There are studies conducted in our institution since last 18 years, preliminary attempts to investigate any possible relationship between fluorosis and periodontal disease. These research projects are directed to find the role of dental fluorosis on periodontal tissues [1]. However, the toxic effect of fluorides on human periodontal tissues remains to be researched extensively to confirm the data and there exists a need to evaluate the biochemical changes in a fluorosed periodontium which may show any possible role of fluorosis in the pathogenesis of periodontitis.

Thus, estimating the levels of Sialic acid (SA) and Glycosaminoglycans (GAG) particularly Chondroitin sulfate (CS) in the different grades of dental fluorosis (jacksons fluorosis index) with and without periodontitis were considered.

Our study results are presented below, A first attempt is made to assess the clinical and biochemical parameters of dental fluorosis grade wise as well as the overall groupwise. The comparison results of the study are discussed as follows: The percentage of occurrence of JFI grade was found to be highest of F grade (35.81%) and least in the A grade (3.34%).

In the current study, the plaque and gingival bleeding were found to be similar amongst different grades in both FH and FD groups. The oral hygiene of the patient plays an important role on the plaque levels which was not recorded in the study. The possible reasons for the above observation requires be ascertained in a larger sample size of each grades of JFI. It is one first attempt by the authors to conduct grade wise assessment of periodontal clinical parameters and requires further studies to comprehend it.

For the overall plaque levels the significant difference in levels in plaque levels was observed in FD group than FH group. The comparison of overall plaque and bleeding levels showed significantly higher values in FD group than FH group.

The main causative factor such as plaque was found to be similar amongst different JFI grades of fluorosis followed by similar levels of GBI grade wise.

The grade wise assessment for PPD and CAL were found to be similar i.e. the occurrence of periodontal destruction measured in terms of PPD and CAL showed no difference. Considering the periodontal parameters such as PI, GBI, PPD and CAL, there was no difference in any of the parameters amongst different grades of fluorosis scores of both dental fluorosed healthy and diseased (PI, GBI) status. This current observation needs to be ascertained in a larger sample size in each JFI grades of dental fluorosis [1].

There are no comparative studies available to compare our study results as it is first of the observation in dental fluorosed subjects.

Only few studies exist in the literature for comparison of the clinical parameters in the dental fluorosed subjects. As the degree of fluorosis increased, periodontitis also increased when gingivitis showed a decreasing trend [1]. Vandana KL and Reddy MS in 2007 reported that as the degree of fluorosis increased, severity of gingivitis reduced and periodontitis increased [2].

The possible reason for increased GBI is speculated to be due to the inflammation caused by fluorosis [14]. The tissue destruction brought about by fluorosis may lead to inflammatory reaction in order to restore homeostasis and the C reactive protein and haptoglobin levels are supportive of inflammatory status [14].

In the current study, GCF SA and CS were assessed for the first time in JFI grade wise dental fluorosis status and as well overall group wise criteria.

The CS a biochemical marker is representation of tissue destruction. In the current study the GCF CS levels were found to be similar suggestive of similar degrees of tissue destruction occurring in both fluorosed healthy and diseased status. This could possibly be attributed to fluorosed induced changes in the hard and soft tissues [1] as discussed earlier.

SA as an indicator of oxidative measure, is a new reported information [15]. It is also reported that the oxidative burst is more in fluorosed subjects [16].

Along with dental fluorosis as a first reason for increased oxidative burst is compounded by the periodontitis in the fluorosed diseased group. This could be the possible reason for significantly increased GCF SA levels (Table 7).

As such there are no studies to compare our study results. The following are the studies pertinent to chondroitin sulfate and sialic acid in periodontitis patients which may serve as an important source to comprehend the objectives of the study.

Saubhik., *et al*. reported higher oxidative markers and in fluorosed and nonfluorosed periodontitis groups [17]. A study on skeletal fluorosis and rheumatoid arthritis by AK Susheela., *et al.* [16] has reported increased serum levels of GAG and sialic acid due to enhanced GAG destruction caused by increased oxidative burst in fluorosis [17] and increased SA levels to overcome the oxidative burst serving as an antioxidant.

Fluorosis does affect the composition of saliva as well as modifying the electrolytes and the antioxidant properties in many ways. Gavriliuk., *et al.* in their study pointed to dose-dependent fluoride intoxication and metabolic imbalance. This changed antioxidant capacity of saliva can influence the integrity of periodontal tissue as well but it remains scarcely studied. A close association between chronic fluoride toxicity and increased oxidative stress has been previously reported in humans. In erythrocytes of children afflicted with skeletal fluorosis, increases in MDA levels and decrease in SOD activity were reported. Fluoride inhibits the activities of SOD, causing a heavy accumulation of free radicals and hydrogen peroxide resulting in damage to various cells [18]. Wang., *et al.* reported a decrease in antioxidants in patients with skeletal fluorosis [19].

Regarding SA, its antioxidant role is reported by Surekha., *et al* [20]. There is a decrease in the biosynthesis of SA proteins during fluorosis [21]. Both SA and GAG serve as important biochemical markers as mentioned in fluorosis related studies [3,22,23].

The possible reasons for CS levels in fluorosed group could be due to the increased synthesis and deposition of GAG in tooth and bone [3] and diminished excretion of GAG [22].

Otherwise GCF chondroitin sulfate (GAG) is due to high mechanical loads, inflammation and trauma from occlusion [22].

The GCF SA and CS estimation have been studied for the first time in the in dental fluorosed patients. There are no relevant studies available in literature apart from those related to systemic conditions. Further larger samples can be considered to ascertain our study results.

The comparison of clinical and biochemical parameters of dental fluorosed and non fluorosed periodontitis patients has been taken up (Dissertation submitted to RGUHS) [24].

Conclusion

The grade wise dental fluorosis assessment of periodontal clinical and biochemical parameters (GCF- SA and CS) revealed similar features between fluorosed healthy and diseased (periodontitis) patients. However, overall comparison between the fluorosed healthy and diseased patients showed significant changes in periodontitis diseased group.

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