A comparative assessment of clinical parameters, sialic acid, and glycosaminoglycans levels in periodontitis patients with and without dental fluorosis: A clinical and biochemical study

Prasad Sakthidharan Aswin, Kharidi Laxman Vandana

Abstract:
Aim: This study was aimed to evaluate and compare the clinical parameters and the gingival crevicular fluid (GCF) levels of sialic acid (SA) and chondroitin sulphate (CS) in dental fluorosed and nonfluorosed (NF) gingivitis and periodontitis patients. Materials and Methods: A total of 100 patients were divided into two control (healthy) and four test (diseased) groups of gingivitis and periodontitis patients with and without dental fluorosis. The GCF-SA and chondroitin sulphate levels were measured using the conventional method and enzyme-linked immuno sorbent assay, respectively. Results: The plaque levels (2.9 ± 0.44), gingival bleeding levels (2.75 ± 0.55), and clinical attachment loss (0.44 ± 0.45) between dental fluorosed participants with chronic periodontitis (fluorosed periodontitis [FP]) and NF participants with chronic periodontitis (nonfluorosed periodontitis [NFP]) groups showed no statistically significant difference. Higher probing pocket depth by community periodontal index (CPI) scores of 4 and clinical attachment level CPI score of 1 (75%) was found in FP group when compared to a score of 3 (FP: 24.5% and NFP: 73.5%) of the NFP group. The GCF SA levels (679.05 ± 101.06) were significantly higher in FP group than NFP group (553.80 ± 49.40) (P = 0.048). Similarly, the GCF CS showed highly significant levels in fluorosis periodontitis (48.08 ± 18.13) group than the NFP group (26.95 ± 8.69). Conclusion: Increased pocket depth score, GCF–SA, and CS levels in the dental fluorosed group were observed when compared with NF group. The diagnostic ability of clinical examination is most often supported by the relevant biochemical parameters that are applicable in this study. The newer diagnostic ability of SA is found to be contributory in this study. The diagnostic ability of CS representing tissue destruction served as an important GCF marker along with SA. Clinical Relevance: In dental fluorosis, estimation of SA and CS is recommended in periodontitis patients. Key words: Chondroitin sulfate, dental fluorosis, gingival crevicular fluid, periodontitis, sialic acid

INTRODUCTION

A

ccording to the 1999 survey, the endemic fluorosis is reported in at least 25 countries globally. Unfortunately, there is no updated survey as of now. Out of 32 states in India, 15 were identified as endemic fluorosis as per the 1993 survey report. An estimated amount of 62 million people were found to have fluorosis-related health hazards in India. The possible consequences of periodontitis in dental fluorosis participants need to be recognized earlier to prevent the disease and its progression. An institutional studies report by Vandana revealed a higher occurrence of periodontitis in dental fluorosed participants and increased severity of periodontitis as the degree of dental fluorosis increased. Susheela et al. have reported that the sialic acid (SA) and glycosaminoglycans (GAGs) ratio can serve as a biochemical indicator of bone destruction in skeletal fluorosis patients. There are several gingival crevicular fluid (GCF) biochemical markers such as SA and GAGs being...
assessed in periodontitis patients (with or without dental fluorosis) as a measure of periodontal tissue destruction.

SA and chondroitin sulfate (CS) are two biomarkers that are known to be associated with periodontitis as well as fluorosis. The Medline search using the words dental fluorosis, SA, CS, periodontitis, and GCF revealed no studies. Thus, this study was conducted for the comparative assessment of clinical parameters and GCF levels of SA and CS (GAGs) levels in periodontitis patients with and without dental fluorosis for the first time.

MATERIALS AND METHODS

Patients in this study who gave their consent to participate were obtained from the department of periodontics. This study was approved by the Institutional Review Board (IRB) (IRB no: IEC/1802/2016-1207) in accordance with RGUHS.

Both the sexes in the age group of 25–50 years were included according to the inclusion and exclusion criteria for the control (healthy) and test (gingivitis and periodontitis) groups [Table 1a].

The control group composed of systemically healthy dental nonfluorosis patients with mild-to-moderate periodontitis. Periodontally diseased individuals had probing pocket depth (PPD) >3 mm and radiographic evidence of bone loss. Systemically healthy participants with dental fluorosis were selected based on the inclusion criteria and participants lived in the endemic water fluoridated (1.5–3 ppm) area for 5–10 years in and around Davangere with mottled tooth enamel, i.e., dental fluorosis stains. Systemically healthy periodontally diseased individuals had to have at least three teeth with PPD >3 mm in at least two quadrants and radiographic evidence of bone loss. Participants with any metabolic bone diseases (Paget’s disease, hyperparathyroidism, and hypophosphatasia), infectious diseases (tuberculosis, hepatitis, and HIV), autoimmune diseases (rheumatoid arthritis), and diabetes and participants undergoing orthodontic or any antibiotic therapy, participants 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 [Table 1a].

The clinical parameters recorded were plaque index (PI), gingival bleeding index (GBI), and community periodontal index (CPI). The dental fluorosis was assessed using the Jackson’s fluorosis index.

The selected 100 participants were grouped into control and test groups. The control group was further divided into Groups A and B, and the test group was further divided into Groups C, D, E, and F [Table 1b].

In this study, human GCF was collected for the measurement of SA and CS of GAGs by colorimetric analysis of Skoza and Mohos and enzyme-linked immuno sorbent assay kit Kinesis (USA) from Krishgen, Mumbai, India. GCF samples were collected from the three representative pocket sites using paper points and were pooled. After 30 s, the resultant GCF samples were transferred to phosphate buffer solution and stored at −80°C until analyzed.

This study was conducted over a period of 9–12 months. The collected data were subjected to statistical analysis, unpaired t-test of significance to evaluate the results, and the correlation was performed using the Pearson’s correlation test.

RESULTS

The current study included 100 participants which were distributed into two groups of 10 patients each and four groups of 20 patients each.

The results of this study are interpreted in Tables 2-4 and Graphs 1-6.

The demographic data is expressed in Graph 1.

In the fluorosed (F) and nonfluorosed (NF) healthy groups (controls), the plaque and bleeding levels are found to be similar between the groups. The GCF-SA level was higher in nonfluorosed healthy (NFH) (547 ng/ml) than fluorosed healthy (FH) (478 ng/ml). The GCF-CS level was higher in FH (23.6 ng/ml) than NFH (16.76 ng/ml) [Graph 2].

Within the dental fluorosis group, the levels of gingival bleeding levels when compared between the groups, fluorosed gingivitis (FG) (3.9 ± 0.91) showed highly significant values than fluorosis periodontitis group (2.02 ± 0.69) (P = 0.024) [Graph 3].

| Sites | Group  | Inclusion criteria                                                                 | Exclusion criteria                                                                 |
|-------|--------|------------------------------------------------------------------------------------|-----------------------------------------------------------------------------------|
| Control | (A) FH  | Systemically healthy participants presented at least twenty teeth in their mouth with clinically healthy gingiva | Subjects with any metabolic bone diseases (Paget’s disease, hyperparathyroidism, and hypophosphatasia), infectious diseases (tuberculosis, hepatitis, and HIV), autoimmune diseases (rheumatoid arthritis), and diabetes |
|       | (B) NFH | The gingivitis group comprised clinically inflamed gingiva with >30% of gingival bleeding sites without any attachment loss | Patients who had undergone any periodontal treatment 3 months priorly, patients undergoing orthodontics or any antibiotic therapy, patients with other intrinsic dental stains, tetracycline stains or any other dental developmental anomalies such as enamel hypoplasia, amelogenesis imperfecta, and dentinogenesis imperfecta, etc., pregnant or lactating patients, smokers, and alcoholics |
| Test   | (C) FG  | Periodontally diseased individuals had to have at least three teeth with probing pocket depth >3 mm, in at least two quadrants and radiographic evidence of bone loss |                                                                                   |
|       | (D) NFG |                                                                                   |                                                                                   |
|       | (E) FP  |                                                                                   |                                                                                   |
|       | (F) NFP |                                                                                   |                                                                                   |

FH – Fluorosed healthy; NFH – Nonfluorosed healthy; FG – Fluorosed gingivitis; NFG – Nonfluorosed gingivitis; FP – Fluorosed periodontitis; NFP – Nonfluorosed periodontitis

Table 1a: Inclusion and exclusion criteria
Table 1b: Sample distribution

| Patients (100) | Groups | Number of patients |
|---------------|--------|-------------------|
| Control (20)  | (A) FH  | 10                |
|               | (B) NFH | 10                |
| Test (80)     | (C) FG  | 20                |
|               | (D) NFG | 20                |
|               | (E) FP  | 20                |
|               | (F) NFP | 20                |

FH – Fluorosed healthy; NFH – Nonfluorosed healthy; FG – Fluorosed gingivitis; NFG – Nonfluorosed gingivitis; FP – Fluorosed periodontitis; NFP – Nonfluorosed periodontitis; 

Table 2: Correlation of clinical parameters in fluorosed gingivitis and fluorosed periodontitis groups

| Clinical Parameters | GBI | PPD | CAL |
|--------------------|-----|-----|-----|
| PI FG r             | 0.768** | 0.318 | b |
| Significant (two-tailed) | 0.00 HS | 0.171 NS |
| PI FP r             | 0.549*  | 0.222 | 0.202 |
| Significant (two-tailed) | 0.012 HS | 0.347 NS | 0.394 NS |
| GBI FG r            | 0.289 NS | b |
| Significant (two-tailed) | 0.217 NS |
| GBI FP r            | 0.462* | 0.122 |
| Significant (two-tailed) | 0.040 HS | 0.607 NS |

**Correlation is significant at the level ≤ 0.05 (two-tailed). b – Cannot be computed because at least one of the variables is constant; PI – Plaque index; GBI – Gingival bleeding index; PPD – Probing pocket depth; CAL – Clinical attachment loss; r – Pearson correlation; HS – Highly significant; NS – Nonsignificant; FG – Fluorosed gingivitis; FP – Fluorosed periodontitis

Table 3: Correlation of clinical parameters in nonfluorosed gingivitis and nonfluorosed periodontitis groups

| Clinical parameters | PI | GBI | PPD | CAL |
|---------------------|----|-----|-----|-----|
| PI NFG r            | 0.669** | 0.659** | b |
| Significant (two-tailed) | 0.001 HS | 0.002 HS |
| PI NFP r            | 0.636** | 0.587**  | 0.444* |
| Significant (two-tailed) | 0.003 HS | 0.006 HS | 0.050 HS |
| GBI NFG r           | 0.626**  | b |
| Significant (two-tailed) | 0.003 HS |
| GBI NFP r           | 0.677**  | 0.641** |
| Significant (two-tailed) | 0.001 HS | 0.002 HS |

**Correlation is significant at the level ≤ 0.05 (two-tailed). b – Cannot be computed because at least one of the variables is constant; PI – Plaque index; GBI – Gingival bleeding index; PPD – Probing pocket depth; CAL – Clinical attachment loss; r – Pearson correlation; NFG – Nonfluorosed gingivitis; NFP – Nonfluorosed periodontitis; HS – Highly significant

The BI (P = 0.029), CPI–PPD (P = 0.052), and CPI–Clinical attachment level (P = 0) were significantly higher in dental fluorosed periodontitis (FP) patients [Graph 3]. The GCF–SA level was significantly higher (P = 0.003) in FP patients (679 ng/µl) than in FG (496 ng/µl) [Graph 4]. The correlation of plaque levels with gingival bleeding levels was highly correlated (r = 0.768) in FG group.

The correlation of plaque levels with gingival bleeding levels was highly correlated (r = 0.549). However, the plaque levels were not correlated with PPD (r = 0.222) and clinical attachment loss (r = 0.202) in the FP group.

The correlation of gingival bleeding levels with PPD showed highly significant correlation (r = 0.462) in FP group [Table 2].
Within the NF group, the PI ($P = 0.090$), BI ($P = 0.024$), CPI–PPD ($P = 0.023$), and CPI–CAL ($P = 0$) were significantly higher in nonfluorosed periodontitis (NFP) group than nonfluorosed gingivitis (NFG) [Graph 5]. The GCF–SA level in FG patients (695 ng/µl) was significantly higher ($P = 0.040$) than NFP (553 ng/µl) [Graph 6]. The correlation of plaque levels with gingival bleeding levels ($r = 0.669$) and ($r = 0.659$) showed highly significant correlation.

The correlation of plaque levels with gingival bleeding levels ($r = 0.636$), PPD ($r = 0.567$), and clinical attachment loss ($r = 0.444$) showed highly significant correlation in NFP group [Table 3].

The correlation of gingival bleeding levels with PPD ($r = 0.677$) and clinical attachment loss ($r = 0.641$) showed highly significant correlation in NFP group [Table 3].

On comparison of plaque levels between the groups, FG (2.02 ± 0.83) and NFG (2.25 ± 0.55) showed no statistically significant difference ($P = 0.090$).

In the FP and NFP groups, both PI and GBI levels showed nonsignificant values [Graph 9 and Table 4].

**Table 4: Results of percentage of occurrence of community periodontal index - Probing pocket depth scores of 3 (probing pocket depth of 4-5 mm) and 4 (probing pocket depth >6 mm) in community periodontal index - clinical attachment loss score 0 (1-3 mm) and 1 (4-6 mm) in fluorosed periodontitis and nonfluorosed periodontitis groups**

| CPI scores | Groups          | n (%) |
|------------|-----------------|-------|
| CPI score 3 for PPD | FP  | 20 (24.5) |
| CPI score 0 for CAL | NFP | 72 (73.5) |
| CPI score 4 or above for PPD | FP  | 63 (75) |
| CPI score 1 or above for CAL | NFP | 26 (26) |

CPI – Community periodontal index; FP – Fluorosed periodontitis; NFP – Nonfluorosed periodontitis; PPD – Probing pocket depth; CAL – Clinical attachment loss

**Graph 5:** Comparison of plaque index, gingival bleeding index, probing pocket depth, and CAL between nonfluorosed participants with gingivitis (nonfluorosed gingivitis), and nonfluorosed participants with chronic periodontitis (nonfluorosed periodontitis) groups (NFG – Nonfluorosed gingivitis; NFP – Nonfluorosed periodontitis; PI – Plaque Index; GBI – Gingival Bleeding Index; CPI – Community Periodontal Index; PPD – Probing Pocket Depth; CAL – Clinical Attachment Loss; S.D – Standard deviation)

**Graph 6:** Comparison of levels of sialic acid and chondroitin sulfate between the groups FG and fluorosed periodontitis (FG – Fluorosed gingivitis; FP – Fluorosed periodontitis; SA – Sialic acid; CS – Chondroitin sulphate; S.D – Standard deviation)

**Graph 7:** Comparison of plaque index, gingival bleeding index, probing pocket depth, and CAL between fluorosed participants with gingivitis (FG) and nonfluorosed participants with gingivitis (nonfluorosed gingivitis) groups (FG – Fluorosed gingivitis; NFG – Nonfluorosed gingivitis; PI – Plaque index; GBI – Gingival bleeding index; S.D – Standard deviation)
was significantly higher \((P = 0.015)\) in FP \((48 \text{ ng/ml})\) than NFP \((26 \text{ ng/ml})\) [Graph 10].

**DISCUSSION**

The current study objectives were to compare the clinical (plaque, gingival bleeding, PPD, and CAL) and GCF-SA and CS levels in gingivitis and periodontitis patients with and without dental fluorosis for the first time.

The results of the study are discussed as follows:

In the current study, both fluorosed and NF controls showed similar plaque and gingival bleeding levels. However, the SA levels were higher in NFH group; the CS levels were higher in FH group.

The possible reasons for increased CS levels in fluorosed group as compared to NF group could be due to the increased synthesis and deposition of GAG in tooth and bone\(^{[11]}\) and diminished excretion of GAG.\(^{[12]}\)

The possible reason for reduced SA in fluorosis could be due to the inhibition of glycoproteins synthesis by the excess fluoride in fluoride intoxication. The SA has a calcium-binding property which gets deposited in the tissue by calcium bridges and the binding of SA leads to decrease in free SA in GCF. Other reasons are due to the decrease in the biosynthesis of proteins. From the above skeletal fluorosis studies,\(^{[11-13]}\) it appears that reduced SA and increased GAG have followed a similar pattern in the current study on dental fluorosis too.

In the current study, the gingival bleeding levels were highly significant in FP \((P = 0.027)\) than the FG group. The comparison of GCF-SA levels was highly significant \((P = 0.003)\) in FP group as compared to FG group, whereas the CS levels were similar in both the groups \((P = 0.752)\).

Only two studies exist in the literature for the comparison of the clinical parameters between FG and FP groups. As the degree of fluorosis increased, periodontitis also increased when gingivitis showed a decreasing trend.\(^{[14]}\) Vandana and Reddy in 2007 reported that as the degree of fluorosis increased, severity of gingivitis reduced and periodontitis increased.\(^{[15]}\)

The estimation and comparison of biomarkers like SA and CS were done for the first time in FG and periodontitis patients. The GCF-SA levels are influenced by oxidative stress. Hence, the increased oxidative stress in fluorosis participants is discussed here, which may be responsible for GCF-SA levels.

Ghosh et al. reported higher oxidative markers in fluorosed and NFP groups.\(^{[16]}\) A study on skeletal fluorosis and rheumatoid arthritis by Susheela et al.\(^{[17]}\) has reported increased serum levels of GAG and SA due to enhanced GAG destruction caused by increased oxidative burst in fluorosis\(^{[16]}\) and increased SA levels to overcome the oxidative burst serving as an antioxidant.

Excess fluoride in drinking water has an important role in oxidative stress. Going by molecular aspect as well, the excess of fluoride in water not only influence the microbial flora but also...
influence of this possibly altered microbial flora on the already altered/modified oral tissues remains to be studied. Fluorosis does affect the composition of saliva as well as modifying the electrolytes and the antioxidant properties in many ways. Stepko 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 malondialdehyde levels and decrease in superoxide dismutase (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. Wang et al. reported a decrease in antioxidants in patients with skeletal fluorosis.

The possible reason for increased GBI in FG group is speculated to be due to the inflammation caused by fluorosis.

The GCF–SA levels were significantly higher in the NFG group ($P = 0.003$), whereas the GCF CS levels were significantly higher in the FG group ($P = 0.015$).

No studies exist in the literature to compare this result.

The increased SA levels in NFG group need to be further ascertained in a larger sample size, which may be due to the pathogenic mechanism of oxidative burst in these patients.

In the current study, the plaque and bleeding levels were similar in FP and NFP groups ($P = 0.305$ and $P = 0.454$, respectively). The CPI–PPD score was significant in NFP group, and the CPI–CAL score was similar between the FP and NFP groups. The problem with CPI index teeth scoring is that the CPI indexed teeth may or may not be affected by periodontitis while other teeth in the oral cavity would be periodontally affected, thus masking the actual disease state of an individual. Thus, CPI is suitable in a survey than in a clinical observational study as the periodontal status gets underestimated. Hence, the PPD and clinical attachment levels were expressed in the percentages to discuss their occurrence with regard to FP and NFP. From the percentage expression, it was evident that higher PPD–CPI scores of 4 and CAL–CPI score of 1 (75%) was found in FP group when compared to a score of 3 (FP: 24.5% and NFP: 73.5%) of NFP group.

The occurrence of periodontitis in high water fluoride areas has shown a global variation due to the involvement of multiple risk factors in its causation. The fluorosis may play as an environmental factor in causing periodontitis through its effects of hard and soft tissues of the periodontium. As the degree of fluorosis increased, the severity of gingivitis reduced and periodontitis increased.

The GCF–SA and CS were significantly higher in FP group ($P = 0.048$ and $P = 0.015$, respectively) than in NFP group. The effect of fluorosis on CS and SA has been discussed earlier. In FP patients, there is a dual role of fluorosis effect and periodontitis effect on the GCF–SA and CS levels. However, in nonfluorosis cases, only the periodontitis per se will influence GCF SA and CS levels. Regarding the correlation of plaque levels with PPD and CAL, the fluorosis showed a lack of correlation in contrast to NF group, whereas plaque levels significantly correlated with PPD and CAL.

The biochemical parameters such as CS and SA in dental fluorosis serve as two markers and be a good source for the diagnosis of periodontitis in dental fluorosis patients.

In the current study, the clinical parameters such as PI, GBI, PPD, and CAL were compared between fluorosis and nonfluorosis gingivitis and periodontitis groups. The variation in PI and GBI in fluorosed and NF groups was observed due to the host-related response to the main causative factor such as plaque. The consequences of plaque-induced periodontal destruction were common in both fluorosed and NF groups. However, the fluorosis-induced toxic changes coexisted in the fluorosis group exaggerated the clinical and biochemical parameter levels in the current study. The clinically appreciable clinical parameters were enhanced by the estimation of the biochemical parameter in this study i.e., the PPD and CAL.
as measure of periodontal destruction caused due to the plaque and nonplaque induced (fluorosis’ catabolic effect on periodontal tissues) were well supported by the one of the markers of tissue destruction that is CS and host response marker of oxidative burst that is SA levels in dental fluorosed patients.

The effect of fluorosis on periodontal health and disease is scarcely discussed in the literature, and a few reports on this issue are not consistent (institutional study). The comparative and correlative studies of dental fluorosis and nonfluorosis groups using clinical parameters and biochemical parameters have been done for the first time in the literature. The available studies on fluorosed and non fluorosed groups served as a good source of support; the studies on skeletal fluorosis by Susheela et al. were of valuable support, based on which the current study results were discussed. These studies provided the possible reasons for various changes in SA and CS levels in the dental fluorosed periodontitis patients. Hence, Sialic acid and Chondroitin sulphate can serve as a good diagnostic markers in dental fluorosis with and without periodontitis.

An institutional review by Vandana has presented various detrimental effects of fluoride on periodontal structures over the two decades. The need of the hour is to study these fluorosed participants from periodontal perspective using standardized criteria to ascertain, determine, and dissect the role of fluoride in periodontal disease and implement modified treatment measures in FP patients as the treatment outcomes would vary between fluorosed and NF participants.

Further studies can be carried out with large sample size of both fluorosed and non fluorosed participants to ascertain the role of biochemical parameters. Due to self-funding, the site-specific sample was not done. However, the site-specific GCF marker evaluation of SA and CS is a better choice.

CONCLUSION

The comparative and correlative studies of dental fluorosis and nonfluorosis groups using clinical parameters and biochemical parameters (SA and CS) have been done for the first time in the literature. The current study results convey the enhancing role of biochemical parameters such as SA and CS levels in dental fluorosed periodontitis patients. The comparative and correlative studies of dental fluorosis and nonfluorosis groups using clinical parameters and biochemical parameters have been done for the first time in the literature. The available studies on fluorosed and non fluorosed groups served as a good source of support; the studies on skeletal fluorosis by Susheela et al. were of valuable support, based on which the current study results were discussed. These studies provided the possible reasons for various changes in SA and CS levels in the dental fluorosed periodontitis patients. Hence, Sialic acid and Chondroitin sulphate can serve as a good diagnostic markers in dental fluorosis with and without periodontitis.

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Conflicts of interest
There are no conflicts of interest.

REFERENCES

1. Fejerskov O, Larsen MJ, Richards A, Baelum V. Dental tissue effects of fluoride. Adv Dent Res 1994;8:15-31.
2. Vandana KL. Fluorosis and periodontium: A report of our institutional studies. J Int Clin Dent Res Organ 2014;6:7-15.
3. Susheela AK, Das TK, Khurana JS, Jayaswal A, Dave PK. Circulating levels of sialic acid and glycosaminoglycans: A diagnostic test for ankylosing spondylitis. Ann Rheum Dis 1988;47:833-7.
4. Inasu S, Thomas B, Kumari S, Ramesh A, Rao A. Evaluation of serum and salivary sialic acid and nitric oxide levels in chronic periodontitis patients. Int J Appl Dent Sci 2016;2:74-6.
5. Embery G, Waddington R. Gingival crevicular fluid: Biomarkers of periodontal tissue activity. Adv Dent Res 1994;8:329-36.
6. American Academy of Periodontology Task Force Report on the Update to the 1999 Classification of Periodontal Diseases and Conditions. J Periodontol 2015;86:835-8.
7. O’Leary TJ, Drake RB, Naylor JE. The plaque control record. J Periodontol 1972;43:38.
8. Ainamo J, Bay I. Problems and proposals for recording gingivitis and plaque. Int Dent J 1975;25:229-35.
9. Murray JJ. Gingivitis and gingival recession in adults from high-fluoride and low-fluoride areas. Arch Oral Biol 1972;17:1269-77.
10. Tewarson SL, Mittal VP, Singh M, Gupta GP. Serum sialic acid – An important cancer marker. Indian J Cancer 1993;30:125-31.
11. Jha M, Susheela AK. In vivo chondrogenesis and histochemical appearance of dermatan sulphate in rabbit cancellous bone. Differentiation 1982;22:235-6.
12. Jha M, Koacher J, Susheela AK. Urinary excretion of glycosaminoglycans, hydroyxproline and hydroxlysine in rabbits after excessive ingestion of fluoride. Clin Exp Pharmacol Physiol 1983;10:615-9.
13. Miller G, Suzuki N, Ciftci-Yilmaz S, Mittler R. Reactive oxygen species homeostasis and signalling during drought and salinity stresses. Plant Cell Environ 2010;33:453-67.
14. Dalvi PJ, Vandana KL, Ghosh S, Joshi VM, Bhat K, Prakash VH. Fluorosis: Environmental Risk Factor for Periodontal Disease? J Postgraduate Med Educ Res 2017;51:157-61.
15. Vandana KL, Reddy MS. Assessment of periodontal status in dental fluorosis subjects using community periodontal index of treatment needs. Indian J Dent Res 2007;18:67-71.
16. Ghosh S, Vandana KL, Thimmasetty J, Miskin N, Bhat KG, Sharma N. Tinospora cordifolia in the treatment of chronic and aggressive periodontitis patients with and without dental fluorosis: A clinical, microbiological, and biochemical study. Int J Oral Health Sci 2017;7:16-23.
17. Gavriliuk LA, Stepko EA, Spinei IJ, Vartichan AJ, Lysyi LT. Impact of antioxidative therapy on the activity of salivary glutathione-dependent enzymes in patients with fluorosis. Klin Lab Diagn 2007;22:22, 35-7.
18. Wang ZC, Fu D, wang YP, Guan DH, Yan JL, Wu Y. Effect of free radicals on the development of fluorosis and the protective effects by SOD and Vit E. Proceedings of the 1Xth Conference of ISFR, Beijing, China: 1994. p. 145.
19. Rathod SR, Khan F, Kolte AP, Gupta M. Estimation of salivary and serum total sialic Acid levels in periodontal health and disease. J Clin Diagn Res 2014;8:ZC19-21.
20. Last KS, Stanbury JB, Embery G. Glycosaminoglycans in human gingival crevicular fluid as indicators of active periodontal disease. Arch Oral Biol 1985;30:275-81.
21. Khongkhunthian S, Sirimeuang N, Krisanaprakornkit S, Pattanaporn K, Om-Chai S, Kongtawealert P. Raised chondroitin sulphate WF6 epitope levels in gingival crevicular fluid in chronic periodontitis. J Clin Periodontol 2008;35:871-6.