Original Article

Relationship between dental fluorosis, dental caries and salivary levels of *Streptococcus mutans*

Neeharika Ravuru, B. Ajay Reginald, Basireddy Siva Reddy, Meda Samatha

Department of Oral and Maxillofacial Pathology, Narayana Dental College and Hospital, Nellore, Andhra Pradesh, India

Abstract

Background and Objective: Worldwide, dental caries is an endemic infection and a significant public health problem. Fluoride reduces caries by helping to prevent demineralisation, by remineralising early carious lesions, and by decreasing the activity and growth of *Streptococcus mutans (S. mutans)*. Fluoride intake above the optimal levels leads to a condition known as dental fluorosis, which causes hypo-mineralisation of the tooth. Some studies have revealed that the severity of fluorosis is directly proportional to caries, but some showed opposite results. Hence, with these contradicting results, this study was undertaken to identify the relationship between different grades of dental fluorosis, dental caries and the most common cariogenic bacteria *S. mutans* in saliva.

Materials and Methods: A total of 90 subjects within 14- to 16-year age range were selected and categorised depending on the severity of fluorosis into three groups: group I (mild), group II (moderate) and group III (severe) based on modified Dean's fluorosis index criteria (1942). Unstimulated whole saliva samples were collected using the oral rinse technique and processed for quantification of *S. mutans* using Mitis Salivarius Bacitracin Agar medium. The number of colony-forming units (CFUs) was determined using a colony counter and expressed as $\geq 10^2$, $\geq 10^3$, $\geq 10^4$ and $\geq 10^5$ CFUs per ml of the sample, in accordance with the scale recommended by the manufacturer (HiMedia Laboratories). The severity of the caries was measured using decayed, missing or filled teeth (DMFT).

Results: Observations revealed that the overall DMFT was directly proportional to the level of S. *mutans* titres. It was observed that 67 to 73% of participants showed fewer colony counts (10^2 and 10^3) with minimal DMFT scores and 27 to 33% showed higher counts (10^4 and 10^5) with higher DMFT scores in all the groups. 10^5 CFUs of group III showed the highest mean DMFT scores (2.9) with an increased number of involved subjects than group II (2.3) and group I (1.5). In all, male participants had higher bacterial titres and DMFT scores than females.

Conclusion: Fluoride in the drinking water served as anticariogenic agent regardless of the severity of fluorosis. Severe fluorosis with a higher incidence of caries and increased CFUs of S. *mutans* clearly indicates the importance of preventive measures and early treatment to reduce the severity of fluorosis and prevalence of dental caries.

Keywords: Dental caries, dental fluorosis, *Streptococcus mutans*

Address for correspondence: Dr. Basireddy Siva Reddy, Department of Oral and Maxillofacial Pathology, Narayana Dental College and Hospital, Chinthareddypalem, Nellore - 524 003, Andhra Pradesh, India.
E-mail: sivabasireddy13@gmail.com

Submitted: 06-Feb-2023, **Revised:** 03-May-2023, **Accepted:** 19-Jun-2023, **Published:** 12-Sep-2023

Access this article online

Quick Response Code:

Website: https://journals.lww.com/JPAT/

DOI: 10.4103/jomfp.jomfp_59_23

How to cite this article: Ravuru N, Reginald BA, Reddy BS, Samatha M. Relationship between dental fluorosis, dental caries and salivary levels of *Streptococcus mutans*. J Oral Maxillofac Pathol 2023;27:603.

INTRODUCTION

Fluoride, a highly effective anticarious agent, is known for its prevention and control of dental caries. While at the same time endemic fluorosis, which results from high fluoride concentration in groundwater, is a public health problem, the association between fluorosis and dental caries is an extensively researched subject in epidemiological studies. The first studies on fluoride, fluorosis and dental caries have suggested that there is an inverse relationship between fluoride, fluorosis and dental caries.[1] This decline in dental caries with increased fluoride levels is due to its anticarious action that is related mainly to its effects on mineral phases of teeth and on the process of remineralisation that results in the formation of fluorapatite, which is more acid resistant to pH changes induced by bacteria. Fluoride also has essential effects on the bacteria of dental plaque, which are responsible for the acidification of plaque that results in demineralisation.

Hamilton IR[2] (1990) stated that a high fluoride concentration in the oral cavity might inhibit acid production by bacteria and thereby reduce the numbers of certain species. Marquis RE[3] (1995) said that fluoride can affect bacterial metabolism through a set of actions with fundamentally different mechanisms and can act directly as an enzyme inhibitor. In particular, the levels of *Streptococcus mutans* (*S. mutans*) are said to be reduced in high fluoride concentrations, but little is known about the inhibitory effect of fluoride, on *S. mutans*, in varying intensities of dental fluorosis. Hence, this study aimed to estimate the levels of salivary *S. mutans* in individuals with varying severities of dental fluorosis. This intern helps to know the importance of preventive measures and early treatment to reduce the prevalence of dental caries caused by dental fluorosis.

MATERIALS AND METHODS

Source of data

The study population consisted of 90 healthy participants in the age range of 14 to 16 years drawn from a local school, an area with high fluoride concentration in the drinking water. The 90 participants were divided equally into three groups (group I—mild fluorosis, group II—moderate fluorosis and group III—severe fluorosis) based on modified Dean's fluorosis index criteria (1942). The study was approved by the Institutional ethical committee of Narayana Dental College and Hospital, Nellore.

Method of collection of data

The study protocol was explained, and written informed consent was obtained from the parent and school principal. A case history with particular emphasis on age, gender, dental fluorosis and dental caries status was obtained. Dental fluorosis status was determined using Dean's fluorosis modified index, and the severity of the caries was measured using decayed, missing or filled teeth (DMFT).

Sample (saliva) collection

For a minimum of an hour, the participants were instructed not to eat, drink or rinse the mouth before saliva collection. Here, unstimulated whole saliva was collected. Each subject was provided with 10 ml of sterile 0.1 M phosphate buffer saline and instructed to rinse their mouth (oral rinse technique) thoroughly for 60 seconds. The rinse was then retrieved back into the universal container. All samples were properly labelled and transported to the laboratory within 2 hours.

Culture method

The salivary samples were transferred to 15-ml sterile screw-capped conical plastic centrifuge tubes and centrifuged at 1250 rpm for 10 minutes. The supernatant was discarded, and the sediment was re-suspended in 1 ml of 0.1 M phosphate buffer solution and vortexed for 30 seconds. A loop full of the solution was streaked or inoculated onto Mitis Salivarius Bacitracin Agar medium, and the culture was incubated, at 37°C for 48 hours under selective anaerobic condition in a candlelit jar (5% CO_2).

Analysis of culture

After 48 hours, colonies of growth were observed in the culture plates. Gram staining and biochemical tests (catalase, mannitol and sorbitol fermentation tests) were conducted to confirm the presence of *S. mutans*. The number of colony-forming units (CFUs) was determined using a colony counter and expressed as $\geq 10^2$, $\geq 10^3$, $\geq 10^4$ and $\geq 10^5$ CFUs per ml (CFU/ml) of the sample, in accordance with the scale recommended by the manufacturer (HiMedia Laboratories).

RESULTS

The frequency distribution of *S. mutans and DMFT scores* in all three groups (groups I, II and III) is presented in Tables 1–3, respectively. The salivary levels of *S. mutans* of the participants were related to their DMFT scores. Observations revealed that the overall DMFT was directly proportional to the level of S. *mutans* titres.

10^2 and 10^3 CFUs did not show a marked increase in DMFT score in all three groups, whereas a notable change in DMFT scores of 10^4 and 10^5 CFUs, in increasing order from group I to group III, was seen. While 10^5 CFUs of group III showed the highest mean DMFT scores (2.9), the

Table 1: Correlation between dental caries experience and salivary *S. mutans scores* in group I (mild fluorosis)

S. mutans count	Males		Females		Total	
	n=15	DMFT (mean)	*n*=15	DMFT (mean)	*n*=30	DMFT (mean)
10^2	3	0	5	0	8 (26.7%)	0
10^3	7	0.14	7	0	14 (46.7%)	0.07
10^4	2	1	0	0	2 (6.7%)	1
10^5	3	2	3	1	6 (20%)	1.5

Table 2: Correlation between dental caries experience and salivary *S. mutans scores* in group II (moderate fluorosis)

S. mutans count	Males		Females		Total	
	n=15	DMFT (mean)	*n*=15	DMFT (mean)	*n*=30	DMFT (mean)
10^2	3	0	4	0	7 (23%)	0
10^3	7	0.14	8	0.12	15 (50%)	0.13
10^4	1	2	1	1	2 (6.7%)	1.5
10^5	4	2.5	2	2	6 (20%)	2.3

Table 3: Correlation between dental caries experience and salivary *S. mutans scores* in group III (severe fluorosis)

S. mutans count	Males		Females		Total	
	n=15	DMFT (mean)	*n*=15	DMFT (mean)	*n*=30	DMFT (mean)
10^2	1	0	5	0	6 (20%)	0
10^3	8	0.4	6	0.2	14 (46.7%)	0.29
10^4	1	2	1	1	2 (6.7%)	1.5
10^5	5	3.2	3	2.67	8 (46.7%)	2.9

lowest was seen in group I (1.5). Male participants showed higher bacterial titres and DMFT scores than females in all the study groups.

DISCUSSION

Dental caries is one of the most common preventable diseases, which is recognised as the primary cause of oral pain and tooth loss. Though several risk factors have been implicated in the pathogenesis of dental caries, the acid produced from *S. mutans* is said to be a typical primary risk factor responsible for demineralisation in dental caries.[4,5] Many risk modifiers have been discovered to reduce caries activity.[6]

Fluoride is one such widely used risk modifier that has played an essential role in the prevention of dental caries since the introduction of water fluoridation in the 1940s, by forming stronger fluorapatite crystal than the original hydroxyapatite.[7-9] It reduces caries by helping to prevent demineralisation, by remineralising early carious lesions and also by antimicrobial action.[10] Fluoride intake above the optimal levels leads to a condition known as dental fluorosis, which causes hypo-mineralisation of the tooth, rendering both enamel and dentin prone to destruction and plaque accumulation.[1]

Studies on children, in areas with high concentrations of fluoride in drinking water (>1 p.p.m), have revealed a higher prevalence and severity of dental fluorosis and lower caries prevalence.[11-15] While studies have also shown the opposite results, an increase in the severity of dental fluorosis revealed higher occurrence of dental caries.[16]

Hence, with these contradicting results, this study was undertaken to identify the relationship between different grades of dental fluorosis, dental caries and the most common cariogenic bacteria *S. mutans* in saliva.

Studies conducted in areas such as South Africa,[17] Ethiopia,[18] Sudan,[19] Puerto Rico[20] and Saharan region[16] with endemic fluorosis owing to high fluoride levels in drinking water have presented results similar to our findings, where higher the fluorosis higher the DMFT. The possible explanation is that severe dental fluorosis causes pitting of the tooth surface leading to the accumulation of food debris and plaque formation contributing to an increased susceptibility to caries.[21]

It was observed that 67 to 73% of participants in all three groups showed fewer colony counts (10^2 and 10^3) with minimal DMFT scores and only 27 to 33% revealed higher counts (10^4 and 10^5) with higher DMFT scores. This decreased count can be attributed to the antimicrobial effect of fluoride in drinking water irrespective of the severity of fluorosis. Earlier studies[1,3] reveal that fluoride would reduce the activity and growth of *S. mutans* by enolase inhibition and also form more acid-resistant fluorapatite, thereby decreasing the occurrence of dental caries as reflected in our study.

In the present study, 10^5 CFUs of group III showed the highest mean DMFT scores (2.9) with an increased number of involved subjects than group II (2.3) and group I (1.5). Severe fluorosis causes a loss of outer enamel or the formation of pits in teeth.[21] Retention of plaque and food debris in these areas leads to the growth of cariogenic bacteria and contributes to increased susceptibility to caries as reflected in our study. Similarly, the low immune status and adverse hygiene habits of the subjects in mild and moderate fluorosis would have contributed to growing the organisms and further increasing caries as shown in our study.

CONCLUSION

The results of our study clearly state that fluoride in the drinking water showed an anticariogenic effect of up to 75% of the subjects irrespective of the severity of fluorosis

by reducing the CFUs. A certain percentage of subjects showed higher DMFT scores in all the groups. Loss of surface enamel and pitting in severe fluorosis, low immune status and poor oral hygiene in mild and moderate fluorosis could be the cause of higher DMFT scores in these subjects. Hence, our study emphasises the importance of optimal intake of fluoride and defluoridation of water in areas with high fluoride concentration to prevent dental fluorosis. It also highlights the importance of appropriate treatment like resin infiltration, followed by bleaching for mild and moderate fluorosis, crown and laminated veneers for severe fluorosis to reduce the prevalence of dental caries.

Acknowledgement

We acknowledge the assistance of our laboratory technician Mr Padmanabha Reddy.

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

REFERENCES

1. Simone ML, Abreu MH, Vargas AM, Vasconcelos M, Ferreira e Ferreira E, Castilho LS. Dental caries and endemic dental fluorosis in rural communities, Minas Gerais, Brazil. Rev Bras Epidemiol 2013;16:1021-8.
2. Hamilton IR. Biochemical effects of fluoride on oral bacteria. J Dent Res 1990;69:660-7.
3. Marquis RE. Antimicrobial actions of fluoride for oral bacteria – Review. Can J Microbiol 1995;41:955-64.
4. van Ruyven FO, Lingström P, van Houte J, Kent R. Relationship among mutans streptococci, "low-pH" bacteria, and lodophilic polysaccharide-producing bacteria in dental plaque and early enamel caries in humans. J Dent Res 2000;79:778-84.
5. Russell RR. How has genomics altered our view of caries microbiology? Caries Res 2008;42:319-27.
6. García-Godoy F, Hicks MJ. Maintaining the integrity of the enamel surface: The role of dental biofilm, saliva and preventive agents in enamel demineralization and remineralization. J Am Dent Assoc 2008;139(Suppl):25S-34S.
7. CDC. Engineering and administrative recommendations for water fluoridation, 1995. MMWR 1995;44:1-40.
8. Ripa LW. A half-century of community water fluoridation in the United States: Review and commentary. J Public Health Dent 1993;53:17-44.
9. Marinho VC, Higgins JPT, Logan S. Fluoride Toothpastes for Preventing Dental Caries in Children and Adolescents (Cochrane Review); The Cochrane Library, Issue 2. New York: John Wiley and Sons; 2004.
10. Ogaard B. The cariostatic mechanism of fluoride. Compend Contin Educ Dent 1999;20 (1 Suppl):10-7.
11. Heller KE, Eklund SA, Burt BA. Dental caries and dental fluorosis at varying water fluoride concentrations. J Public Health Dent 1997;57:136-43.
12. Whelton H, Crowley E, O'Mullane DM, Donaldson M, Kelleher V, Cronin M. Dental caries and enamel fluorosis among the fluoridated and non-fluoridated populations in the Republic of Ireland in 2002. Community Dent Health 2004;21:37-44.
13. Angelillo IF, Torre I, Nobile CGA, Villari P. Caries and fluorosis prevalence in communities with different concentrations of fluoride in the water. Caries Res 1999;33:114-22.
14. Narbutaite` J, Vehkalahti MM, Milciuviené S. Dental fluorosis and dental caries among 12-yr-old children from highand low-fluoride areas in Lithuania. Eur J Oral Sci 2007;115:137-42.
15. El-Nadeef MA, Honkala E. Fluorosis in relation to fluoride levels in water in Central Nigeria. Community Dent Oral Epidemiol 1998;26:26-30.
16. Almerich-Silla JM, Montiel-Company JM, Ruiz-Miravet A. Caries and dental fluorosis in a western Saharan population of refugee childre. Eur J Oral Sci 2008;116:512-7.
17. Grobler SR, Louw AJ, Van W Kotze TJ. Dental fluorosis and caries experience in relation to three different drinking water fluoride levels in South Africa. Int J Paediatr Dent 2001;11:372-9.
18. Olsson B. Dental findings in high fluoride areas in Ethiopia. Community Dent Oral Epidemiol 1979;7:51-6.
19. Ibrahim YE, Bjorvatn K, Birkeland JM. Caries and dental fluorosis in a 0.25 and a 2.5 ppm fluoride area in Sudan. Int J Paediatr Dent 1997;7:161-6.
20. Elías-Boneta AR, Psoter W, Elías-Viera AE, Jiménez P, Toro C. Relationship between dental caries experience (DMFS) and dental fluorosis in 12-year-old Puerto Ricans. Community Dent Health 2006;23:244-50.
21. Ekanayake L, Van Der Hoek W. Dental caries and development defects of enamel in relation to fluoride levels in drinking water in an arid area of Sri Lanka. Caries Res 2002;36:398-404.