Original Article

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Biochemical Analysis of Normal and Fluorosed Human Permanent Teeth

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Abstract

Aims: The present investigation was carried out to study biochemical analysis on human permanent teeth in Dental Fluorosis, a condition caused by excess intake of fluoride.

Methodology: In present study 45 human fluorosed posterior teeth were obtained from department of oral surgery and clinically classified according to Dean's index into mild, moderate and severe forms. Fifteen posterior normal tooth samples were also collected from patients who opted for denture. The samples were than biochemically analyzed for fluoride and calcium contents by **Ion Meter and Atomic Absorption Spectrophotometer** respectively.

Results: Biochemical analysis showed that increase in fluoride content and decrease in calcium content in fluorosed human teeth when compared to normal.

Conclusion: The present study showed that pitting, perforation and tooth getting fractured (chipped off) in dental fluorosis were mainly due to accumulation of fluoride, depletion of calcium in the matrix.

Keywords: Dental fluorosis; Ion meter; Atomic absorption spectrophotometer.

Introduction

Fluoride is a double – edged sword. Fluoride at optimal level, decreases the incidence of dental caries and is also necessary for maintaining the integrity of oral tissues but at the same time when taken in excess during developmental stages, can cause adverse effects like dental fluorosis and skeletal fluorosis.[1]

Fluorine is a member of the halogen family. It is the most electronegative of all the elements, which makes it extremely reactive. It combines with almost every element and also reacts with organic radicals. It is rarely found in Free State in nature, but is widely distributed in the earth's crust, ranking 17th in abundance (0.06% to 0.09%).[2] The high natural concentration of fluoride in the rocks, irregularly distributed in the world, is responsible for endemic fluorosis. It is from the original igneous rocks that fluoride is released in high amount in hydrogeochemical environment that is in ground waters, sea waters, subsoil waters, surface dusts and soils.[3]

India is one among the 23 nations around the globe where health problems have been reported due to excessive fluoride in drinking water. An estimated 62 million people in India in 17 out of 28 states are affected with dental, skeletal and or non skeletal fluorosis.[3]

In recent years the prevalence of dental and skeletal fluorosis in India is increasing due to, population overgrowth necessitating for more and more water, indiscriminate digging of tube wells leading to more usage of fluoridated water and total unawareness of the importance of water quality assessment and drinking water from any and every source.[4]

In addition to diet, modern sources of ingestion of fluoride included a variety of dental products, some of which have been identified as risk factors for fluorosis. A highly

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significant association was found between the estimated fluoride ingestion from toothpaste and fluorosis. In fluoridated toothpaste users, especially children in the age group 5–10 years and 10–14 years, even after rinsing their teeth satisfactorily with water, fluoride level in circulation was enhanced within a few minutes. [5]

Clinical dental fluorosis is characterized by lusterless, opaque white patches in the enamel, which may become striated, mottled or pitted, or may be stained yellow to dark brown. With the increasing degree of severity the teeth are more likely to be discolored.[6]

As revealed by studies in humans[7,8] and several other mammalian species[9,10], these alternations arise from fluoride effects on both secretary and maturation stages of amelogenesis. Studies on various animal models and in humans support the view that the early maturation stage is the most critical developmental period for dental fluorosis, but sufficiently high concentration of fluoride might effect the enamel at all stages of its formation.[11]

Biochemically calcium content is found depleted in fluorosed teeth and the teeth matrix becomes demineralized. Studies conducted on fluorosed human teeth matrix molecules revealed that one of the sulphated isomers of glycosaminoglycans, i.e. dermatensulphate accumulates as a result of fluoride ingestion and thus results in demineralization of teeth matrix. It is also a fact that the demineralized loci in teeth are unlikely to get remineralized due to the presence of dermatansulphate in the matrix. Embery *et al* as told in the article of sushella, has shown that in rat incisor teeth on exposure to fluoride, dermatansulphate accumulates and provides evidence to suggest that, fluoride ingestion in high amounts causes a major imbalance to the ground substance components of mineralized tissues. In the animal model it has also been reported that long-term fluoride administration leads to structural alteration on the enamel surface and adverse effects on biochemical constituents.[5]

Hence the present study was undertaken to estimation and inter-comparison of fluoride and calcium levels in normal and fluorosed human teeth and intra-comparison of fluoride and calcium levels in fluorosed human teeth according to different grades given by Dean's fluorosis index.

Material and Method

The study group composed a total of 60 posterior human teeth both including premolar and molars, which were extracted for periodontal reasons (without carious), collected from department of oral surgery. They were further categorized in to 45 fluorosed and 15 normal human teeth according to diagnostic criteria and weighing system of Dean's index (1942). However in the present study the questionable category were excluded and very mild and mild category were combined as mild degree of fluorosis, in order to avoid confusion.

Subjects aged 30 to 65 years who had been lifelong residing there and were consumers of moderate- to high-fluoride groundwater (> or = 0.64 ppm) were selected for the study. Detailed visual observations were made of the gross appearance and structure. Detail medical and dental history was recorded and excluded the subjects who were having systemic illness, and carious tooth.

All the human teeth were kept in hydrogen peroxide for 24 hours, washed in 0.1 M phosphate buffer (p^H 7.4) and rinsed with deionized water in order to remove all the external debris and calculus.

The samples were than biochemically analyzed for fluoride and calcium contents by Ion Meter and Atomic Absorption Spectrophotometer respectively. The samples were cut in two equal halves. Only one half were used for biochemical analysis.

The teeth were cut into small pieces and defatted for 3 days with a 12-hourly change of diethyl ether and acetone in the ratio of 1:1 and dried in acetone. Dried defatted pieces of teeth kept in pestle and were ashed for 48 h at 550° C in Muffle Furnace. The ash were weighed and used for estimation of fluoride

and calcium contents and it was statically analysed by ANOVA.

Fluoride Estimation

A known amount of ash from individual normal and fluorosed teeth was digested in 1 ml of 0.25 M HCl and neutralized by 0.125 M NaOH diluted by deionized water . The concentration of fluoride was determined using an Ion Meter. The values are expressed as mg/kg ash wt.

Calcium Estimation

A known amount of ash from individual normal and fluorosed teeth was digested in perchloric acid. After dilution with deionized water, the calcium content was estimated using an Atomic Absorption Spectrophotometer. The values are expressed as mg/g ash wt.

Results

Result showed that the comparison between fluorosed and normal group for calcium and fluoride level were highly significant difference ($p < 0.001$). Calcium level was found to be higher in normal group (mean 448.6) than in fluorosed group (mean 278.8) and fluoride level was found to be higher in fluorosed group (mean 649.4) than in control group (mean 114.9) (Table 1).

The inter-comparison of calcium and fluoride level in mild–moderate fluorosed group showed that the mean value of calcium was greater in mild group (297.533) in comparison of moderate fluorosed group (276.813) and the mean value of fluoride was lower in mild group (mean 549.067) in comparison of moderate group (mean 667.750)

Table 1: Comparison between fluorosed group and normal group for calcium and fluoride levels

Calcium

Group	No.	Mean	SD	SE	t	Df	Sig.
Fluorosis	45	278.867	15.405	2.296	33.708	58	***
Normal	15	448.600	20.880	5.391			

Fluoride

Group	No.	Mean	SD	SE	t	Df	Sig.
Fluorosis	45	649.400	79.304	11.822	25.901	58	***
Normal	15	114.933	8.972	2.317			

Table 2: Intercomparison of calcium and fluoride levels in mild–moderate fluorosed group

Calcium

Degree	No.	Mean	SD	SE	t	Df	Sig.
Mild	15	297.533	3.720	0.960	12.780	29	***
Moderate	15	276.813	5.141	1.285			

Fluoride

Degree	No.	Mean	SD	SE	t	Df	Sig.
Mild	15	549.067	22.908	5.915	15.455	29	***
Moderate	15	667.750	19.821	4.955			

Table 3: Intercomparison of calcium and fluoride levels in mild–severe fluorosed group

Calcium

Degree	No.	Mean	SD	SE	t	Df	Sig.
Mild	15	297.533	3.720	0.960	25.779	27	***
Severe	15	261.214	3.867	1.033			

Fluoride

Degree	No.	Mean	SD	SE	t	Df	Sig.
Mild	15	549.067	22.908	5.915	26.645	27	***
Severe	15	735.929	13.211	3.531			

Table 4: Intercomparison of calcium and fluoride levels in severe–moderate fluorosed group

Calcium

Degree	No.	Mean	SD	SE	t	Df	Sig.
Moderate	15	276.813	5.141	1.285	9.279	28	***
Severe	15	261.214	3.867	1.033			

Fluoride

Degree	No.	Mean	SD	SE	t	Df	Sig.
Moderate	15	667.750	19.821	4.955	10.912	28	***
Severe	15	735.929	13.211	3.531			

(Table 2). In mild –severe fluorosed group mean values of calcium were greater in mild group (297.533) in comparison of severe fluorosed group (261.214) and fluoride was lower in mild group (549.067) in comparison of severe group (735.929) (Table 3). In severe–moderate fluorosed group mean values of calcium were greater in moderate group (276.813) in comparison of severe fluorosed group (261.214) and fluoride were lower in moderate group (667.750) in comparison of severe fluorosed group (735.929) (Table 4).

Discussion

The first systematic description of enamel changes caused by toxic levels of fluoride was given by Black and Mckay who introduced the term "mottled enamel". They described mottled enamel as "characterized by minute white flecks or yellow or brown spots or streaks over the surface of the tooth, or it may be a condition where the entire tooth surface is of a dead paper-white, like the color of a china disk". A special type was noted as having a "pitted" surface structure.[5]

Further they demonstrated the endemic nature of the "disease" by stating that mottled teeth were only observed in definite geographic areas. In addition, he suggested that the disease was related to the water supply in the endemic areas. In order to improve the understanding of relationship between the amount of fluoride ingested and dental fluorosis, Dean (1934 and 1942) classified each person into one of seven categories ranging from "normal" to "severe".[5]

The severity of dental fluorosis depends on when and for how long the overexposure to fluoride occurred, the individual response, weight and degree of physical activity, nutritional factors and bone growth. Other factors that may increase the individual susceptibility to dental fluorosis are altitude, malnutrition and renal insufficiency.[6]

Fluorosed enamel is characterized by retention of amelogenins in the early maturation stage of development and the formation of more porous enamel with a subsurface hypo-mineralisation. Secretory stage of amelogenesis is believed to be more susceptible to acute fluoride exposure. The transition or early maturation stage of enamel formation is the most susceptible to chronic fluoride ingestion above the threshold

levels.[7,8]

In present study human permanent teeth showed an increase in fluoride content and a decrease in calcium content compared to normal permanent teeth.

The elevated fluoride content was found in fluorosed enamel in all stages of enamel development. Due to long-term fluoride administration, marked reduction in calcium content in mature enamel was noted. Surface enamel that exhibits dental fluorosis contains higher concentration of fluoride than unaffected enamel and the fluoride content generally increases with severity of the condition.[9]

It is evident that long-term exposure to fluoride therefore interferes with the process of mineralization and structural alterations of ameloblastic layer results in the retardation of enamel matrix formation and its mineralization. Calcium deficiency and generalized malnutrition disturbs the physiological conditions that affect amelogenesis in humans and can lead to variations in clinical appearance of dental fluorosis at similar levels of fluoride intake. Nevertheless deviations in the natural stages of mineralization of human teeth enamel and the histopathology of fluorosed enamel have led to the hypothesis that dental fluorosis is the result of impairment of enamel mineralization. Unlike normal teeth the fluorosed teeth readily get fractured which suggests, gross structural alterations and decrease in mineral content in the teeth.[3,10]

The present study investigating the biochemical composition of human teeth and has provided evidence to suggest that pitting, perforation, and tooth getting fractured (chipped off) in Dental Fluorosis are mainly due to accumulation of fluoride, depletion of calcium in the matrix.

References

1. Tewari A. Fluorides and Dental caries. *JADA.* 1986; 1: 9-10.
2. Hiremath SS. Textbook of Preventive and Community Dentistry. 1st ed. India: Elsevier Publication; 2007.
3. Chakraborti D, Chanda CR, Samanta G, Chowdhury UK, Mukherjee SC. Fluorosis in Assam, India. *Curr Sci.* 2000; 78(12): 1421-3.
4. Janakiram C, Krishnarao PA. Prevalence of dental fluorosis in rural areas of Davangere, India. *Int Dent J.* 2004; 54: 235-9.
5. Susheela AK, Bhatnagar M, Gnanasundram N, Saraswathy TR. Structural aberrations in fluorosed human teeth: Biochemical and scanning electron microscopic studies. *Curr Sci.* 1999; 77: 1677-80.
6. Berhane Y, Demissie B, Zerihun L, Makonnen E, EsheteB. Preventing Dental Fluorosis. *Ethiop J Health Dev.* 2002; 16(2): 225-6.
7. Fejerskov O, Sliverstone ML, Melsen B, Moller IJ. Histological features of fluorosed human dental enamel. *Caries Res.* 1975; 9: 190-210.
8. Thylstrup A, Fejerskov O. A scanning electron microscopic andmicroradiographic study of pits in fluorosed human enamel. *Scand J Dent Res.* 1979; 87: 105-14.
9. Richard A, Kragstrup J, Josephsen K, Fejerskov O. Dental fluorosis developed in post-secretory enamel. *Scand J Dent Res.* 1986; 65: 1406-09.
10. Kierdorf H, Kierdorf U. Disturbances of the secretory stage of amelogenesis in fluorosed deer teeth: A scanning electron microscopic study. *Cell Tissue Res.* 1997; 289: 125-35.
11. Evans WR, Stamm JW. An epidemiologic estimation of the critical period during which human maxillary central incisor are most susceptible to fluorosis. *J Public Health Dev.* 1991; 51: 251-9.