Chapter 2  

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2.1 Dental fluorosis

Dental fluorosis is a disturbance in the tooth formation caused by the excessive ingestion of fluoride during the formative period of the dentition (62). The dental fluorosis has been classified by World Health Organization under nomenclature of International classification of diseases in the Chapter XI: Diseases of the digestive system, sub-chapter: Diseases of oral cavity, salivary glands and jaws (K00-K14)

(K00.3) Mottled teeth
Dental fluorosis
Mottling of enamel

2.1.1 History of dental fluorosis

The history of dental fluorosis can be divided into three distinct periods. The first period is from 1901-1933 which was concerned with the cause of a developmental enamel defect, described in the United States initially by Frederick McKay and was called “Colorado Brown Stain”. This defect was later identified as “mottled enamel” or more specifically, chronic endemic enamel fluorosis (63). The second period from 1933-1945, encompassing, the classical epidemiological studies of H T Dean, focused on the relationship between naturally occurring fluoride concentration in drinking water, enamel fluorosis and dental caries. Later, on considering the preventive benefits achieved by fluoride and risks of dental fluorosis, the limits of optimal fluoridation were set between 0.7 and 1.2 ppm fluoride in drinking. The third period, designated as the “moment of truth in fluoridation history” by Frank McClure began on January 25th, 1945, till
date, when Grand Rapids, Michigan, USA, became the first city in the world to adjust its water fluoride concentration to a level expected to promote dental health (63).

2.1.2 MECHANISM OF DENTAL FLUOROSIS

It has been established that the hypo mineralized alterations of fluorotic enamel are not due to general effects of fluoride on the calcium metabolism, or due to the poisoning effects that depress the fluoride metabolism, but are primarily due to in-situ effects of the fluoride in the local environment (tooth bud). The fluoresced enamel retains a relatively high proportion of immature matrix proteins (high proline contents) (3, 4). An incomplete removal of amelogenin proteins under influence of fluoride during tooth development leads to fluorosis. Whitford (64-70) stated that "although several other fluoride-induced effects might be involved in the etiology of fluorosis, it now appears that inhibition of enzymatic degradation of amelogenins, which delays, their removal from the developing enamel, is the most accepted reason.

Proposed molecular mechanism involved in formation of fluorotic enamel

Effect of fluoride on enamel crystals

Enamel apatite is primarily consists of hydroxyapatite crystals, which is formed from the reaction.

\[
\text{Ca}_{10} (\text{PO}_4) _6(\text{OH})_2 + 10 \text{Ca}^{++} + 6\text{PO}_4^{--} + 2\text{OH} = 2 \{\text{Ca}_{10} (\text{PO}_4) _6(\text{OH})_2\}
\]

In the presence of elevated levels of fluoride ions in the forming of enamel, a significant amount of hydroxyapatite is converted to fluoroapatite. The reaction
releases hydroxyl group, which could limit the pH drop, accompanied by rapid crystal growth (64-70).

\[ 2\text{NaF} + \text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2 = \text{Ca}_2(\text{PO}_4)_6(\text{F})_2 + 2\text{OH}^- + 2\text{Na}^+ \]

This change in pH could cause amelogenins to aggregate and prevents the diffusion of the protein out of the maturing enamel (enamel proteins are more soluble in acidic conditions but form insoluble aggregates in neutral solutions). The second effect of fluoride on enamel is the promotion of hydroxyapatite crystal growth having more thickness than height to trap matrix protein and hence prevent their efficient removal during maturation (64-70).

Figure 2.1 Schematic illustrations of the events relevant to early enamel mineralization. (70)
Effect of fluoride on enamel proteinases

In mature enamel, crystals replace the enamel matrix. Proteolytic enzymes are believed to be important in removal of enamel matrix protein. Enzymes such as metallo and serine proteinases are responsible for degradation of enamel proteins. Fluoride inhibits these enzymes, which are active only in mature enamel.

Effect of fluoride on ameloblast and its metabolism

The significant proportions of enamel proteins are removed by endocytosis involving lysosomal enzyme. These enzymes (dipeptidase) are inhibited by fluoride, thereby causing the amelogenins to accumulate in the extracellular spaces of enamel matrix. Fluoride also acts by stimulating the secreting ameloblast, resulting in an excess production of enamel matrix protein, which is then retained in the mature enamel.

2.1.3 Clinical Features of Dental fluorosis

The most critical period for the development of fluorosis in permanent dentition is during the latter stages of pre-eruptive tooth development. Ishil and Suckling (9) have observed that the critical exposure period for the development of permanent dentition is between 11 months and seven years of age. Excessive ingestion of fluoride after seven years will not cause dental fluorosis.

Fluoride has been assigned as the single most factor for causing enamel mottling (71). Thus, the differential diagnosis of enamel fluorosis from non-fluorotic enamel defects is critical for accurate assessment of the prevalence of dental fluorosis (72-76). The clinical appearance of milder forms of the enamel fluorosis is characterized by narrow white lines following the perikymata, cuspal snow-
capping, and a snow flaking appearance that lack a clear border with unaffected enamel (73, 77).

The corresponding enamel lesion is featured histopathologically as a subsurface hypo mineralized lesion covered by a well-mineralized outer enamel layer (78-79). Electron microscopy further confirmed that the structural arrangement of the crystals appears normal; however widening gaps between the enamel rods and enlarged inter-crystalline spaces are noticed, leading to porosity in the enamel structure (78).

With increasing severity, the subsurface enamel all along the tooth becomes more porous. As the fluoride content increases the lesion extends towards the inner enamel. After eruption, the opaque areas may become stained yellow to dark brown, and the more severe forms are subjected to extensive mechanical breakdown (attrition of the surface) (80-82). It is now widely accepted that pitting and larger surface destructions of enamel, are post-eruptive features, and not true hypoplasia of the teeth (75, 77, 83-87).

It has been shown that there are individual variations in fluorosis manifestation for similar water fluoride intake between and within the populations (88). The manifestations of dental fluorosis depend upon the amount ingested, the duration of exposure and the age of the subject (62, 88). The teeth which form and mineralize early in life are those that are least affected especially mandibular incisors, permanent first molars and deciduous teeth, whereas the teeth which mineralize later in life are the most severely affected.
Post eruptive changes of fluorosis

In milder forms of dental fluorosis, mechanical attrition will occur over time which causes an apparent “remissions” of the lesions. These lesions occur most likely, due to the surface enamel demineralization (89). This may give the impression that incisal area is more affected than the remaining surfaces, which in reality is equally porous. Hence, this incisal/cuspal tip part is more susceptible to attrition and has a strong tendency to absorb stains (90). The uptake of stain will depend on the degree of porosity and this discoloration in itself, is not an appropriate measure of severity. This post eruptive damage may increase over the time depending on the degree of severity at the time of eruption.

2.1.4 Classification of Dental Fluorosis

Dean (91) described the grades of endemic mottled enamel, which were associated with varying levels of fluoride in the drinking water. The classification was based upon the degree of severity of the defects.
1. Normal enamel: The enamel is translucent, smooth and presents a glossy appearance.

2. Questionable mottling: Normal enamel, but translucency is varied by a few white flecks or white spots.

3. Very mild mottling: Small, opaque, paper-white areas are scattered over the teeth, involving less than 25 percent of the tooth surface. Summits of the cusps of bicuspids and second molars are commonly affected.

4. Mild mottling: The white opaque areas are more extensive but do not involve more than 50 percent of the surface and faint brown stains are sometimes apparent.

5. Moderate mottling: All enamel surfaces are affected and those which are subjected to attrition, show marked wear. Brown stain is a frequent disfiguring feature.

6. Severe mottling: Severe hypoplasia affects the form of the tooth. Stains are wide spread and vary in intensity from deep brown to black and the teeth often present a corroded appearance.

Figure 2.2 Different grades of dental fluorosis
2.1.5 ENDEMIC AREAS OF WORLD

Endemic dental fluorosis is prevalent in areas where the drinking water contains elevated levels of fluoride, seen in most parts of Africa and Asia (92). It is extremely difficult to state exactly how many people are affected, but an approximate estimate would be about 100 million, worldwide. However, concentrations as high as 95 ppm have been recorded in Tanzania and the highest natural fluoride concentration in water ever found was in Lake Nakuru in the Rift Valley in Kenya at 2800 ppm. The soil at the Lake shore contained up to 5600 ppm and the dust in the huts of local inhabitants contained 150 ppm.

Figure 2.3 World map showing endemic areas of fluorosis (92)

From the original igneous rocks, fluoride is leached in high amount in ground water and soils. The fluoride finds its way to the human body through drinking water, cereals and vegetables. The geographical endemic belt extends from :- (5-6)

- Turkey via Syria, Jordan, Egypt, Libya, Algeria to Morocco and from Egypt and Sudan through the Rift valley to Kenya, Tanzania, Mozambique and South Africa.
- Another belt is the one stretching from Turkey through Iran, Iraq, and Afghanistan to India, Northern Thailand, China and Japan.
In the Americas, a similar belt stretches from USA (Texas, New Mexico), Mexico and Central America along the foothills of the Andes to Northern Chile and Argentina.

### 2.2 Diagnosis of Dental Fluorosis

#### 2.2.1 Indices used to diagnose dental fluorosis

Fluoride opacities are distinct and there is little epidemiologic evidence that opacity characteristic of dental fluorosis, can be caused by other factors. Trace elements other than fluoride, like strontium (93) and zinc (94) have shown an association with fluorosis like opacities, but these associations were found to be weak (87).

The accuracy of fluorosis diagnosis may be as high as 95%, for the experienced examiners using proper attention to examination method, and the use of differential diagnostic criteria. Two distinct groups of indexes have been proposed for measuring dental fluorosis (87).

a) Specific fluorosis indexes - specifically measures the fluoride induced enamel changes in order to reflect increasing severity of fluorosis of lesions (79, 89).

b) Descriptive indexes - including all types of defects. These indexes includes all defects of enamel are recorded based solely on descriptive criteria, regardless of causative factors. It is based on the principle that examiner should record what he sees and do not presume the etiology (93).
2.2.2 Dean’s Index

Dean (91) provided a standard classification system for clinical conditions as described by McKay on 2000 subjects in endemic areas of six states in USA (Table 2.1). The “moderately severe” and the “severe” categories were combined into a single “severe” category providing the six-point measurement ordinal scale (95).

Table 2.1 Diagnostic criteria and weighting system for Dean’s Index (91)

<table>
<thead>
<tr>
<th>CLASSIFICATION AND WEIGHT</th>
<th>ORIGINAL CRITERIA (DEAN, 1934)</th>
<th>MODIFIED CRITERIA (DEAN, 1942)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>The enamel presents the usual translucent semi-vitriform type of structure. The surface is</td>
<td>The enamel presents the usual translucent semi-vitriform type of structure. The surface is</td>
</tr>
<tr>
<td></td>
<td>smooth and glossy and usually of a pale creamy white color.</td>
<td>smooth, glossy and usually of a pale creamy white color.</td>
</tr>
<tr>
<td>Questionable</td>
<td>Slight aberrations in the translucency of normal enamel, ranging from a few white flecks to</td>
<td>The enamel discloses slight aberrations from the translucency of normal enamel, ranging from</td>
</tr>
<tr>
<td>0.5</td>
<td>occasional white spots, 1 to 2 mm in diameter</td>
<td>a few white flecks to occasional white spots. This classification is utilized in those instances</td>
</tr>
<tr>
<td>Very Mild</td>
<td>Small, opaque, paper-white areas are scattered irregularly or streaked over the tooth surface.</td>
<td>Small, opaque, paper-white areas scattered irregularly over the tooth but not involving as</td>
</tr>
<tr>
<td>1.0</td>
<td>It is principally observed on the labial and buccal surfaces, and involves less than 25% of</td>
<td>much as approximately 25% of the tooth surface. Frequently included in this classification are</td>
</tr>
<tr>
<td></td>
<td>the tooth surfaces of the particular teeth affected. Small pitted white areas are frequently</td>
<td>teeth showing no more than about 1-2 mm of white opacity at the tips of the summits of the</td>
</tr>
<tr>
<td></td>
<td>found on the summits of the cusps. No brown stain is present in the mottled enamel of this</td>
<td>cusps of the bicuspid or second molars.</td>
</tr>
<tr>
<td></td>
<td>classification.</td>
<td></td>
</tr>
<tr>
<td>Mild</td>
<td>2.0</td>
<td>The white, opaque areas on the surfaces of the teeth involve at least half of the tooth surface. The surfaces of molars, bicuspid, and cuspids subject to attrition show thin white layers worn off and the bluish shades of underlying normal enamel. Faint brown stains are sometimes apparent, generally on the upper incisors.</td>
</tr>
<tr>
<td>------</td>
<td>-----</td>
<td>--------------------------------------------------------------------------------------------------------------------------------------------------</td>
</tr>
<tr>
<td>Moderate</td>
<td>3.0</td>
<td>No change is observed in the form of the tooth, but generally all of the tooth surfaces are involved. Surfaces subject to attrition are definitely marked. Minute pitting is often present, generally on the labial and buccal surfaces. Brown stain is frequently a disfiguring complication. It must be remembered that the incidence of brown stain varies greatly in different endemic areas, and many cases of white opaque mottled enamel, without brown stain, are classified as “moderate” and listed in this category.</td>
</tr>
<tr>
<td>Moderately Severe</td>
<td></td>
<td>Macroscopically, a greater depth of enamel appears to be involved. A smoky white appearance is often noted. Pitting is more frequent and generally observed on all the tooth surfaces. Brown stain, if present, is generally deeper in hue and involves more of the affected tooth surfaces.</td>
</tr>
<tr>
<td>Severe</td>
<td>4.0</td>
<td>The hypoplasia is so marked that the form of the teeth is at times affected, the condition often being manifest in older children as a mild pathologic incisal occlusal abrasion. The pits are deeper and often confluent. Stains are widespread and range from a chocolate brown to almost black in some cases.</td>
</tr>
</tbody>
</table>

**Shortcoming of Dean’s Index**

- Single score is given to a tooth rather than, a separate score to each tooth surface. Hence differences in the severity of fluorosis in different tooth surfaces cannot be ascertained.
- An individual has been classified according to the tooth most affected by fluorosis which may be located in the mouth that has little cosmetic value.
- Questionable diagnostic category (score 0.5) in Dean’s Index is difficult to define and interpret precisely.
- The distinctions between some of the diagnostic categories in Dean’s system are unclear, imprecise or lack sensitivity.

2.2.3 Thylstrup and Fejerskov Index (TFI)

The Thylstrup and Fejerskov developed an index in order to refine, modify, and extend the original concepts established by Dean (91). The primary aim was to develop a more sensitive classification system for recording enamel changes associated, with increasing level of fluoride in water. The basis of TFI is the classification scale, closely to the histological changes that occur (79) and fluoride content found in the enamel (81). A 10-point ordinal scale is used to classify enamel changes associated with increasing fluoride exposure (Table 2.2). As originally proposed, facial and occlusal surfaces were scored with different criteria. From 1988, onwards the scoring of facial surface was recommended for TFI.

Enamel stains are ignored in assignment of scores throughout the entire scale. Teeth are to be cleaned and dried before examination. The histological and clinical basis is used for scoring fluorosis with this index, have clarified the way in which, fluorosis is distributed over the tooth surface. This scoring also estimates the continuous exposure of fluoride during tooth development and loss of enamel in fluorosis. The approach used in formulating scoring criteria has little scope for subjectivity. Further, cleaning and drying of teeth accentuates the appearance of
fluorotic change, making diagnosis easier in questionable cases. Using TFI, the following methods are used to provide prevalence and severity estimates:

1. Percent distributions of scores for all teeth
2. Percent distributions of scores by tooth type
3. Cumulative percent distributions of subjects indicated by percent of teeth affected at a given TFI score (or greater) per subject.

Table 2.2 Diagnostic criteria and weighing system for T F Index

<table>
<thead>
<tr>
<th>Score</th>
<th>Score Original Criteria (Thylstrup and Fejerskov, 1978)</th>
<th>Modified Criteria (Fejerskov et al. 1988)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>Normal translucency of enamel remains after Prolonged air-drying.</td>
<td>The normal translucency of the glossy, creamy-white enamel remains after wiping and drying of the surface.</td>
</tr>
<tr>
<td>1</td>
<td>Narrow white lines located corresponding to the perikymata.</td>
<td>Thin white opaque lines are seen running across the tooth surface. The lines correspond to the position of the perikymata. In some cases, a slight &quot;Snowcapping&quot; of cusps/incisal edges may also be seen.</td>
</tr>
<tr>
<td>2</td>
<td><strong>Smooth surfaces</strong>&lt;br&gt;More pronounced lines of opacity which follow the perikymata. Occasionally confluence of adjacent lines. <strong>Occlusal surfaces</strong>&lt;br&gt;Scattered areas of opacity &lt; 2 mm in diameter and pronounced opacity of cuspal ridges.</td>
<td>The opaque white lines are more pronounced and frequently merge to form small cloudy areas scattered over the whole surface. &quot;Snowcapping&quot; of incisal edges and cusp tips is common.</td>
</tr>
<tr>
<td>3</td>
<td><strong>Smooth surfaces</strong>&lt;br&gt;Merging and irregular cloudy areas of opacity. Accentuated drawing of perikymata often visible between opacities. <strong>Occlusal surfaces</strong>&lt;br&gt;Confluent areas of marked opacity worn areas appear almost normal but usually circumscribed by a rim of opaque enamel.</td>
<td>Merging of the white lines occurs, and cloudy areas of opacity occur spread over many parts of the surface. In between the cloudy areas, white lines can also be seen.</td>
</tr>
<tr>
<td>4</td>
<td><strong>Smooth surfaces</strong>&lt;br&gt;The entire surface exhibits marked opacity or appears chalky white. Parts of surface</td>
<td>The entire surface exhibits a marked opacity or appears chalky white. Parts of the surface exposed to attrition or wear</td>
</tr>
</tbody>
</table>
Critics of TFI

The TFI has received widespread use for both descriptive and analytical studies, with 18 studies (77, 81, 80, 96-98) (more than 30 different populations and 275 subjects per study), reporting percent agreement scores for examiner reliability were in the range from 65% to 100%. The prevalence and severity
estimates are generally reported at the individual or tooth level, typically, a frequency distribution of scores (82, 98-99).

The TFI seems more appropriate than Dean’s Index for use in clinical trials or analytical epidemiologic studies, primarily because teeth are dried and fluorosis can be identified in its milder forms. The resulting increased sensitivity provides statistical and practical advantages from the possible detection of effects with smaller samples. This feature of the TFI is a particular advantage when potential fluoride effects are small, or when the exposure may be widespread.

Granath et al (99) on comparing the Dean and TF indexes, concluded that the latter was more detailed and sensitive, because it was based on biological aspects. Thylstrup and Fejerskov (80) considered that the Dean’s index was not capable of distinguishing the different aspects of the severity of dental fluorosis in areas of high fluoride concentrations in the water (above three ppm) and presented difficulties in evaluating questionable and very mild categories (100-103).

2.3 Magnitude of fluorosis in India and Karnataka

Fluorosis history in India

Fluorosis has been prevalent in India for seven decades. It was first noticed in early 1930s, among cattles by the farmers of Andhra Pradesh. They noticed inability of the bullocks to walk due to painful and stiff joints (5, 6). The episode was repeated within six months when new pairs of bullocks were acquired. It was during the year later, the same disease was detected in human beings and Short et al published the first report on endemic fluorosis in India. During the period from 1960 to 1986, nine states in India had been identified as endemic fluorosis areas.
Presently, the 17 states India have been classified as endemic fluorosis areas. The abnormal high natural concentration of fluoride bearing minerals are found in the rocks which are irregularly distributed in India, is responsible for endemic fluorosis (5-6). These natural fluoride bearing minerals identified in India are:

A) Fluoride: Fluoride (CaF$_2$) and Cryolite (Na$_3$AlF$_6$)
B) Phosphates: Fluorapatite Ca$_5$(PO$_4$)$_3$F
C) Silicates: Topaz Al (F.OH) 2 SiO$_4$
D) Mica Group: Magnesium mica and lithium mica

Figure 2.4 Fluorosis statuses in India (5,6)
Endemic areas in Karnataka

The Rajiv Gandhi National Drinking Water Mission (RGNDWM) (5, 6) survey identified many parts of Karnataka as fluoride affected areas. The population in over eight districts continues to drink water with excess fluoride. Many parts of the state reportedly have alarmingly high levels of fluoride ranging from 1.3 to 8 ppm (5, 6). According to studies conducted by (D.R.D.W.S.S.P) DANIDA Assisted Rural Drinking Water Supply Sanitation Programme and Central Ground Water Board (CGWB), have identified Dharwad, Kolar, Raichur, Gulbarga, Davangere, Chitradurga, Gadag, Bagalkot and Bijapur in North Karnataka to be endemic fluoride areas (5, 6).
2.4 Studies on Dental Fluorosis and Fluoride in Drinking Water

Dean (104) conducted an investigation on caries and dental fluorosis on 7,257, 12 to 14 year children from 21 cities in four states. This study showed with startling clarity, the association between increasing fluoride concentration in the drinking water and decreasing caries experience in the population. They stated that, near maximal reduction in caries experience occurred with a concentration of 1 ppm fluoride in the drinking water. At this concentration, fluoride caused only sporadic instances of the mildest forms of dental fluorosis of no aesthetic significance. They further stated that between 0.5 and 1 ppm fluoride levels, five to 10 percent of the children showed mild fluorosis, characterized by small opaque white areas scattered irregularly over the tooth. With intake of two ppm, about 10 percent were graded moderate dental fluorosis in which, the whole enamel surface showed either the opaqueness or the brown stain. Fewer than 25 percent of the teeth were free from some defect. With four ppm, only five percent of the teeth were normal and about 25 percent showed moderate fluorosis. About 12 percent were graded “severe”, in which all the enamel surfaces were affected either by opacity or brown stain, in addition to pitting. At about six ppm, no teeth were free of dental fluorosis and above 50 percent showed severe response. Higher levels upto 14 ppm did not make the condition appreciably more than what was observed at six ppm.

Nanda (105) conducted an investigation to evaluate the prevalence of dental fluorosis in Lucknow, India, and the factors responsible for the unusually high degrees of fluorosis. 16,565 school children in the age group of six to 17 years from Lucknow city and from 23 villages of Lucknow district were examined for
dental fluorosis using Dean’s Index (1942). The fluoride concentration in drinking water was in the range of 0.0 to 1.21 ppm and 54% showed no fluorosis, 28% had questionable fluorosis, 18% had definite fluorosis. In 0.4 to 0.8 ppm of fluoride definite fluorosis was seen in 24% of children. To determine the factors responsible for the unusually high degree of fluorosis a complete dietary history on 444 children were collected at three distinct times during the year. Drinking water was the principle source of fluoride intake and constituted for more than half of total fluoride, as compared to ingested foods and other fluids. They concluded that high intake of fluoride and deficient nutrition plays the most important role in the unusually high degree of dental fluorosis in areas within low concentration of fluoride in the drinking water.

**Chandra** (106) conducted a study to determine optimum fluoride concentration in drinking water in fluorotic zone of western India. The area under study was divided into four parts depending upon the fluoride level in drinking water 0 to 1 ppm, 1.1 to 5 ppm, 5.1 to 10 ppm and 10.1 and above. 1320 school children in the age group of 15-19 years were examined for dental fluorosis using Dean’s Index (1942). They found significant increase in the prevalence of dental fluorosis from 3.8% to 65.4%, with the rise in fluoride level in drinking water. A significant positive correlation was found between fluoride in drinking water and Community Fluorosis Index (CFI). They concluded that 0.8 ppm would be optimum for the fluoride zone of Western India.

**Subba Reddy and Tewari** (107) investigated enamel mottling at various levels of fluoride drinking water in an endemic area in Bathinda district Punjab, India. 1759 school children in the age group of 12-17 years, who are permanent
residents, were examined for dental fluorosis by Dean’s index. The fluoride level in drinking water in six selected areas were (Manimajra) 0.30 ppm, (Sardulgarh) 1.70 ppm, (Budhlad) 2ppm, (Jhandekalan) 3.40 ppm, (Mehraj) 5.40 ppm and Bhikhi (10.40 ppm). In 0.30 ppm fluoride area, not even a single child was affected with enamel mottling. In 1.10 ppm fluoride area, 85% children were affected with enamel mottling. Out of these 24% showed questionable mottling, 31% had very mild mottling, 22% had mild mottling, and 6.19% had moderate mottling. They concluded that for Indian conditions around 0.80 ppm to 1 ppm of fluoride in drinking water may be optimum.

Brouwer (108) conducted a survey to know the prevalence of dental fluorosis among children aged 7-16 years living in regions of Senegal where fluoride concentrations in the drinking water ranged from 0.1 to 7.4 ppm. Milder forms of dental fluorosis were found, the prevalence being 68.5%. In areas where fluoride concentrations exceeded four ppm, the prevalence of dental fluorosis reached 100%. High sweat loss and a high intake of water because of the hot weather may account for the higher prevalence of fluorosis. They concluded that the World Health Organization guideline for the upper limit of fluoride level in drinking water may be unsuitable for countries with a hot, dry climate.
2.4.1 Studies on Dental fluorosis prevalence in low fluoride levels

Ray (109) studied prevalence of dental fluorosis in relation to fluoride concentration of the drinking water in two adjacent villages of Varanasi district, India. In one of the villages, the prevalence of dental fluorosis was 24.33% where fluoride concentration in drinking water was in range of 0.4 to 2.1 ppm. In another village, the prevalence rate was 29.91% where fluoride concentration was between 0.4 to 0.9 ppm. But in a village where the fluoride level was 0.2 to 0.3 ppm, higher prevalence of 35.5% was observed. They concluded that the cause for higher prevalence of dental fluorosis may be due to other fluoride sources.

Manji (110) studied the prevalence of dental fluorosis in an area of Kenya with 2 ppm fluoride in drinking water. 102 school children in the age group 10-15 years were examined for dental fluorosis using Thylstrup and Fejerskov (1978) index (TFI). The prevalence was 100%, 92% of all teeth exhibited a TFI score of four or higher and 50% of the children had pitting or more severe damage. The fluorotic changes showed a high degree of bilateral symmetry, and they concluded that unexpected high prevalence and severity of fluorosis may be due to unknown variables.

Baelum (80) conducted a study to assess enamel changes in two low fluoride areas of Kenya. 317 school children in the age group of 11-15 years in two rural areas of Machokos district Kenya were examined for fluorosis, using Thylstrup and Fejerskov Index (1978). The children were selected from two areas having
fluoride concentration in drinking water: area A = 0.10 to 0.45 ppm, and area B = 0.54 to 0.93 ppm. The prevalence of dental fluorosis among children was 78% and 91.2% was in these areas A and B respectively. The prevalence and severity of enamel changes of each tooth type increased from the lowest to the highest fluoride areas. They concluded that increasing prevalence and severity of dental fluorosis in low fluoride area needed to evaluate relationship between dental fluorosis, cultural habits, living conditions and other relevant factors to fully elucidate the biological effect on the human body.

Sampaio (111) conducted a study to know the prevalence of dental fluorosis in areas with fluoride in the drinking water in Paraiba, Brazil and its relation to nutritional status. 650 lifelong residents (6-11 years old) were examined for dental fluorosis and nutritional status (height for age index WHO Index) was assessed. The sample was divided into three groups according to fluoride levels in the drinking water: low 0.7 ppm (n = 164), medium between 0.7 to 1.0 ppm, n = 360) and high (above 1 ppm (n = 126). Dental fluorosis was observed in 30.5%, 61.5% and 71.4% of the children in these three groups respectively. The prevalence of dental fluorosis was significantly related to water fluoride concentration. They concluded that malnutrition was prevalent in 20% of school children but was unrelated to dental fluorosis.

Mabeya (112) aimed at studying the significance of “Magadi” a type of salt used to tenderize the vegetables as determinant for dental fluorosis. He examined school children 12-17 yrs of 18 villages with fluoride concentration in drinking water at about 0.2-0.8 ppm range. The prevalence of dental fluorosis in
non fluoride village varied from 7% to 46%. In contrast, in Magadi consumed villages at inland altitude, the prevalence of fluorosis was 53% to 100% and 18% to 97% had severe pitting. The villages with highest fluoride content in Magadi samples showed highest level of fluorosis. This provides strong evidence that consumption of Magadi was the major determinant of the observed high prevalence and severity of fluorosis in inland village at 1500 m altitudes. Hence in studies on dental fluorosis, careful attention should be paid to dietary habits and local customs in the preparation of food in order to identify possible additional fluoride sources.

Cao J, Zhao Y, Liu J (15) Dietary fluoride intake and the prevalence of dental fluorosis were investigated in children from three population groups (Mongol, Kazak and Yugu) in Gansu Province, China. The concentration of fluoride in drinking water ranged from 0.11 to 0.32 ppm. There was a high prevalence of dental fluorosis- 52%, 84% and 76% among the Mongol, Kazak and Yugu children respectively. Dental fluorosis was particularly severe among the Kazak population (severity index: 2.00, 3.05 and 2.57 among the three populations, respectively). Each of the population groups had a long tradition of drinking milk tea made from brick tea water. This milk tea was found to contain high concentrations of fluoride (2.58-3.69 ppm). The daily fluoride consumption was 1.36-2.42-times the US RDA of 2.5 mg for children. Regression analysis showed that fluorosis was significantly correlated with the consumption of milk tea made from brick tea water, but not with any other dietary component (including milk).
2.5 Dental Fluorosis and Urinary Fluoride Levels

*W Czarnowski and J Krechniaka* (113) conducted a study to determine the concentration of fluoride in urine of 1240 children (635 boys, 605 girls), aged 7-14 years, living in Gdańsk, Poland, and to examine whether any correlation exists between age, gender, school location, and fluoride level in the urine. The mean urinary fluoride concentration in children attending two schools near a fluoride-bearing phosphate fertilizer waste disposal site with 1.0 - 1.5 ppm in the drinking water, was 2.16 ± 1.14 ppm. At other three schools near a phosphate fertilizer plant, with 0.2 - 0.5 ppm in the drinking water, the mean urinary fluoride concentration was 1.05 ± 0.49 ppm. In the first two schools the urinary fluoride concentration in boys was significantly higher than in girls. No age-dependent differences were found in children in any of the schools.

*Yadav JP and Lata S* (114) A study was conducted in the Jhajjar, Haryana, India, to assess the fluoride excretion in the population exposed to fluoride in drinking water and to correlate with dental fluorosis. The mean fluoride concentration in drinking water samples of Bahadurgarh, Beri, Jhajjar, Matanhail and Sahalawas blocks of Jhajjar district were 2.05 ppm, 2.14 ppm, 2.05 ppm, 2.14 ppm and 1.93 ppm respectively. The mean urinary fluoride concentration was 1.58 ppm in Bahadurgarh, 1.48 ppm in Beri, 1.50 ppm in Jhajjar, 1.51 ppm in Matanhail and 1.56 ppm in Sahalawas. The mean prevalence of fluorosis on the basis of stage of dental fluorosis was highest for, yellowish brown type. On the basis of TSIF score, score 4 type fluorosis was highest in most of the blocks of Jhajjar district. The
study revealed that more than 50% of the individuals were found to be affected with fluorosis in this district.

S Ramsubhag RS Naidu D Narinesingh S Teelucksingh (115) conducted a study to determine urinary fluoride levels in school children in a non-fluoridated area in Trinidad and Tobago. Morning urine samples were collected from 750 children aged 5 to 14 years, attending a primary school in the area of St Joseph, North West Trinidad. Overall mean fluoride concentration was 0.5 ± 0.27 ppm; males (n = 263) were 0.58 ± 0.28 ppm and females (n = 237) 0.55 ± 0.27 ppm. Children in the 5 to 7-year age group had the highest levels (0.64 ± 0.33 ppm). Mean urinary fluoride levels in this sample of school children were low indicating a fluoride intake below the optimum level for caries prevention.

2.6 Studies on Jowar and Fluorosis

Chandrashekar and Anuradha (17) conducted a cross sectional survey to assess the prevalence and severity of dental fluorosis and its relationship with fluoride levels in drinking water. Twelve villages with similar climatic, dietary, socioeconomic conditions and altitudes were selected from rural areas of Davangere district, Karnataka, India. The fluoride levels in drinking water of selected villages were in the range of 0.22–3.41 ppm. A stepwise increase in the prevalence of dental fluorosis with corresponding increase in water fluoride content was found. Even at low fluoride (0.73 ppm & 0.98 ppm) concentration; the objectionable fluorosis (severity of dental fluorosis) was prevalent (12.3% to 16%) which was attributed to local dietary habits like jowar consumption. Such prevalence (52.8%) and severity of dental fluorosis could not be explained alone
by water borne fluorides. Although other factors like, tropical climate, have been attributed, the severity was not comparable with other endemic regions in India, with similar climatic conditions. High incidence of dental fluorosis even with low content of fluoride in water is probably, related to, dietary (jowar) factors aggravating the condition. There was a significant positive linear correlation (r=0.99) between Community fluorosis index (CFI) and water fluoride level. The occurrence of objectionable dental fluorosis in low fluoride may be related to dietary practices and fluoride in drinking water.

R Harikumar (24) conducted a cross-sectional study in north western districts of Tamil Nadu, India to assess severity of dental fluorosis. The nine villages selected were grouped on the basis of fluoride levels in drinking water as less than 2 ppm, 2-4 ppm and 4-6 ppm. The clinical examination of dental fluorosis was carried out by Smith’s classification 1986 and community Index of dental fluorosis was used to assess the severity between districts. A total of 8700 individuals, including 1745 children between five and 14 years of age were selected. A cross sectional study in Tamil Nadu state, India observed 50.8% prevalence of dental fluorosis among children in the age group of 5 to 14 years, when the water fluoride level was 1.2 ppm whereas in another village, with a fluoride level of 1.3 ppm the prevalence was only 41.1%. The prevalence of dental fluorosis showed no strong association with fluoride levels in drinking water. This observation was attributed to variations in dietary habits like jowar and Ragi (millets).

Lakshmaiah and Srikantia (116) conducted a clinical trial to assess whether jowar and rice, the major cereal components of the diets consumed in areas of
endemic fluorosis have any influence on the retention of fluoride. For ten days, each group was given diets of rice and jowar and 10mg of sodium fluoride, to both groups, in divided doses through water. Representative samples of cooked diet and water analyzed for fluoride content. Urine and feces of subjects were analyzed for fluoride. The study was conducted in two parts. In the first experiment, a cross over design was employed while in the second, a double cross over design was adopted. In the first experiment, eight apparently healthy subjects were chosen. Four of them were given a jowar based diet for a period of 10 days and switched to rice based diet for another 10 days. The other 4 subjects were first on rice diet for 10 days and then changed over to a jowar based diet for 10 days. The second experiment was carried out on four subjects. The whole experiments were divided into four periods in ten days. Two subjects received jowar based diets during I & III periods and rice based diets during II & IV periods. The other two subjects received rice based diets during periods I and II and jowar based diet during periods II and IV. The pooled data for percent fluoride retention were subjected for ANOVA. Significant difference was observed between jowar and rice based diets. Nearly 30 to 45 % of (10.92 mg jowar group and 10.64 mg rice group) fluoride was retained in body in the jowar group as compared to rice based diet group. The diets used in both groups did not vary much in calcium content. The extent of absorption of fluoride was not different in rice and jowar diets. Hence it is the lower urinary excretion that contributed to the greater fluoride retention with jowar diets. It seems reasonable to postulate that the metabolism of fluoride differs on jowar based diets. It could be that less fluoride is
available for the kidney to filter or that renal tubular reabsorption of fluoride is greater when jowar is the staple food.

**Krishnamachari** (117) conducted a cross sectional study to assess the extent of endemic fluorosis (skeletal fluorosis (Genu Valgum) and dental fluorosis) in endemic areas of Andhra Pradesh, Karnataka and Tamil Nadu, South India. Of the 90 villages in Andhra Pradesh, 30 villages had 30 percent prevalence of dental fluorosis. The water fluoride level was in the range of 1.5 to 10.8 ppm. The prevalence of dental fluorosis was 5 to 92 % in Tamil Nadu and fluoride level in drinking water was in the range of 0.8 to 3 ppm. The dental fluorosis prevalence was 20 to 90 % of the population in individual villages in north Karnataka where the fluoride level in water was 3 to 7.6 ppm. The prevalence of skeletal fluorosis and dental mottling was four percent among jowar consumers when compared to rice consumers. Marked changes in soil conditions can modify fluoride toxicity, not only by changes in trace elements and the composition of food grains grown on such soil, but also by altering the trace element content of drinking water. It was concluded that trace element-molybdenum acquires in jowar in significantly higher amounts as compared to rice. This accounts for severe dental fluorosis in jowar consumers.

**Shivashankara** (118) The prevalence of dental and skeletal fluorosis was determined among children of Kheru, Nayak, Thanda of Gulbarga India, where the fluoride concentration in drinking water ranges from 0.6 to 13.4 ppm and the water has low levels of copper and zinc. These children were investigated clinically, radiologically and biochemically. The study revealed that 89% of the
children had dental fluorosis and 39% exhibited skeletal fluorosis. Low levels of copper and zinc, and high levels of molybdenum in water and food contribute to the development of the genu valgum syndrome in fluorotic patients.

2.6.1 Effect of different cereals on fluoride deposition in bone

National Institute of Nutrition (119) Fluoride retention in the body and progression of fluorosis are reported to be influenced by the type of cereal used as staple diet. An experiment on rats was initiated to find out the influence of jowar, wheat and rice as staple cereals on fluoride retention and deposition in the bones. The femur samples were analyzed for fluoride, Ca++ and Mg++. The calcium and magnesium contents of femur (mg F/femur) were not statistically significant in different groups. Fluoride content of mandibles (mg F/mandible) was 0.59±0.05 for wheat group, 0.66±0.05 for rice group and 0.7±0.069 for jowar group. Fluoride content of wheat group was significantly lower than that of jowar and rice groups. These results support the earlier observations that fluoride retention on jowar diet is higher than that on rice diet.

Krishnamachari (120) stated epidemiological observations indicated the extent and severity of fluoride toxicity in endemic areas which vary from one part to the other. Apart from these differences in the concentration of fluoride in drinking water, it was observed that the staple food consumed were also different in different areas. Diets based on jowar, resulted in significant increase in retention of fluoride compared to diets based on rice at identical intakes of fluoride. The increased retention of fluoride was not due to, changes in the intestinal absorption
or excretion. It was concluded that jowar based diets retains more fluoride in body as compared to other diet and molybdenum may be related to this fluoride retention in the body.

2.6 Fluoride interactions with Molybdenum

Khandare (30) conducted an experimental study on the role of molybdenum (Mo) in the deposition of fluoride in bone. For this purpose, four groups of rabbits were used [control (C), fluoride (F), fluoride + molybdenum (F + Mo), and fluoride + molybdenum + copper (F + Mo + Cu)]. Bone mineral content (BMC), bone mineral density (BMD) [by dual energy X-ray absorptiometry (DXA)], and strength of femur bones were also assessed. Fluoride content in the femur was significantly higher in all experimental groups compared to the control group. Mo supplementation increased fluoride deposition in femur bone in the F + Mo group. Serum PTH, alkaline phosphatase, and urinary hydroxyproline and Ca were significantly higher in the F and F + Mo than in the C and F + Mo + Cu groups. However, serum PTH and urinary hydroxyproline were higher in the F + Mo group than the F group. Alkaline phosphatase was significantly higher in the F + Mo group than the F and F + Mo + Cu groups. Results of the study showed that, ingestion of Mo with F does not create secondary Cu deficiency (due to increased excretion of Cu through urine). Deposition of fluoride in femur bone was more (22%) when it was given along with Mo, as compared to fluoride alone.

Quarterman (27) conducted animal experimental study to assess, the variations in dietary content of copper and molybdenum, on the composition and
mechanical properties of the bones and teeth, offered with diets with and without fluoride. Ten groups of weaning rats were offered, a semi synthetic diet for 3 months, which contained 2.5mg Cu/Kg with or without sodium fluoride (100 mg F/Kg) and Mo (3mg Mo /Kg) as ammonium tetrathiomolybdate. The breaking stress and Young’s modulus of freshly dissected femurs and tibias and fluoride content of bone was measured. When fluoride and molybdenum were administered simultaneously, the fluoride content of bone increased significantly when compared to fluoride given alone.

Stookey and Muhler (28) conducted an experimental study to assess the relationship between skeletal retention of fluoride in the presence of molybdenum. 24 weaning rats (Sprague–Dawley strain bred rats) were divided into 4 groups according to their initial body weight. Group I received fluoride low drinking water (F = 0.01 ug/ml) and Group 2 received 50 ug/ml Mo in drinking water. Group 3 received 50 ug/ml F in water and Group 4 received Mo and F at the same as group 2. Animals were killed after 30 days and femur retrieved for fluoride analysis. During the 30 day experimental period, the animals that received low fluoride water and 50 ppm Mo gained on the average of 87 g. Those which received 50 ppm F gained less (70g) but those receiving both MO and F gained weight (98g). 145ug of fluoride was present in control animals while animals receiving 50 ppm Mo had 8200 ug of F and when both Mo and F was fed 9820 ug of F was found. The total fluoride retention was found to be 32% increase higher when 50 ppm Mo is added to low fluoride as compared to the group receiving no Mo. When both Mo and F are added to the drinking water there is approximately
17% more fluoride than when only fluoride is present. The presence of Mo is definitely associated with increasing retention of fluoride in the rat by two mechanisms. 1. It increases fluoride retention by apparently increasing availability of, fluoride, both, present as a constitute of the diet and also that small fraction present in drinking water. 2. It increases retention of that available fluoride added directly to drinking water. These data suggest that Mo may act metabolically to increase the availability of fluoride ion.

Kruger (121) conducted an experimental study to assess the effects of fluoride and molybdenum interaction and its effect of dental morphology. Eleven pregnant uniform breeds of rats were used. 2 by 2 factorial designs were used with low levels of fluoride F and molybdenum set at zero. The treatments were administered during the major period of amelogenesis of the rat molars, postnatal from 4 through 19 days inclusively. The F (fluoride) and Mo (molybdenum) treated rats (the controls rats) received injections of normal saline solutions containing zero levels of F and Mo. The FoMl received 7 mg/kg Ammonium molybdate. F1Mo received sodium fluoride 7mg/kg body weight. The F1Mo1 received 7mg/kg of NAF and 7mg/kg of Mo. The rats were weighed at intervals of 3 days from day 4. At the end of 21 days the rats were sacrificed. The interaction of fluoride and molybdenum altered the widths of the fissures and the thickness of the enamel and the dentin in F1 and Ml fed rats when compared to others.

Buttner (122) reported that 25 ppm of Mo (molybdenum) in the drinking water decreased caries by 18 per cent, 25 ppm of fluoride decreased caries by 32 per cent, and the interaction of 25 ppm of fluoride plus 25 ppm of Mo decreased
caries by 52 per cent. In a second study, these workers demonstrated that the administration of molybdenum increased the retention of fluoride in the bones of rats. But these elevations in fluoride retention were not directly proportional to the increase in the amount of molybdenum that had been administered. They found that the synergistic effect of molybdenum and fluoride was much more prominent in older rats.

2.8 **Justification of Study**

A population based data on risk of dental fluorosis and its association with jowar consumption would validate the experimental and point prevalence estimates. Jowar as a local factor may contribute to the unexplained observation of higher severity of dental fluorosis in these endemic communities. This study aims to find out the presence of any association between jowar consumption and dental fluorosis.

**Rationale of the study**

1. Dental fluorosis is major public health problem in North Karnataka, India.
2. Several studies have demonstrated waterborne fluorides to be the main causative factor for dental fluorosis.
3. However, the association between jowar consumption and severe dental fluorosis has not been documented in literature, hence this study was conducted.