CHAPTER - II

REVIEW OF LITERATURE
2. REVIEW OF LITERATURE

Fluorine, a gaseous element is a halogen which being most electronegative and reactive of all elements does not occur in free form in nature. Fluoride combines directly with most elements and indirectly with few to form fluorides. Fluorine does not occur in the elemental state because it is a highly reactive electronegative non-metal with an oxidation potential greater than ozone. (Greenwood and Earnshaw, 1984; Cerkleswski et al., 1997; Parker, 1992).

Fluorides are ubiquitous in nature and are present in rocks, soil, water, plants, food and even in air. The geological survey of India reported that topaz, apatite, rock phosphate, phosphate nodules, and phosphorite are widespread in the earth’s crust in India and contain high percentages of fluoride. As a result of the rich mineral content and high rainfall, fluoride leaches out and contaminates the water and the soil affecting mainly teeth, bones and joints (WHO technical report series, 846, 1994).

2.1 Fluoride in environment:

The atmosphere has very low fluoride content and in 97 per cent of non-urban areas fluoride is hardly detectable. The fluoride content of atmosphere is seen to have risen wherever there is volcanic action or industrial activity. Volcanic fumarole’s vapors have high concentration of fluoride and industrial emissions from those engaged in mining or manufacture of fluoride containing minerals may be hazardous. Low-grade coal has high levels of fluoride and smoke may be a source of fluoride pollution. Coal is the most abundant fossil fuel resource worldwide, therefore, a huge amount of hazardous chemical components including fluorides have been released into the environment by combustion of coal (Ando et al., 1995, 1998). Some of the industrial activities with which fluoride gain access into the environment as particulate emission are manufacture of fluoride containing fertilizers, aluminum smelting, nuclear power plants, electric power industry and petroleum refining industry. Fluoride finds its way into seawaters, surface water, underground water and vegetation (WHO technical report series, 846, 1994).
2.1.1 Fluoride in lithosphere:

Fluorine is chemically the most active non-metallic element and gives raise to fluoride the most reactive electro negative ion. In its elemental form, fluorine is a pale yellow, highly toxic and corrosive gas. Fluorine is seventeenth in the order of frequency of occurrence of elements and represents about 0.06-0.09 per cent of the earth's crust. Volcanic and hypabyssal rocks also contain significant amount of fluoride up to 2500 mg/Kg (WHO technical report series, 846, 1994).

2.1.2 Fluoride in water:

Owing to the universal presence of fluorides in the earth's crust, all water contains fluoride in varying concentrations. The bulk of the water normally available to humans is involved in the hydrological cycle, which means that it originates in the sea. Sea water itself contains significant quantities of fluoride at levels of 0.8-1.4 mg/L. The fluoride content of water obtained from lakes, rivers or wells is the most part below 0.5mg/L, although concentrations as high as 95mg/L have been reported in the United Republic of Tanzania. (WHO Technical Report Series, 846, 1994).

Concentration of fluoride in ground water varies depending up on the following factors:

- Availability and solubility of the parent rock
- Porosity of rocks or soils
- Speed at which the water flows
- Temperature of interaction between water and rocks
- Alkalinity of soil and water
- Complexation of fluoride with other ions such as aluminum, beryllium, silicon, boron and iron (Anasuya Das, 1998).

Apart from the naturally occurring minerals containing fluoride, several other factors such as use of fluoride containing fertilizers, pesticides, gaseous and particular emissions from industries also add to the fluoride content of soils and water (Anasuya Das et al., 1996). The general geological formation is not an indicator of the concentration of fluoride in the ground water. There are significant variations in the distribution of rocks with readily leaching fluoride. It has been observed that even
within one village community different wells often show widely divergent fluoride contents apparently as a result of differences in the local hydrological conditions. Ground water may show variations in fluoride content depending on the presence of fluoride containing formations at different depths.

Based on the hydrological investigations the individual states have been further categorized into four groups showing the concentration of fluoride in the range 0-1.5 ppm, 1.5-4.0 ppm, 4.0-8.0 ppm and above 8.0 ppm in arid and semi arid belts and west-central India (Raghava Rao, 1974). In southern states, the groups are arranged in the ranges 0-1.5 ppm, 1.5-5.0 ppm and above 5.0 ppm (data collected during 1965-1974 except for Tamilnadu). Toxic levels of fluoride present in water sources in Andhra Pradesh are indicated in Map-1.

2.1.2.1 Recommended levels of water fluoride:

The recommended fluoride content in water is 1.0 ppm (WHO Technical Report Series, 846, 1994). The recommended level of fluoride varies with the temperature as water requirement increases in hot climates. Intake of fluoride recommended for tropical and temperate climate is 0.5 and 1.0 ppm, respectively.

2.1.3 Fluoride in foods:

Fluorine is widespread in nature resulting in its ingestion through various sources, mainly the diet. It has also been observed that agricultural crops grown in areas endemic for fluorosis absorb fluoride from soil and water and there is a growing concern that fluoride finds entry into the body through the food chain (Susheela, 1993). The fluoride content of foods and beverages is also one of the major factors affecting the stage of fluorosis. (Sangh et al., 1996, Dilnawaz et al., 1973). Nearly all foods contain small quantities of fluoride and the total daily intake through any average human diet is small except in endemic regions. In certain endemic regions of India, the fluoride content of vegetables and food may be very high (Chari et al., 1974). The contribution of food to the total daily intake of fluoride varies from region to region. Staple diets rich in sorghum, ragi or bajra containing high silicon besides fluoride seem to aggravate fluoride toxicity in some endemic areas of India. Parboiled rice had higher fluoride content than other varieties of rice. Parboiling of rice using fluoride rich water causes higher fluoride levels in rice (Pandit et al., 1940; Anasuya et al., 1996).
Map-1: Toxic levels of fluoride present in water sources in Andhra Pradesh
Extensive reviews on food-borne-fluoride showed that the concentration in unprocessed food such as rice, wheat and pulses with brand is usually low (0.1-25.0 mg/Kg). However, meat products in which skeletal tissue has been intentionally added during processing can have high fluoride concentrations.

Among beverages tea has an exceptionally high fluoride content which varies in different brands from 122-260 ppm or more. Each cup of tea may supply 0.3-0.5 mg of fluoride. Bottled beverages, which are increasingly being consumed around the world, have a variable and some have high content of fluoride and should be considered as additional sources of fluoride. Certain foods and beverages like seafood and teas contain high concentrations of fluoride "naturally" because of the environment in which they are grown (Raja Reddy, 1979).

In geographical areas, naturally low in fluoride, the total daily adult intake is likely to be <1.0 PPM (Singer et al., 1985). Adults living in a city served by fluoridated water, either naturally occurring or adjusted to 1 ppm, will have a total daily fluoride intake of about 2 ppm. In either situation, water will account for the major source of fluoride intake for an adult, because most foods are low in fluoride except seafood, sea salt and some teas. Preparation of food in the home with fluoride-containing water adds fluoride to the total daily dietary intake of fluoride. (Taves, 1983, Kumpulainen and Koivistoinen, 1977). Fluoride content of some foods is presented in Table-1.

2.1.3.1. Recommended level of fluoride intake per day:

The fluoride contents from all the sources determine the human intake of fluoride. In majority of endemic areas around the world, the main contribution is from water and significant amounts come from foods. Recommended levels of fluoride intake are presented in Table-2.

2.2 Metabolism of Fluoride:

Fluorine naturally occurs in the earth's crust, water and food as fluoride ion. The metabolic fate of the fluoride ion ingested through food in adult is shown in Flow chart-1.
### Table - 1: Fluoride Content of Some Foods

<table>
<thead>
<tr>
<th>Food items</th>
<th>Fluoride levels (ppm)</th>
<th>Food items</th>
<th>Fluoride levels (ppm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>A*</td>
<td>B**</td>
<td>A*</td>
</tr>
<tr>
<td>Cereals</td>
<td></td>
<td>Cashew nut</td>
<td>4.1</td>
</tr>
<tr>
<td>Rice</td>
<td>4.6</td>
<td>2.59-3.3</td>
<td>Coconut</td>
</tr>
<tr>
<td>Wheat</td>
<td>5.9</td>
<td>3.27-14.03</td>
<td>Mustard seeds</td>
</tr>
<tr>
<td>Maize</td>
<td>5.6</td>
<td>-</td>
<td>Groundnut</td>
</tr>
<tr>
<td>Pulses and</td>
<td></td>
<td>Beverages</td>
<td></td>
</tr>
<tr>
<td>Legumes</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bengal gram</td>
<td>6.2</td>
<td>3.84-4.84</td>
<td>Tea (Dry leaves)</td>
</tr>
<tr>
<td>Green gram dal</td>
<td>2.5</td>
<td>2.34-4.84</td>
<td>Tea infusion</td>
</tr>
<tr>
<td>Red gram dal</td>
<td>3.7</td>
<td>2.34-4.84</td>
<td>Aerated drinks</td>
</tr>
<tr>
<td>Soya bean</td>
<td>4.0</td>
<td>-</td>
<td>Coconut water</td>
</tr>
<tr>
<td>Leafy Vegetables</td>
<td></td>
<td>Spices and</td>
<td></td>
</tr>
<tr>
<td>Mint</td>
<td>4.8</td>
<td>Condiments</td>
<td></td>
</tr>
<tr>
<td>Spinach</td>
<td>2.0</td>
<td>0.77-4.14</td>
<td>Cumin Seeds</td>
</tr>
<tr>
<td>Amaranth</td>
<td>5.8</td>
<td>4.91-7.14</td>
<td>Garlic</td>
</tr>
<tr>
<td>Other vegetables</td>
<td></td>
<td>Ginger</td>
<td></td>
</tr>
<tr>
<td>Cucumber</td>
<td>4.1</td>
<td>2.57-3.58</td>
<td>Tamarind Pulp</td>
</tr>
<tr>
<td>Tomato</td>
<td>3.4</td>
<td>1.00-2.08</td>
<td>Turmeric</td>
</tr>
<tr>
<td>Brinjal</td>
<td>1.2</td>
<td>1.62-2.48</td>
<td>Animal Sources</td>
</tr>
<tr>
<td>Ladies finger</td>
<td>4.0</td>
<td>2.2-3.62</td>
<td>Mutton</td>
</tr>
<tr>
<td>Snake Gourd</td>
<td>2.3</td>
<td>2.16-3.44</td>
<td>Beef</td>
</tr>
<tr>
<td>Fruits</td>
<td></td>
<td>Pork</td>
<td></td>
</tr>
<tr>
<td>Banana</td>
<td>2.9</td>
<td>0.84-1.58</td>
<td>Fish</td>
</tr>
<tr>
<td>Mango</td>
<td>3.7</td>
<td>0.8-1.80</td>
<td>Roots and Tubers</td>
</tr>
<tr>
<td>Apple</td>
<td>5.7</td>
<td>1.05-2.2</td>
<td>Carrot</td>
</tr>
<tr>
<td>Guava</td>
<td>5.1</td>
<td>0.24-0.52</td>
<td>Potato</td>
</tr>
<tr>
<td>Nuts and Oil</td>
<td></td>
<td>Onion</td>
<td></td>
</tr>
<tr>
<td>Seeds</td>
<td></td>
<td>Sweet Potato</td>
<td></td>
</tr>
<tr>
<td>Almond</td>
<td>4.0</td>
<td>-</td>
<td>Beet root</td>
</tr>
</tbody>
</table>

A* Sengupta and Pal (1971); B** Lakadawala and Punekar (1973)

### Table-2: Safe and adequate daily fluoride intake of different age groups

<table>
<thead>
<tr>
<th>Age</th>
<th>Recommended intake of fluoride per day</th>
</tr>
</thead>
<tbody>
<tr>
<td>1-3 years</td>
<td>0.50-1.5mg</td>
</tr>
<tr>
<td>3-5 years</td>
<td>Maximum of 2.5mg</td>
</tr>
<tr>
<td>6-17 years</td>
<td>Maximum of 2.5mg</td>
</tr>
<tr>
<td>Adults</td>
<td>1.5-4.0mg</td>
</tr>
</tbody>
</table>

(National Academy of Sciences, 2003)
2.2.1 Absorption:

Fluoride, an abundant geologic mineral, is rapidly absorbed from the stomach and proximal intestine and becomes incorporated into calcified tissues. (Whitford, 1994). Ingested fluoride can be absorbed from both the stomach and intestinal mucosa. The low pH in the stomach favors formation of highly diffusible hydrogen fluoride (pKₐ = 3.4) and the absorption is very rapid (Whitford et al., 1984). Conditions of high gastric acidity, therefore, favor fluoride absorption whereas alkalinity decreases fluoride absorption (Whitford, 1996). Fluoride absorption from the small intestine occurs as the fluoride ion by non-pH dependent diffusion (Nopakun et al., 1989). Under conditions where fluoride is ingested as part of a total diet most of the ingested fluoride will be absorbed from the small intestine (Ophaug, 1990). A greater proportion of fluoride is likely to be absorbed from the stomach that indicated in Fig.1 when fluoride is ingested in the fasting state its absorption is 100 per cent. The presence of food reduces efficiency of fluoride absorption to 50 to 80 per cent (Parkins et al., 1966).

![Flow chart-1: Fate of fluoride ingested with food in an adult. Interrupted line shows a minor pathway (Florian, 1997).](image)

The mechanism of fluoride absorption is by passive diffusion by way of membrane channels (Parkins et al., 1966). The possibility that fluoride might be absorbed by an active process (Stookeey et al., 1964) was strongly challenged by the observation that fluoride transfer across the intestinal membrane was unaffected by the
metabolic inhibitor sodium cyanide, sodium iodoacetate, and by 2,4-dinitrophenol (Ekstrand, 1978).

Absorption of fluoride from the diet can vary from one individual to another and in the same individual from one occasion to another. Apart from the systemic disorders absorption of fluoride is hindered if calcium, magnesium, or aluminum ions are present, because these cations can bind fluoride (Whitford, 1986).

Conditions of high gastric acidity, therefore, favor fluoride absorption whereas alkalinity decreases fluoride absorption (Whitford, 1996).

Fluoride absorption from the small intestine occurs as the fluoride ion by non-pH dependent diffusion (Nopakun and Messer, 1990).

When ingested directly, fluoride compounds are readily absorbed by the intestines.

Under conditions where fluoride is ingested as a part of a total diet, most of the ingested fluoride will be absorbed from the small intestine (Nopakun and Messer, 1989).

A greater proportion of fluoride is likely to be absorbed from the stomach than indicated in Flow chart-1, when fluoride is ingested while in fasting. During fasting, fluoride absorption is almost 100 per cent, whereas the presence of food reduces efficiency of fluoride absorption to 50-80 per cent. There is no evidence to indicate that fluoride absorption is regulated (Florian, 1997).

The mechanism by which fluoride is absorbed is by passive diffusion by way of membrane channels (Ophaug, 1990).

The possibility that fluoride might be absorbed by an active process (Parkins et al., 1966) was strongly challenged by the observation that fluoride transfer across the intestinal membrane was unaffected by the metabolic inhibitors sodium cyanide, sodium iodoacetate and by 2,4-dinitrophenol (Stookey et al., 1964).

The placenta does not form a barrier to fluoride which, to a limited extent, penetrates the fetus. (Forestier et al., 1990).
2.2.2 Transport and Tissue uptake:

Generally fluoride intake and absorption rate, plasma fluoride concentration ranges from 10 to 20 μg/L or 0.5 to 1.0 μM (Forbes, 1990). Ionic fluoride in the plasma is not protein-bound in contrast to the small metabolically unimportant fluorocarbon fraction (Whitford, 1996). Fluoride is rapidly removed from plasma by mineralized tissue in exchange with other anions such as hydroxyl ion, citrate and carbonate in contrast to soft tissue, which does not accumulate fluoride. Ninety five percent of total body fluoride is found in bones and teeth in an adult. Total body fluoride has been estimated to be about 2.6 gm, which is second only to the trace element iron (Nielsen, 1996; Forbes, 1990).

The most efficient uptake of fluoride into bones and teeth occurs during periods of rapid growth because of high bone turnover. Thus, mature bone takes-up fluoride considerably slower than newly forming bone. Higher concentrations of fluoride are also found in surface layers of mineral structure than in deeper layers and fluoride released during bone remodeling is largely redeposited (Guo et al., 1988). Fluoride is deposited to a greater extent in trabecular bone compared to compact bone (Rao et al., 1995).

In children maximum fluoride deposition occurs on teeth and unlike bone it is not subject to resorption associated with the remodeling process, except for surface demineralization-remineralization, because of the severed blood supply following tooth eruption (Depaola et al., 1994). Unlike bone, tooth is not subject to resorption and remodeling process. Low permeability within tooth structure also restricts ionic mobility. As in bone, the deposition of fluoride into teeth follows a gradient. Thus, the outer layers of surface enamel may contain 300 μg F/gram in contrast to 100 μg F/gram at the dentin-enamel junction (Maheswar et al., 1981). The concentration of fluoride in the outer enamel layer can also be influenced post-eruptively by oral fluoride exposure by way of surface demineralization-remineralization events.

The fluoride absorbed through the gastrointestinal tract is rapidly distributed to all the tissues by simple diffusion. Fluorine, the most electronegative element, can rapidly cross the cell membrane, skeletal and cardiac muscle, liver, skin and the
erythrocytes. Under certain conditions, the absorbed fluoride can affect virtually every phase of human metabolism. (Chinoy et al., 1992).

2.2.3 Excretion:

Over time, the compound is excreted through the urine, and the half life for concentration of fluorine compounds is on an order of hours. Urine tests are a good indication of high exposure to fluoride compounds in the recent past. In infants, as much as 90 per cent of the fluoride intake may be retained (Bergmann and Bergmann, 1995). This proportion decreases with age (Ekstrand et al., 1984). In growing children and adults, more than 90 per cent of the fluoride ingested is excreted via the urine, and only minor proportions are retained in the skeleton. Urinary fluoride excretion reflects total fluoride intake (Hodge and Smith, 1981).

Under normal conditions, about 90 per cent of total fluoride is excreted through urine (Hodge et al., 1970). Loss of fluoride in perspiration is considered to be negligible except under unusual circumstances (Schiff and Binswanger, 1982; Spac et al., 1985). Renal clearance of fluoride is linear with glomerular filtration rate and about 60 per cent of filtered fluoride is reabsorbed (Spac et al., 1985; Ekstrand et al., 1978). The rate of fluoride clearance is lower in children than adults, which allows for greater fluoride retention at a time when bone and teeth are rapidly developing in children. Cessation of bone growth is associated with an increase in the amount of fluoride excreted in the urine.

2.2.3.1 Feces:

Fluoride present in feces results from two sources: the ingested fluoride that is not absorbed and the absorbed fluoride that is excreted into the gastrointestinal tract. About 10 to 25 per cent of daily intake of fluoride is excreted in the feces.

2.2.3.2 Sweat:

Some fluoride is also lost from the body through sweat and so appreciable amounts may be lost in situations marked by excessive sweating. Sweat fluoride concentrations are similar to plasma.
2.3 Biochemical functions of fluoride:

2.3.1 Mineralized tissue:

*Hydroxylapatite,* also frequently called hydroxyapatite, is a mineral. It is a naturally occurring form of calcium apatite with the formula $\text{Ca}_5(\text{PO}_4)_3(\text{OH})$, but is usually $\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$ to denote that the crystal unit cell comprises two molecules. Hydroxylapatite is the hydroxyl end member of the complex apatite group. The $\text{OH}^-$ ion can be replaced by fluoride, chloride or carbonate. It crystallizes in the hexagonal crystal system. It has a specific gravity of 3.08 and is 5.0 on the Mohs hardness scale. Hydroxylapatite is the main mineral component of bone. Carbonated-calcium deficient hydroxyapatite is the main mineral of which dental enamel and dentin are comprised. (Wopenka and Pasteris, 2005).

*Fluoroapatite,* often with the alternate spelling of fluorapatite, is a mineral with the formula $\text{Ca}_5(\text{PO}_4)_3\text{F}$. Fluoroapatite is a hard crystalline solid. It is an important constituent of tooth enamel. Fluoroapatite crystallizes in a hexagonal crystal system. It is often combined as a solid solution with hydroxylapatite in biological matrices.

Fluoroapatite can be synthesized in a two step process. First, calcium phosphate is generated by combining calcium and phosphate salts at neutral pH. This material then reacts further with fluoride sources (often sodium monofluorophosphate or calcium fluoride $[\text{CaF}_2]$) to give the mineral. This reaction is integral in the global phosphorous cycle (Holleman, 2001).

$$3\text{Ca}^{2+} + 2\text{PO}_4^{3-} \rightarrow \text{Ca}_3(\text{PO}_4)_2$$

$$3 \text{Ca}_3(\text{PO}_4)_2 + \text{CaF}_2 \rightarrow 2 \text{Ca}_5(\text{PO}_4)_3\text{F}$$

The defects that result with the toxic effects of fluoride interfere with the mineralization process and are generally irreversible (WHO, 1984). Because the fluoride ion is similar in size and charge to the hydroxyl ion, the normal component of hydroxyapatite, it can substitute to form fluorapatite. The resulting crystal lattice is more compact, less soluble and more stable than hydroxyapatite and causes resistance to bone remodeling (Kleerekoper, 1996; Yildiz *et al.*, 2003). For these reasons, once fluoride is incorporated into skeletal architecture, especially trabecular bone (Riggs *et al.*, 1990), its presence is prolonged (Whitford, 1994). Furthermore, fluoride is an
anabolic agent that uncouples the remodeling process with stimulation of bone formation in the absence of prior resorption (Kleerekoper, 1996).

The formation of calcium fluoro-apatite and its deposition in the tissue matrix of bone results in functional derangement. As a result of laying down of apatite crystals and perhaps due to other changes, the bones become thicker with increased bone mass and density. X-rays reveal such changes in the bone, which become visible through radiographs. Besides the calcified tissues, extremely thin membranous connective tissue, viz. ligaments, calcify due to prolonged exposure to fluoride and become visible through radiographs. Changes in bone mass, density and the presence of calcified ligaments are considered as the main structural derangements characteristics of the disease. Fluoride action on cancellous and cortical bone matrix, effect on glycosaminoglycans, sulphated isomers, charge density, molecular configuration of disaccharides and occurrence of dermatan sulphate, disappearance of dermatan sulphate from ligament and other tissues. The role of dermatan sulphate is a detrimental factor in the pathogenesis of fluorosis (Susheela, 1993).

Fluoride action on cancellous (spongy) bone and bone cortical (compact) matrices is of great significance. The matrix besides having a large proportion of collagen protein, contains glycosaminoglycans and glycoproteins and provides an ideal milieu for calcification. The two types of bones distinctly different in structural and biochemical profile (Susheela and Jha Mohan, 1981).

The major crystalline salt of mature bone and teeth is predominately composed of calcium and phosphorus in the form of hydroxyapatite with the chemical formula \( \text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2 \). Additional forms of calcium and phosphorus, such as \( \text{CaHPO}_4.2\text{H}_2\text{O} \) (Calcium Biphosphate) and \( \text{Ca}_3(\text{PO}_4)_2.3\text{H}_2\text{O} \) (Calcium Phosphate), represent a fraction of salts called the amorphous phase that can be readily mobilized (Eanes, 1983). In bone, the amorphous fraction may be as much as 20-30 per cent of the total salts.

Substitution of the fluoride ion into the hydroxyapatite crystal structure in place of hydroxyl and carbonate ions (fluorapatite) promotes crystal formation, which is associated with increased apatite crystal size, reduced crystal distortion and reduced solubility (Okazaki, 1992). The unique function of fluoride is attributed to high degree
of reactivity of fluoride ion coupled with its small ionic radius. (Greenwood et al., 1984).

The chemical structure of fluorapatite $\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2\cdot 2x\text{F}_{2x}$ indicates that there are various degrees of fluoridation of the mineral matrix denoted by “x” (Simonen and Laitinen, 1985).

Fluoride distribution in bone and teeth is not homogeneous. The degree of fluoridation of the hydroxyapatite crystal is inversely related to the solubility of the crystal. The significance of the substitution of fluoride into the hydroxyapatite crystal is much more pronounced in teeth than in bone.

Fluoride ions replace hydroxide ions in calcium hydroxyapatite, $\text{Ca}_8(\text{PO}_4)_3\text{OH}$, in teeth, forming calcium fluoroapatite, $\text{Ca}_8[(\text{PO}_4)_3\text{F}]$, which is more chemically stable and dissolves at a pH of 4.5, compared to 5.5 pH for calcium hydroxyapatite.

It has been observed that bone modified by fluoride has decreased elasticity and increased crystallinity (Eanes et al., 1965).

2.4 Biomarkers of fluoride exposure:

A fluoride biomarker is of value primarily for identifying and monitoring deficient or excessive intakes of biologically available fluoride. Knowledge of fluoride availability during pre-eruptive periods of tooth formation allows assessment of the potential for later development of fluorosis. While knowledge of its post eruptive availability provides a guide to the potential level of protection from caries. It also serves to assess the impact of water fluoridation on bone quality and other physiological conditions (WHO, 846, 1994).

2.4.1 Historic markers - Bone and Teeth:

The body's burden of fluoride is best reflected in the calcified tissues, though enamel is not the tissue of choice because most of its fluoride was taken up during tooth formation. After tooth eruption, exposure to widely fluctuating concentrations of fluoride in the oral cavity significantly affects fluoride levels in the surface layers of enamel, where the highest concentrations of fluoride are found. Bone fluoride concentrations are much better indicators of long-term fluoride exposure and body
burden, though fluoride is not uniformly distributed throughout bone. For example, cancellous bone has higher fluoride concentrations than cortical bone. Dentin, especially coronal dentin, may be the best marker for the estimation of chronic fluoride intake and the most suitable indicator of the body burden. The tissue does not normally undergo resorption; it is more easily obtained than bone (WHO, 846, 1994). Osteoblasts proliferate, either because fluoride enhances mitogenic signals or growth factors in bone (Gruber and Baylink, 1991) or because it inhibits phosphotyrosine phosphatase activity, thereby increasing cellular tyrosyl phosphorylation and stimulating bone cell proliferation (Lau and Baylink, 1998). Excess skeletal mass would still contain substantial amounts of fluoroapatite and be brittle and more prone to fracture than skeletons containing only hydroxyapatite (Kleerekoper, 1996). In fact, long-term fluoride therapy for osteoporosis was associated with more fractures likely for this reason (Riggs et al., 1990).

2.4.2 Contemporary markers - Plasma, Urine and Saliva:

There are several fluids that may be used to determine the amount of fluoride in various compartments of the body. Some of these are readily accessible and are useful for determining the current availability of fluoride. The values obtained are not a direct measure of fluoride accumulation in the body, but they are indicative of the body burden due to an incompletely defined relation between fluoride concentrations in bone and in the extracellular fluids. These fluids include urine, plasma and ductal saliva. Ductal salivary fluoride is related to the concentration in plasma by a factor of about 0.8. Samples taken from these two fluids are influenced significantly by intake during recent hours. Urinary fluoride excretions, as well as concentrations, are also related to those of plasma, but they are more variable than those of ductal saliva because of variations in urinary flow and pH (WHO, 846, 1994).

Urinary fluoride, which reflects resorption of bone fluorapatite that continues for many years, seems to be an optimal test for detecting prior (and active) fluoride exposure. Eliminating the source of fluoride toxicity leads to a reversible disorder, albeit one that is likely to linger and may, perhaps, inflict the patient for decades. Patients should be monitored for hypercalciuria and treated for impending nephrolithiasis. Prophylactic therapy for this complication, including hydration, limiting calcium intake and use of thiazide diuretics could be beneficial. With the
removal of fluoride, patients can achieve a fairly rapid amelioration of clinical symptomatology and forestall the development of more severe skeletal morbidity (Etah et al., 2007).

2.4.3 Recent markers - Nails and Hair:

The concentrations of fluoride in nails and hair appear to be proportional to intake over longer periods of time. Nails grow at about 0.1 mm/day so the average level of fluoride intake over a 1 to 3 week period can be estimated. Fluoride in hair could be used to estimate intake over longer periods (Czarnowski et al., 1996) conducted a study on 58 workers employed in the superphosphate production plant in Skawina. The results revealed that the fluoride content in the urine, hair and nails increased above the control level (WHO, 846, 1994).

2.5 Biochemical changes in fluorosis:

Salient biochemical changes in fluorosis of composition of bone, urine, and plasma.

2.5.1 Bone matrix:

Thirty per cent of bone is made up of organic matter. It consists of two components-collagen and mucopolysaccharides. Bone is rich in collagen. Collagen has a high turnover capacity. When toxic amounts of fluoride enter into collagen, its structure changes (Anasuya Das, 1998). Increased blood fluoride levels are observed in people living in endemic regions and in persons dwelling around contaminated industrial emissions as well as in those suffering from severe poisonings.

Chronic exposure to fluoride is known to produce new bone formation in the form of exostoses and of calcification of ligaments, muscular attachments, interosseous membranes, tendons, and arteries. Thus, osteosclerosis has been the well-documented form of the skeletal phase of fluoride toxicity. When fluoride enters the bones, a basic change in the chemical composition occurs which is, therefore, understandable that in fluorosis there is a certain amount of osteomalacia. There is evidence of hormonal changes in children with genu valgum such as elevated levels of circulating immunoreactive parathyroid hormone, increased growth hormone levels and decreased thyrocalcitonin. There is also an increased $^{47}$Ca turnover in these children with
increased accretion as well as resorption rate. In addition to radiological evidence of osteoporosis, there is biochemical evidence of bone collagen destruction in the form of elevated urinary hydroxyproline excretion. Some studies indicate a coexistence of osteomalacia and hyperparathyroidism. (Krishnamachari, 1982).

The diagnosis of fluorosis relies mainly upon X-ray changes in bone. But, minor changes of bone are often nonspecific. The diagnosis of fluorosis, therefore, is still difficult. When the X-ray changes become significant, the disease has advanced too far from recovery (Jei Wang et al., 1993).

2.5.1.1 Gross changes in the skeleton:

Skeletal changes involving overall increase of 2 to 3 times in bone mass are a characteristic feature of fluorosis (Weatherell and Weidmann, 1959; Singh et al., 1962; Reddy et al., 1969). The changes are first noticed in the vertebral column and pelvis and thereafter in the rib cage and limb bones. The bones become whitish and occasionally mottled like the teeth. Effects of moderate fluoride intake on the axial and appendicular skeleton may not be identical. It has been shown that trabecular bone, typified as being more metabolically active, is more likely to accumulate fluoride than cortical bone.

A clear indication of chronic fluorosis is the calcification and ossification of ligaments and interosseous fasciae occurring along with periosteal new bone formation and development of exostoses on long bones and osteophytes in the spine. It is in the muscular attachments and tendinous insertions that new bone formation occurs, as a result of which there is a thickening of the cortex and narrowing of the medullary cavity. The effect on the vertebral column is seen in roughening of pedicles, laminae, spinous and transverse processes. The osteophytes projecting into the spinal canal and intervertebral foraminae may press upon the cord and spinal roots and thus, account for the radiculomyelopathic features in chronic fluorosis. The spine is converted into a single rigid bone as a result of ossification of spinal ligaments and fusion of the adjacent bony structures. The bones of pelvis exhibit changes essentially similar to those found in the spine. The skull is rarely involved, although there may be thickening of the calvaria and a roughening of the outlines of the foramen magnum.
2.5.1.2 Histopathology of bone:

Johnson et al. (1965) worked on fluorosis, spells out the mechanisms underlying the development of skeletal changes caused by fluorosis in man and animals. There are three successive stages of development of fluorosis in bones, viz., fluoridation, mottling and abnormality. Bone fluoridation or chemical fluorosis is indicated if the fluoride content is less than 25 ppm- the stage at which no gross or microscopic abnormalities occur in the bones. In second stage bone motting is seen at fluoride levels ranging between 25-50 ppm. The mottled osteone is signified by brownish discoloration and increase in the number of osteocytes found in a tangled mass on its periphery synchronizing with a reduction of osteocytes in the rest of the osteone. The final stage is reached when the fluoride levels exceed 5000-6000 ppm, a stage at which even the naked eye could detect abnormality in the formation of the bone. These changes cause impairment of mechanical properties of bones.

Bone histomorphometric studies have indicated that the effect of fluoride to increase bone mass was due to increase in bone formation and not to a reduction in bone resorption, and that the stimulation of bone formation was mediated through an increase in the osteoblast number (Briancon, 1981; Harrison et al., 1981). This indicates that fluoride is an anabolic agent for bone cells and that the bone-forming effect of fluoride is mediated by an increase in osteoblast proliferation. In this regard, fluoride therapy has an unfavorable benefit-to-risk profile, which is believed to be related to the high incorporation of fluoride in bone and to the fluoride-associated osteomalacia (Lau and Baylink, 1998).

*In vitro* studies proved that fluoride at clinically relevant concentrations (5-30 \( \mu \)M) significantly increased the \( ^{3}H \) thymidine incorporation and cell doubling in normal human bone cells *in vitro*. Mitogenic doses of fluoride *in vitro* also stimulated transient calcium uptake (Farley et al., 1993; Zerwekh et al., 1990) and sodium-dependent phosphate transport (Selz et al., 1991) in bone cells.

2.5.2 Teeth:

Fluorosis occurs when fluoride interacts with mineralizing tissue, causing alterations in the mineralization process in the enamel of the teeth. Fluorosis causes subsurface hypo-mineralization or porosity, which extend toward the dentinal-enamel
junction as severity increases. This subsurface porosity is most likely caused by a delay in the hydrolysis and removal of enamel proteins, particularly, amelogenins as the enamel matures. This delay is either due to the direct effect of fluoride on the ameloblast or due to an interaction of fluoride with the proteins or proteinases in the mineralizing matrix. The specific mechanisms by which fluoride causes the changes leading to enamel fluorosis are not well defined; though the early maturation stage of enamel formation appears to be particularly sensitive to fluoride exposure. The risk of enamel fluorosis is lowest when exposure occurs in both secretory and maturation stages. Dental fluorosis is best correlated with the total cumulative fluoride exposure to the developing dentition (Den, 1999).

Dental mottling was thought to occur with ingestion of water containing more than 2.0 ppm of fluoride whereas skeletal fluorosis was not believed to occur even at 8.0 ppm in the Western countries. In India and Japan, however, dental fluorosis at less than 1.0 ppm and crippling skeletal fluorosis at 2.0 to 4.0 ppm fluoride in water have been well-documented. Drinking water requirements and dietary habits also differ in different populations and may profoundly influence the daily fluoride intake (Jolly, 1976).

Fluoride is also known to have antibacterial effects (Clarkson, 2000). It inhibits cariogenic bacteria which metabolise the carbohydrates to produce acid that leads to the development of dental caries. When a low concentration of fluoride is constantly present, Streptococcus mutants produce less acid (Marquis, 1990; Bowden, 1990).

2.5.3 Blood:

In fluorosis, metabolism of bone related minerals are altered to a varied extent. These metabolic changes are related to serum levels of the minerals, calcium and phosphorus levels. Fluoride ion replaces hydroxyl ions of hydroxyapatite in the bone to form fluoroapatite. It is a large crystal which being relatively more stable may not allow an easy exchange of calcium between the bone and blood (Havivi and Guggenheim; 1966; Zipkin et al., 1963). Such an abnormality may lead to decrease in serum calcium levels. Serum alkaline phosphatase activity is an indicator of biochemical abnormality of bone metabolism in fluorosis. Significant elevated levels (2-5 folds) of alkaline phosphatase seen in fluorosis. It is related to degree of bone
mineralization (Anasuya Das, 1998). The alkaline Phosphatase is often found to be elevated in fluorotic cases, which may be due to increased turnover rather than any specific effect of fluoride on the enzyme (Rosenquist 1974). A significant increase in serum alkaline phosphatase activity has been considered as an indicator of biochemical abnormality of bone mineral homeostasis in fluorosis. Studies reported that fluoride is a potent enzyme poison. It injures all the cells in bone (osteoblast and osteocytes). The cell initiates a repair response and results in increased serum alkaline phosphatase (SAP) production in both of the cell population. The repair response in osteoblasts results in increased proliferation, matrix production and SAP production. When the repair process in osteoblast fails, the osteoblast undergoes either apoptosis or necrosis and is replaced by proliferation of osteoprogenitor cells. These new osteoblasts will be injured and increases repair and cell death would be repeated. This activation of a repair response in osteoblasts would contribute to increase SAP (Farely et al., 1983; Marie and Hott, 1986; Tomkinson et al., 1997; Noble et al., 1997).

Serum Fluoride does not always indicate the chronicity of fluoride toxicity (Anasuya Das, 1998). The plasma fluoride accounting for the three-fourths of the total amount of fluoride found in the whole blood and cells for the rest. Fluoride in plasma exists in free ionic and bound forms, the latter bound to the serum albumin forming about 85 per cent of the total amount fluoride in plasma (Taves, 1968). Plasma fluoride in normal individuals in non-fluoridated areas ranges from 0.14 to 0.19 ppm and is higher in fluorotic patients (Singer and Armstrong, 1969). However, abnormally high intake of fluoride through water and food may disturb the regulatory mechanism, which in turn may lead to elevated levels of fluoride in plasma (Anasuya Das, 1998). If the fluoride level in blood is more than the double amount in the control group, hypocalcemia sets in and symptoms of tetany may be observed. (Kierst, 1964).

2.5.4 Urine:

Usually fluoride excretion in urine is higher in subjects with fluorosis (1.0 to 2.0 ppm/day). A positive correlation between fluoride intake and urinary fluoride levels are observed in fluorosis (Anasuya Das, 1998). The elimination of absorbed fluoride occurs almost exclusively via the kidneys. Urinary levels of fluoride are higher in individuals exposed to higher intake of fluoride. The renal clearance of fluoride is directly related to urinary pH, and under some conditions to urinary flow
rate. In general, the higher the fluoride content in drinking water the higher the level of urinary fluoride in the population. All urinary calculi, even those constructed of organic compounds, contain fluoride (Machoy-Mokrzynska, 1993).

In areas where the water and foodstuffs are low in fluoride, it is always present in the urinary stones. Quantitatively, more fluoride is present in calculi with high calcium content (Editorial. Fluoride, 1980). Similarly, a somewhat less intense reaction is demonstrated by phosphorus and still less by magnesium. The role of calcium in fluoride binding in urinary calculi has been stressed earlier by other authors (Editorial. Fluoride, 1980).

2.5.5 Hormonal changes:

In fluorosis the basic abnormality is calcification of dental and skeletal tissues, as well as ligaments. The circulating hormone namely PTH, calcitonin, growth hormone are directly involved in calcium and bone metabolism (Anasuya Das, 1998). Vitamin D status of the individual modifies the expression of skeletal fluorosis. Teotia et al. (1978) found increased levels of calcitonin in the plasma of fluorotic subjects. Growth hormone levels are normal in fluorotic subjects (Sivakumar, 1977). High fluoride ingestion has a definite relationship with increased PTH secretion, which may be responsible for maintaining serum calcium levels and may have a role in toxic manifestations of fluorosis (Gupta et al., 2001).

2.6. Interactions between calcium, vitamin D and parathyroid hormone:

2.6.1 Calcium:

Calcium is the most abundant mineral in the human body. About 99 per cent of the total body calcium is found in the bones and teeth. It is essential in maintaining bone health and plays an integral role in the homeostatic balance between blood and bone calcium levels. The functions of calcium in bones include: bone formation and growth, maintenance of bone density, bone strength and structure, and preventing disease such as osteoporosis (Swaminathan, 1993).

The presence of calcium in each cell is the factor that attracts fluoride, which results in CaF₂ formation. The element is deposited in various forms in cells and tissues (Machoy-Mokrzynska, 1993).
One of the most characteristic features of the biological action of fluoride is its strong affinity to calcium. Calcified tissues (bones, teeth and nails) are the major target organs of fluoride. Not only is the absorption and retention of fluoride affected by dietary calcium, but calcium metabolism itself altered by the presence of fluoride. Acute fluoride intoxication is associated with low serum calcium. In chronic intoxication, calcium metabolism is often disturbed; the calcium content of the blood may be either elevated or reduced (Fluoride and soft tissue calcifications).

In general, calcium ions have relatively poor affinity toward proteins (Machoy, 1987). Calcium binding by certain proteins fails to influence fluoride binding, since protein-bound calcium is short of free valencies to form new chemical bonds. The main sources of calcium binding in proteins are dicarboxylic amino acids. However, the tendency to bind fluoride is exhibited by proteins that contain alkaline amino acids of lysine, arginine (Wieczorek et al., 1992), which would point to their electrostatic interaction.

Calcium ions are generally not specific activators of enzymatic reactions. However, some enzymes such as peptidases, alpha-amylases, phosphatases, ATP-ases are activated by calcium ions and are inhibited by added fluoride. One of the possible mechanisms of enzyme inhibition in these reactions may be calcium binding to fluoride in the catalytic center (Machoy, 1987).

Calcium is also the principal inorganic component of fingernails (1600 ppm) and may be what binds fluoride in them (Machoy-Mokrzynska, 1993). Fingernails easily absorb fluoride from the environment, even up to hundreds of parts per million and with the lapse of time, fluoride is desorbed. One of the factors accelerating the process of superficial desorption is certainly frequent hand washing with the use of alkaline agents.

2.6.2 Phosphorus:

Orthophosphate is known to appear in all living cells. Phosphate esters act as mediators in numerous metabolic processes.
The inhibitory action of fluoride was thought to arise from a magnesium-fluorine-phosphate complex (Machoy-Mokrzynska and Machoy, 1992).

2.6.2 Vitamin D:

Vitamin D is primarily responsible for maintaining blood calcium and phosphorus levels assisting in calcium absorption, which builds and maintains strong bones. It is necessary for the calcification of bone. In the absence of vitamin D, Ca absorption from the gut is diminished. Cartilage cells of matrix and the osteoid matrix are not calcified. When fluoride intoxication occurs in a vitamin D deficient individual the clinical expression of the disease varies. Defective ossification causes the typical deformities in bone such as knock-knees, bowing of legs, spinal curvature and also malformations of the chest and pelvis (Chatterjee, 1985).

2.6.3 Parathyroid hormone:

Mechanistically, parathyroid hormone preserves blood calcium by several major effects; stimulates production of the biologically active form of vitamin D within the kidney; facilitates mobilization of calcium and phosphate from bone; prevents detrimental increase in phosphate and maximizes tubular reabsorption of calcium within the kidney. This activity results in minimal loss of calcium in urine.

Serum calcium levels are closely regulated in the body so as to maintain optimal muscle contractility and cellular function. Several hormones are involved in this regulation. 1,25(OH)₂ vit. D₃ produced by the kidney, parathyroid hormone released by the parathyroid gland, and thyrocalcitonin released by the thyroid C cells. Each has a specific function with respect to serum calcium levels and all three are independent. 1,25(OH)₂ vit. D₃ increases blood calcium by increasing intestinal calcium absorption and decreases blood calcium by increasing calcium deposition in the bone. In the relative absence of active form of vitamin D₃, parathyroid hormone increases serum calcium levels by increasing the activity of the kidney 1α hydroxylase with the result of increasing blood levels of 1,25(OH)₂ vit. D₃. Parathyroid hormone in the presence of vitamin D has the reverse action on the bone. When both hormones (parathormone and 1,25(OH)₂ vit. D₃) are present, bone mineralization is stimulated. Even though the parathyroid hormone stimulates the production of 1,25(OH)₂ vit. D₃, the later does not stimulate parathyroid hormone release. Thyrocalcitonin serves to lower blood calcium
levels through stimulating bone calcium uptake and its effect is independent of parathyroid hormone still dependent on the availability of calcium. If serum calcium levels are elevated through a calcium infusion, thyrocalcitonin is released, which stimulates bone calcium uptake even in animals lacking both parathyroid hormone and vitamin D₃ (Carolyn, 1994).

Flow chart-2: Showing a possible mechanism of secondary hyperparathyroidism due to high fluoride ingestion (Gupta et al., 2001)

In fluorotoxic metabolic bone disease (FMBD), the PTH mid molecule (MM) values were found to be within the normal limits (Harinarayan et al., 2006).

2.7 Toxic effects of fluoride:

In high concentrations, as with almost all substances, fluoride compounds are toxic. Five grams of full strength of sodium fluoride will kill most adult humans, the lethal dose being 75 mg per kilogram (approx.) of body mass. The acute toxic dose of fluoride is believed to be from 2.0 to 8.0 mg per kilogram of body weight. Airborne fluoride can easily enter human respiratory tract and inhaled fluoride has been directly associated with respiratory failure. (Chen et al., 1999). Intratracheally instilled fluoride
in experimental animal activates alveolar macrophages, enhances the production of chemokines and proinflammatory cytokines, and causes polymorphonuclear leukocyte infiltration in the lung (Hirano and Ando, 1996; Hirano et al., 1999).

Some sources of fluoride poisoning:

- Air pollution
- Anesthetics
- Antibiotics
- Automobile wheel-cleaning products
- Contaminated beverages and food products
- Dietary supplements that contain sodium fluoride
- Fluoridated milk
- Fluoridated salt
- Fluoridation of public water supplies
- Fluoride tablets
- Glass-etching or Chrome-cleaning agents like ammonium bifluoride
- Groundwater pollution
- Household products
- Industrial exposure to fluxes used to lower melting points of metals.
- Insecticides containing sodium fluoride
- Mattresses emitting fluoride gases
- Scotchgard
- Soy products
- Rodenticides containing sodium fluoride
- Tea
- Teflon
- Toothpaste or other oral dental products containing sodium monofluorophosphate
- Vaccine contamination
Fluoride hampers the escape of calcium from bone tissue by increasing the mineralization of bones and activation of osteoblasts and in that way slows the pace of osteoporotic processes. This fails, however, to improve the physical properties of bone, e.g., strength (Editorial, 1983; Editorial, 1987).

In vitro studies have shown that fluoride is essential to initiate the deposition of calcium phosphate in “matrix vesicles” and thus, it facilitates the nucleation process prior to bone mineralization. Fluorides play an essential role in the formation of enamel in teeth. Some amount of fluoride is considered to be essential for the metabolism of bone and teeth, large doses of fluoride are known to lead to toxic effects. These toxic effects are of two types (Anasuya Das, 1998).

1. Acute toxicity
2. Chronic toxicity.

1. Acute toxicity:

This is as a result of single massive dose of fluoride. Fluoride toxicity is characterized by a variety of signs and symptoms. The onset of symptoms can be seen within minutes of exposure. Severity of symptoms can depend on the amount of fluoride compounds ingested.

In high concentrations, fluoride compounds are toxic and can cause death. An individual report involving fatality following the accidental administration of fluoride ion to a child at 5 mg/Kg was cited by Whitford (1987), while after experimenting on himself Baldwin (1899) reported symptoms of acute toxicity (e.g. gastrointestinal upset) occurred at doses as low as 0.1 to 0.3 mg per Kg body weight.

Skin or eye contact with many fluoride compounds in high concentrations is dangerous. In case of accidental swallowing, milk, calcium carbonate, or milk of magnesia is given to slow the absorption. Eye or skin contact is treated by removing the contamination by clothing and flushing with water.

The signs and symptoms of acute fluoride toxicity are as follows (Newbrun, 1987):

- **Gastrointestinal:** Nausea, vomiting, diarrhea, abdominal pain, and cramps.
- **Neurological:** Paresthesia, paresis, tetany, central nervous system depression, and coma.
Cardiovascular system: Weak pulse, hypotension, pallor, shock, cardiac irregularities and ultimately failure.

Blood chemistry: Acidosis, hypocalcemia, and hypomagnesaemia.

2. Chronic toxicity:

It is due to cumulative effect of consuming relatively high levels of fluoride continuously over several years. One of the manifestations of this type of toxicity is a disease called fluorosis.

The following are the factors, which could influence the severity of fluorosis (Teotia and Teotia, 1994):

1) Fluoride concentration in the drinking water;
2) daily intake of fluoride;
3) duration of fluoride exposure;
4) continuity of residence in the endemic area;
5) fluctuations in the fluoride intake;
6) age at the time of fluoride ingestion;
7) nutritional status, particularly the dietary intakes of calcium and vitamin D
8) physical hard work in a hot environment.

Symptoms for early detection of fluorosis:

Any discolouration of the enamel surface in front row of teeth of the patient (central and lateral incisors of the upper and lower jaw) may be due to dental fluorosis. This is an important clue for follow-up.

Aches and pains in the joints, viz. neck, back, hip, shoulder and knee without visible signs of fluid accumulation. These may be due to fluoride toxicity manifestations besides other reasons (Susheela and Majumdar, 1992; Susheela, 1998).

Non-ulcer dyspepsia, viz. nausea, vomiting, pain in the stomach, bloated feeling/gas formation in the stomach and constipation followed by diarrhea may be due to fluoride toxicity manifestations besides other reasons (Susheela and Majumdar, 1992; Gupta et al., 1992; Das et al., 1994; Dasarathy et al., 1996).
Polyurea (tendency to urinate more frequently) and polydipsia (excessive thirst), if detected, may be due to fluoride toxicity manifestations besides diabetes and/or other diseases (Susheela, 1997).

Muscle weakness, fatigue, anemia with very low hemoglobin levels may be due to fluoride toxicity besides other reasons (Susheela and Jain, 1986; Kaul and Susheela, 1976).

Complaints of repeated abortions/stillbirth and if the patient hails from an endemic area, one may suspect fluoride toxicity besides other reasons as fluoride is known to harden/calcify blood vessels and blood flow to the growing foetus is hampered (Susheela and Kharb, 1990).

Complaints of male infertility with abnormality in sperm morphology, oligospermia (deficiency of spermatozoa in the semen), azoospermia (absence of spermatozoa in the semen) and low testosterone levels and if the patient hails from an endemic area, one ought to suspect fluoride toxicity, besides other reasons (Susheela et al., 1996).

Toxic effects of fluoride released by bone resorption:

The fluoride content of bone increases with age according to the amount of fluoride absorbed and varies considerably from bone to bone and even in different parts of the same bone.

Fortunately, fluoride deposition in bone ordinarily prevents excessive fluoride accumulating in the blood. Detoxifying bone storage also occurs with other substances such as, Strontium 90. Deposited fluoride, however, is ultimately released during normal, constantly-occurring bone resorption.

The fluoride acquired from the mother in the developing bones of the foetus is gradually released in to the infant's blood by resorption of those bones. In breast-fed infants as efficient physiological barrier almost completely prevents passage of fluoride from the mothers blood into her milk. As a result, breastfed babies are normally prevented from ingesting more than very small amounts of fluoride.
Storage of fluoride in bone is a progressive process. Many small intakes of fluoride from drinking and using fluoridated water accumulate in the bone, where the concentration can increase to many times the level in the ingested water. Furthermore, the concentration of fluoride in the bone surrounding the lacunae containing osteocytes and in the walls of the canaliculi is considerably higher than in bone, in general. This high-fluoride bone surrounding the resorbing osteocytes is degraded during bone resorption, producing relatively high local concentrations of fluoride.

Killing or inactivating resorbing osteocytes not only disrupts normal resorption of bone but also interferes with its remodelling. Proper bone remodelling is especially necessary for the full development of the jaw and its alveolar processes in order to permit eruption of the teeth into their normal positions. Partial failure of alveolar bone remodelling during childhood is therefore likely to be an important factor in producing the increased malocclusion that has been associated with elevated fluoride ingestion.

Mode of action:

Ingested fluoride initially acts locally on the intestinal mucosa. It can form hydrofluoric acid in the stomach, which leads to gastrointestinal irritation or corrosion. After ingestion, the gastrointestinal tract is the earliest and most commonly affected organ system.

Fluorosis is of two types.
1. Industrial fluorosis.
2. Endemic fluorosis.

1. Industrial fluorosis:

It is observed in factory workers chronically exposed to fluoride gases emanating from industries based on fluorine containing ores eg. cryolite industries. Chronic exposure to fluoride in aluminium works may cause industrial fluorosis, which is characterized by increased mineral content of the bone tissue. Decreased bone mineral density was found in aluminium workers compared to the control group particularly in age groups of 40-44 and 50-54 years (Czerwinski and Lankosz, 1977).
2. Endemic fluorosis:

This is as a result of consumption of water and food containing high concentration of naturally occurring fluoride over prolonged periods. In both these types of fluorosis, dental and skeletal tissues are the primary targets and show most significant changes. The most prominent features of endemic fluorosis are dental and skeletal abnormalities, widely known as dental fluorosis and skeletal fluorosis, respectively.

In patients with fluorotoxic metabolic bone disease, the major clinical manifestations found are bone pain, tetany and dental mottling and the radiological findings included osteosclerosis, pseudo-fracture and ligamentous calcification. Recent studies have pointed to a primary tubular dysfunction leading to secondary nephronal loss and chronic renal failure eventually supervening in patients with fluorosis. The study also reveals that fluoride intoxication plays an important role in the pathogenesis of fluorotoxic metabolic bone disease, which is a unique osteo-renal syndrome (Harinarayan et al., 2006).

Hydrogen bonding by Fluoride ion - Mechanism of Toxicity:

Hydrogen bonds have the structure X-H-Y, in which X-H is a normal covalent bond and H-Y is the weaker hydrogen bond. Elements X and Y that participate best in hydrogen bonding are strongly electronegative ones like oxygen, nitrogen, and fluoride (as F). It is the O-H-O hydrogen bond that keeps water liquid at room temperature and makes it so high boiling for a molecular weight of only 18 atomic mass units. In macromolecules, N-H-O and N-H-N hydrogen bonds between amide groups and between thymine-adenine and cytosine-guanine base pairs bind protein and nucleoprotein chains together, respectively, as in the double helix of deoxyribonucleic acid.

According to Emsley (1981), the hydrogen bond between fluoride ion and the nitrogen-hydrogen linkage of amides, N-H-F, has a bond strength of 148 KJ/mol (35 kcal/mol) which makes it "the second strongest type of hydrogen bond and the strongest hetero nuclear hydrogen bond".
The strongest hydrogen bond is that of the bifluoride ion, F-H-F, estimated to have a bond energy of 214 KJ/mol (51 Kcal/mol). By contrast, the strength of intermolecular amide hydrogen bonds, N-H-O, is only about 20-40 KJ/mol (5 - 10 Kcal/mol). It therefore appears likely that in the presence of fluoride the N-H-O hydrogen bond can be replaced by the much stronger N-H-F hydrogen bond.

The fluoride ion is comparatively stable in aqueous solution and not very reactive in normal covalent bond-forming and bond-breaking reactions.

2.8 Biomarkers of Fluorosis:

Epidemiological studies by Dean in the 1942s clearly demonstrated the relationship between dental fluorosis in humans and the level of fluoride in water supplies. These and other studies have shown that in a population there is a direct relationship between the degree of fluorosis, the plasma and bone fluoride levels on the one hand and the concentration of fluoride in drinking water on the other hand. Fluorosis can be used as a biomarker for the level of fluoride exposure, though dental fluorosis is a reflection of fluoride exposure only during the time of enamel formation (WHO, 846, 1994).

2.8.1 Dental fluorosis:

Dental fluorosis is a condition of permanent hypomineralized change, with increased surface and sub-surface enamel porosity resulting from excess fluoride reaching the developing tooth prior to eruption (Fejerskov et al., 1990). It is a dose-response condition, so that higher intakes during the critical period of tooth development will result in more severe fluorosis (Dean, 1942; Larsen et al., 1987). It is an aesthetic and social problem besides being a health problem. The changes in enamel induced by fluoride were first described by Black and Mc Kay (1916) as mottled enamel. Teeth exhibit the first sign of chronic fluoride toxicity. Ameloblast (the enamel forming cells) is the most sensitive cell to fluoride toxicity. Enamel exhibits the first clinical changes in chronic fluoride toxicity. These dental changes help in the diagnosis of fluorosis. The characteristic feature of dental fluorosis is dental mottling. In this condition, the structure of the enamel is affected during formative stages resulting in hypoplasia known as mottled enamel. Minute abnormal white flecks, yellow flecks
characterize the mottling or brown spots or striations scattered irregularly over the tooth surface (Fig.1). The discoloration of teeth may change the colour from white, yellow brown to black. The discoloration may be in spots, streaks invariably horizontal in orientation as it is during the development of new layers of the matrix horizontally added.

The outermost covering of the tooth is the hard structure in the body with inorganic compounds mainly with the calcium salts. Enamel protects the tooth besides giving colour and luster. Normally, healthy dental enamel is semi-transparent, smooth and milky white in appearance. However, appearance of white opaque patches on the enamel may be indications of initial phases of dental fluorosis. In extreme cases of fluorosis not only does the entire dental enamel turn opaque white to brown, but also the teeth tend to break off easily and even their shape may begin to be affected.

In dental fluorosis the discoloration will be away from the gums and on the enamel surfaces and it can never be removed, as it is an integral part of tooth matrix. Calcium rich constituents of teeth, viz., enamel and dentin have strong affinity for fluoride during formation of teeth. Fluoride combines with calcium during the mineralisation of teeth forming calcium fluoro-apatite crystals. Enamel matrix is laid down in incremental lines before and after birth. Hence, dental fluorosis is invariably seen as horizontal lines or bands on the surface of teeth and never as vertical bands. It may also appear as spots.

Teeth commonly affected are 1) central incisors 2) lateral incisors and 3) molars of the permanent dentition. Fluorosis affects both the inner and outer surfaces of teeth. Teeth affected by fluorosis being poorly calcified (hypo-mineralized) loose enamel under the normal masticatory stress.

Enamel has no regenerative capacity. Once it is lost, it is lost forever. The Dentin is then exposed. Cavities formed in Dentin spread much faster and involves the pulp easily, leading to loss of teeth. The teeth once affected by dental fluorosis cannot be reverted to normal. But, the disclosed teeth can be masked by bleaching and or by other methods.
Fig. 1: Manifestation of Fluoride Toxicity in Teeth – Dental Fluorosis (Dean and Elvove, 1935)
For years it was assumed that fluoride absorbed during the secretary phase of tooth development had the greatest effect on fluorosis, but the research with animals (Johnson and Bawden, 1987; Richards et al., 1986) and humans suggests that the later maturation phase is more important.

Dental calculi are 70-80 per cent inorganic substances, wherein calcium makes up 40 per cent. Some of the compounds in tartar appear in the form of Ca_{10}(PO_4)_6F_2 and CaF_2. From a chemical viewpoint the formation of such salts is understandable. Electropositive calcium reacts with electronegative anions, such as fluoride and phosphate. Fluoride activity is so great that it may evict oxygen from a phosphate group, forming the anion PO_3F_2^- . These changes are not ionic bonding, since both compounds present reticular structure with durable stability.

The mechanisms of dental calculus formation may be depicted in the following manner: cytotoxic fluoride exerts, in the oral cavity, influence on degeneration and decomposition of many bacterial cells, speeding up their calcification, thereby, creating conditions for progressive calcification involving dental calculus. The possibility for CaF_2 formation is based on the existence of calcium ions as well as fluoride ions in saliva and dental plaque (Borysewicz-Lewicka, 1989). Earlier formed tartar is richer in fluorine compounds than recently formed calculi.

Based upon the severity of changes, attempts have been made to classify the degree of dental fluorosis into different grades (Fig. 2). This gradation of classification is based on an index of fluorosis developed by Dean and Elvove (1935) for use to relate the severity of fluorosis to the level of fluoride exposure.

2.8.1.1 Classification of dental fluorosis (WHO, 1997)

Stage 0 — Normal: The enamel surface is smooth, glossy and usually a pale creamy-white colour.

Stage 1 — Questionable: The enamel shows slight aberrations from the translucency of normal enamel, which may range from a few white flecks to occasional spots.
Fig. 2: Different Grades of Dental Fluorosis (Based on Dean’s index criteria, WHO, 1997)
Stage 2—Very mild: Small, opaque areas scattered irregularly over the tooth but involving less than 25 per cent of the label tooth surface.

Stage 3—Mild: The white opacity of the enamel of the teeth is more extensive than for code 2, but covers less than 50 per cent of the tooth surface.

Stage 4—Moderate: The enamel surfaces of the teeth show marked wear and brown stain is frequently a disfiguring feature.

Stage 5—Severe: The enamel surfaces are badly affected and hypoplasia is so marked that the general form of the tooth may be affected. There are pitted or worn areas and brown stains are widespread; the teeth often have a corroded appearance.

Stage 8—Excluded: (e.g. a crowned tooth).

Stage 9—Not recorded.

Galagan (1953) concluded that the higher incidence of fluorosis in individuals living in warmer climates was secondary to an increased level of fluoride exposure, probably due to consumption of larger amounts of water. In this way, enamel fluorosis was used as a biomarker to relate the optimum level of fluoride in the drinking water to the environmental temperature.

2.8.2 Skeletal fluorosis:

Skeletal fluorosis is a manifestation of fluoride toxicity caused by chronic ingestion or inhalation of fluoride (Krishnamachari, 1986). It is a para metabolic bone disease.

Fluoride is a cumulative toxin, which can alter accretion and resorption of bone tissue. It also affects the homeostasis of bone mineral metabolism. The total quantity of ingested fluoride is the single most important factor, which determines the clinical course of the disease.

Skeletal fluorosis often results in osteosclerosis of the skeleton with significant long-term difficulties, including impaired neck and lumbar mobility, aching of the axial skeleton, kyphosis, and painful lower extremities, ultimately causing crippling and incapacitation (Krishnamachari, 1986; Kleerekoper, 1996).
RADIOLOGICAL PRESENTATIONS
- Osteosclerosis
- Periosteal bone formation
- Calcification of interosseous membrane, ligaments, capsules, muscular attachments, tendons.
- Exostoses
- Osteophytosis
- Associated metabolic bone disease

CLINICAL PRESENTATION
- Heel pain
- Painful and restricted joint movements
- Deformities in Limbs
- Hunch back

IN EXTREME CASES
- Paralysis,
- Muscular wasting,
- Premature aging

Fig. 3: Different Clinical Features of Skeletal Fluorosis
Skeletal fluorosis is not easily recognizable until the disease has developed to an advanced stage. Excessive quantities of fluoride when deposited in the skeleton are more in cancellous bone compared to cortical bone. Changes in the bone will then be revealed through radiographs. Maximum ill effects of fluoride are detected in the neck, spine, knee, pelvic and shoulder joints. It also affects small joints of the hands and feet. The usual complaints of the patients, viz., pain in the neck, back, joints and rigidity begin in regions where cancellous bones predominate. With increased severity of skeletal fluorosis pain is associated with rigidity and restricted movement of cervical and lumbar spine, knee and pelvic joints as well as shoulder joints. Clinical features are presented in Fig. 3 (Mithal et al., 1993; Gupta et al., 1993; Wang et al., 1994).

2.8.2.1 Clinical features:

Stage 1: This is a relatively asymptomatic stage. It is usually encountered in young adults whose only complaints are vague pain in the small joints of hands and feet, knee and spinal joints.

Stage 2: In this stage, obvious stiffness of the spine with limitation in movement is observed.

Stage 3: At this stage the development of kyphosis is obvious. The subjects experience difficulty in walking and suffer from the following problems. Stiffness and limitation of the movements of various joints, especially inability to bend downwards, backwards and neck rigidity. This loss of movements is because of the calcification of intervertebral ligaments, which renders the vertebral column rigid. This abnormal calcification also results in narrowing of intervertebral foramen and spinal canal and compression of vertebral disc (Anasuya Das, 1998). Manifestation of fluoride toxicity in skeletal fluorosis is presented in Fig. 4 (Susheela et al., 1993).

The stage at which skeletal fluorosis becomes crippling usually occurs between 30 and 50 years of age in the endemic regions. Newcomers to a hyper endemic region may sometimes develop symptoms of skeletal involvement within 4 years of their arrival (Siddiqui, 1955). Men suffer more than women from severe effects of the disease presumably because their work is usually more strenuous than that of women (Siddiqui, 1955; Jolly et al., 1968). The factors which govern the development of skeletal fluorosis are (a) the prevalence of high levels of fluoride intake, (b) continual
Normal healthy individual
A. Can bend body and touch the floor/toes
C. Can touch chest with chin
E. Can stretch hands, fold arms and touch back of head

Fluoride toxicity manifestation
B. Unable to bend without folding knees
D. Unable to bend neck-touching chest with chin not possible
F. Unable to stretch hands fold arms and touch back of head

Fig. 4: Manifestation of fluoride toxicity in skeleton – Skeletal fluorosis
exposure to fluoride, (c) strenuous manual labour, (d) poor nutrition and (e) impaired renal function due to disease (Pandit et al., 1940; Daver, 1945). In regions with very high fluoride content, the disease may affect younger age groups including children. The longer the exposure to fluoride the higher will be its incidence. Epidemiological observations revealed that nutritional status might influence chronic fluoride toxicity.

The incident of fluorosis is higher in tropical and subtropical countries, probably because of higher drinking water consumption. It is observed that in adults who are exposed to high fluoride ingestion, the hydroxyl bonds of the hydroxy apatite material in bone are partly replaced by fluorides. It is surmised that in order to immobilize fluorine from the circulating fluoride phase in the body (blood and cellular fluids), the body’s defense mechanism fixes excess fluorides into hydroxy apatite material of the bone by replacement of OH\(^-\) by F\(^-\) (Teotia and Teotia, 1994) irreversibly till the exposure continues. In the process, the rate of synthesis of bone material (hydroxy apatite) is considerably increased leading to excessive bone formation or Osteosclerosis, a basic symptom of subjects suffering from skeletal fluorosis.

2.8.3 Genu valgum:

A new and serious dimension to the problem of skeletal fluorosis was added about a 3 decades ago when the National Institute of Nutrition at Hyderabad discovered that in parts of Andhra Pradesh where fluorosis has been long known to be endemic large numbers of adolescents and young adults had developed a deformity characterised by the outward bending of the legs from the knees down known as genu valgum or knock knees (Krishnamachari and Krishnaswamy, 1973). Most patients were between the ages of 10 and 30 years. All had dental changes. NIN studies reported that the disease is related to environmental changes consequent to the construction of the massive Nagarjunasagar dam. Briefly stated, the view is as follows: Construction of dams leads to an elevation in the level of subsoil water in the dam vicinity. The soil alkalinity rises and influences the concentration of trace elements in food grains grown in that area. The concentration of one trace element molybdenum goes up. Consumption of molybdenum-rich foods causes deficiency of copper in the body, which may lead to osteoporosis, a possible cause of genu valgum.
Map-2: Endemic Status of Fluorosis in India
The syndrome occurred mostly among young male adults and adolescents belonging to the poor socio-economic groups. This disease is not only crippling but also has socio-economic implications. The classical features of endemic fluorosis are dental and skeletal changes characterized by osteosclerosis of the spine, pelvis and other bones and calcification of interosseous membrane, of ligaments and muscular attachments. The cardinal feature of this syndrome is the occurrence of genu valgum and osteoporosis of the bones of the lower extremities in subjects suffering from endemic fluorosis. Genu valgum makes its appearance among children around 8 to 10 years of age residing in endemic fluorosis villages. The deformity develops slowly and progresses insidiously over many years, before the full-fledged clinical picture is established. The disease is painless, the deformity usually bilateral. The distance between the two medical malleoli when measured while the child is standing upright with the knees gently touching each other gives a measure of the degree of genu valgum. In advanced cases, the deformity restricts physical movements and impedes walking. When extreme, the lower extremities are so distorted that the knees actually cross over while the subject walks. Obvious shortening of stature occurs within a few years (Krishnamachari and Sivakumar, 1976).

2.9 Prevalence of Fluorosis in India:

Fluorosis is a crippling and painful disease caused by intake of fluoride. Fluorosis can affect young and old. Fluorosis is an endemic disease prevalent in 17 states. In 1960 only four states of India were identified for endemic fluorosis — namely Andhra Pradesh, Punjab, Tamil Nadu and Uttar Pradesh. In 1980 the problem was also identified in Delhi, Gujarat, Haryana, Karnataka, Madhya Pradesh and Rajasthan. Today there is clear epidemiological and clinical evidence that the problem exists in India. About 25 million people from these areas were found to be seriously affected with endemic fluorosis and about 40 million are at risk (ITRC, 1986-95).

Depending on the seriousness of problem, Indian states have been categorized into 3 groups (Map-2):

**Highly endemic:** Seventy to 100 per cent districts are affected in Andhra Pradesh, Gujarat and Rajasthan.
Table 3: Drinking water fluoride levels in different endemic areas

<table>
<thead>
<tr>
<th>Authors</th>
<th>Area</th>
<th>Water Fluoride levels (ppm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saralakumari and Ramakrishna Rao (1993)</td>
<td>Ralla Ananthapuram, Ananthapuram (Dt.), Andhra Pradesh</td>
<td>7.2 to 10.70</td>
</tr>
<tr>
<td>Sivaparvathi (1999)</td>
<td>Varikuntapadu mandal, Nellore (Dt.)</td>
<td>1.25 to 3.20</td>
</tr>
<tr>
<td>Silpa (2001)</td>
<td>Madanapalli division, Chittoor (Dt.)</td>
<td>1.6 to 2.1</td>
</tr>
<tr>
<td>Baby Devaki and Ramalakshmi (2001)</td>
<td>Raphadu mandal, Ananthapur (Dt.)</td>
<td>2 to 4</td>
</tr>
<tr>
<td>Shivasankara et al., (2000)</td>
<td>Gulbarga (Dt.), Karnataka</td>
<td>0.6 to 13.4</td>
</tr>
<tr>
<td>Pushpa Bharathi and Meera Rao (2003)</td>
<td>Dharwad (Dt.), Karnataka</td>
<td>4.0 to 13.5</td>
</tr>
<tr>
<td></td>
<td>Hirapur village, Mandla (Dt.)</td>
<td>1 to 13.5</td>
</tr>
<tr>
<td>Moudgil et al., (1986)</td>
<td>Faridabad-town, Haryana</td>
<td>7.3 to 29.0</td>
</tr>
<tr>
<td>Susheela et al., (1993)</td>
<td>Faridabad (Dt.), Haryana</td>
<td>0.25 to 8.00</td>
</tr>
<tr>
<td>Susheela et al., (1996)</td>
<td>Palem and mega city of Delhi</td>
<td>1.2 to 32.5</td>
</tr>
<tr>
<td>Sharma, (2003)</td>
<td>Jammu and Kashmir</td>
<td>&lt; 0.2 to 18</td>
</tr>
<tr>
<td></td>
<td>Himachal Pradesh</td>
<td>&lt; 0.2 to 6.5</td>
</tr>
<tr>
<td></td>
<td>Rajasthan</td>
<td>&gt; 1.5</td>
</tr>
<tr>
<td></td>
<td>Bihar</td>
<td>0.35 to 15</td>
</tr>
<tr>
<td></td>
<td>West Bengal</td>
<td>an average 12</td>
</tr>
<tr>
<td></td>
<td>Chattisgarh</td>
<td>15 to 20</td>
</tr>
<tr>
<td></td>
<td>Orissa</td>
<td>8.2 to 13.2</td>
</tr>
<tr>
<td></td>
<td>Maharashtra</td>
<td>0.7 to 6.0</td>
</tr>
<tr>
<td>Choubisa et al., (1997)</td>
<td>Dungarpur (Dt.), Rajasthan</td>
<td>0.50 to 10.8</td>
</tr>
<tr>
<td>Misra et al., (1992)</td>
<td>Lucknow, Uttar Pradesh</td>
<td>0.55 to 12.0</td>
</tr>
</tbody>
</table>
Moderately endemic: Forty to 70 per cent districts are affected in Bihar, Delhi, Jharkhand, Karnataka, Maharashtra, Madhya Pradesh, Orissa, Tamil Nadu and Uttar Pradesh.

Less endemic: Ten to 40 per cent districts are affected in Assam, Jammu & Kashmir, Kerala, Chattisgarh and West Bengal (Susheela, 2003).

In Andhra Pradesh, the fluorosis affected areas are Nalgonda, Ranga Reddy and Mahaboobnagar in Telangana region; Prakasam, Guntur and Krishna in coastal region and Anantapur, Kurnool and Cuddapah in Rayalaseema region (Lingeswara Rao, 2003). Rajgopal and Tobin (1991) reported that fluoride content increased with the depth of water source. The concentration of fluoride in drinking water is said to vary from one geographical region to another. According to Sangh et al. (1996) the fluoride content of drinking water varied from 0.5 to 25.0 ppm in different parts of India.

Ahuja Bhavadeep (2001) the fluoride content in drinking water samples varied from 1.6 to 20.6 ppm in different areas of Andhra Pradesh. In Telangana region, water fluoride level was reported to be 20.6 ppm in Nalgonda district, in Medak district the fluoride level was 3.0 ppm, in Mehboobnagar district the fluoride level was 6.4 ppm, in Warangal district the fluoride level was 5.8 ppm, in Kareemnagar district the fluoride level was 4.9 ppm, in Hyderabad district the fluoride level was 4.8 ppm, in Nizamabad district the fluoride level was 3.0 ppm and in Adilabad district the fluoride level was 2.8 ppm.

In Coastal region, water fluoride level was 12.0 ppm in Prakasam district, in Visakhapatnam district the fluoride levels above 5.0 ppm, in Guntur district the fluoride level was 10.0 ppm, in Nellore district the fluoride level was 8.0 ppm, in Srikakulam district the fluoride level was 3.0 ppm, in East Godavari district the fluoride level was 1.6 ppm.

In Rayalaseema region, the reported water fluoride level was 10.1 ppm in Anantapur district, 9.6 ppm in Kurnool district, 4.6 ppm in Cuddapah district and 3.0 ppm in Chittoor district. Based on research studies drinking water fluoride levels in different endemic areas are presented in Table-3.
## Table 4: Prevalence of Dental Fluorosis in Different Endemic Areas

<table>
<thead>
<tr>
<th>Authors</th>
<th>Area</th>
<th>Age group (years)</th>
<th>n</th>
<th>Dental fluorosis (per cent)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saralakumari and Ramakrishna Rao (1993)</td>
<td>Ralla Ananthapuram, Ananthapuram (Dt.), Andhra Pradesh</td>
<td>&lt; 20</td>
<td>162</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td></td>
<td>&gt; 20</td>
<td>156</td>
<td>92.3</td>
</tr>
<tr>
<td>Baby Devaki and Ramalakshmi (2001)</td>
<td>Raphadu mandal, Ananthapur (Dt.)</td>
<td>7 to 15</td>
<td>268</td>
<td>94.7</td>
</tr>
<tr>
<td></td>
<td></td>
<td>16 to &gt; 50</td>
<td>587</td>
<td>71.8</td>
</tr>
<tr>
<td>Silpa (2001)</td>
<td>Madanapalli division, Chittoor (Dt.)</td>
<td>16 to &gt; 50</td>
<td>396</td>
<td>15</td>
</tr>
<tr>
<td>Sivaparvathi (1999)</td>
<td>Varikuntapadu mandal, Nellore (Dt.)</td>
<td>7 to 15</td>
<td>230</td>
<td>52.7</td>
</tr>
<tr>
<td></td>
<td></td>
<td>16 to &gt; 50</td>
<td>314</td>
<td>80.8</td>
</tr>
<tr>
<td>Shivasankara et al., (2000)</td>
<td>Gulbarga (Dt.), Karnataka</td>
<td>3 to 10</td>
<td>46</td>
<td>89</td>
</tr>
<tr>
<td>Pushpa Bharathi and Meera Rao (2003)</td>
<td>Dharwad (Dt.), Karnataka</td>
<td>7 to 9</td>
<td>224</td>
<td>32.5</td>
</tr>
<tr>
<td></td>
<td></td>
<td>10 to 12</td>
<td></td>
<td>43.7</td>
</tr>
<tr>
<td></td>
<td></td>
<td>13 to 15</td>
<td></td>
<td>19</td>
</tr>
<tr>
<td></td>
<td></td>
<td>&lt; 15 to 80</td>
<td>133</td>
<td>25</td>
</tr>
<tr>
<td>Misra et al., (1992)</td>
<td>Lucknow, Uttar Pradesh</td>
<td>3 to 12</td>
<td>69</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td></td>
<td>13 to 90</td>
<td>78</td>
<td>96.1</td>
</tr>
<tr>
<td>Choubisa et al., (1997)</td>
<td>Dungarpur (Dt.), Rajasthan</td>
<td>&lt; 16 years</td>
<td>1224</td>
<td>62.7</td>
</tr>
<tr>
<td></td>
<td></td>
<td>&gt; 16 years</td>
<td>1364</td>
<td>69.7</td>
</tr>
<tr>
<td>Mukta Agrawal and Purva Johri (1998)</td>
<td>Banasthali village, Rajasthan</td>
<td>2 to 18</td>
<td>198</td>
<td>100</td>
</tr>
<tr>
<td>Chakma et al., (2000)</td>
<td>Tilapalpani village</td>
<td>&lt; 20</td>
<td>84</td>
<td>51.1</td>
</tr>
<tr>
<td></td>
<td>Hirapur village, Mandla (Dt.)</td>
<td>&gt; 20</td>
<td>36</td>
<td>22.2</td>
</tr>
<tr>
<td>Susheela et al., (1993)</td>
<td>Faridabad (Dt.), Haryana</td>
<td>&gt; 16</td>
<td>1953</td>
<td>58</td>
</tr>
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</table>
Table-5: Prevalence of Skeletal Fluorosis in Different Endemic Areas

<table>
<thead>
<tr>
<th>Authors</th>
<th>Area</th>
<th>Age group (years)</th>
<th>n</th>
<th>Skeletal fluorosis (per cent)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saralakumari and Ramakrishna Rao</td>
<td>Rallu Ananthapuram, Ananthapuram (Dt.), Andhra Pradesh</td>
<td>&gt; 20</td>
<td>129</td>
<td>76.3</td>
</tr>
<tr>
<td>Sivaparvathi (1999)</td>
<td>Varikuntapadu mandal, Nellore (Dt.)</td>
<td>16 to &gt; 50</td>
<td>314</td>
<td>27.3</td>
</tr>
<tr>
<td>Baby Devaki and Ramalakshmi</td>
<td>Rapthudu mandal, Ananthapur (Dt.)</td>
<td>21 to 50</td>
<td>476</td>
<td>64.1</td>
</tr>
<tr>
<td>Silpa (2001)</td>
<td>Madanapalli division, Chittoor (Dt.)</td>
<td>&gt; 50</td>
<td>398</td>
<td>13.8</td>
</tr>
<tr>
<td>Shivasankara et al., (2000)</td>
<td>Gulbarga (Dt.), Karnataka</td>
<td>3 to 10</td>
<td>18</td>
<td>39</td>
</tr>
<tr>
<td>Pushpa Bharathi and Meera Rao</td>
<td>Dharwada (Dt.), Karnataka</td>
<td>&gt; 19 to 80</td>
<td>166</td>
<td>31.20</td>
</tr>
<tr>
<td>Choubisa et al., (1997)</td>
<td>Dungarpur (Dt.), Rajasthan</td>
<td>21 to 60</td>
<td>1955</td>
<td>27.8</td>
</tr>
<tr>
<td>Misra et al., (1992)</td>
<td>Lucknow, Uttar Pradesh</td>
<td>13 to 90</td>
<td>78</td>
<td>46.1</td>
</tr>
<tr>
<td>Chakma et al., (2000)</td>
<td>Tilapalpani village</td>
<td>&gt; 20</td>
<td>142</td>
<td>22.0</td>
</tr>
<tr>
<td>Susheela et al., (1993)</td>
<td>Faridabad (Dt.), Haryana</td>
<td>&gt; 16</td>
<td>1953</td>
<td>27</td>
</tr>
</tbody>
</table>
2.10 Fluorosis Prevalence Studies:

2.10.1 Dental Fluorosis:

Based on research studies prevalence of dental fluorosis in different endemic areas is presented in Table-4.

2.10.2 Skeletal Fluorosis:

Based on research studies prevalence of skeletal fluorosis in different endemic areas is presented in Table-5.

2.11 Fluoride Status Studies:

The studies conducted in fluorotic areas of different parts in India such as Delhi (Susheela et al., 1996), Dharwada district of Karnataka (Pushpa Bharathi and Meera Rao, 2003), Varikuntapadu mandal of Nellore district (Sivaparvathi, 1999), Rapthadu mandal of Ananthapur district (Baby Devaki and Ramalakshmi, 2001), Madanapalli division of Chittoor district (Silpa, 2001), Jaipur district of Haryana (Yadav and Latha, 2003), endemic fluorotic areas of Rajasthan (Gupta et al., 2001) showed elevated levels of serum and urinary fluoride.

2.12 Bone Mineral Status Studies as related to fluorosis:

Bone mineral studies revealed that the fluorosis-affected subjects had normal levels of calcium and phosphorus. Alkaline phosphatase levels were significantly elevated in fluorosis subjects.

The studies in endemic areas from Lucknow (Misra et al., 1992), Hyderabad (Raghuramulu et al., 1997), Nalgonda district (Srikantia and Siddiqui, 1965), Kheru Nayak thanda of Gulbarga district (Shivashankara et al., 2000), Mandla district of Rajasthan (Chakma et al., 2000), villages of Rajasthan (Gupta et al., 2001) showed normal serum calcium and phosphorus and elevated alkaline phosphatase levels.

Fluoride produces metabolic changes in bone by enhancing both osteoclastic and osteoblastic activities. The former leads to osteoporosis and the latter to a state of calcium deficiency finally leading to rickets and osteomalacia. (Rao et al., 1968).
Unloading the skeleton of excess mineral seems to increase urinary calcium excretion causing nephrolithiasis and increments in serum creatinine levels (Etah et al., 2007)

2.13. Ca-turnover studies:

An alteration in bone metabolism is a basic feature of this disease. Calcium plays an important role in bone metabolism. National Institute of Nutrition conducted studies on turnover of calcium in fluorosis (Rao et al., 1968; Narasinga Rao et al., 1977). In calcium turnover studies carried out on monkeys, experimental fluorosis was induced by normal diets as well as diets containing low amounts of vitamin C and calcium, which aggravated the conditions (Sriranga Reddy and Srikantia, 1971). Ca$^{45}$ kinetics showed that the cumulative retention of radioactive calcium was high in experimental animals as compared to control group. These data clearly indicated that the level of dietary calcium influences fluoride toxicity (Sriranga Reddy and Narasing Rao, 1977). Radioactive studies with calcium$^{45}$ in skeletal fluorosis subjects indicated that there is increased absorption and retention of calcium due to both increased intestinal absorption and lowered urinary excretion (Rao et al., 1968). Calcium turnover studies using Ca$^{47}$ in subjects with genuvalgum had higher rate of total body calcium turnover as compared to fluorosis subjects (Narasinga Rao et al., 1977).

2.14. Biochemical Status Studies as related to fluorosis:

Biochemical studies reported that the fluorosis-affected subjects had normal or low levels of protein, albumin and globulin i.e., protein levels have not increased.

Srikantia and Siddique (1965) reported normal serum protein and low albumin and high globulin levels in fluorotic adults (n=31) residing in endemic villages of Nalgonda district. Silpa (2001) also reported, normal serum protein levels in fluorotic and non-fluorotic males and females residing in Madanapalli division of Chittoor district. Normally hydroxyproline excreted in urine is a measurement of break down of collagen of the bone. Krishnamachari (National Institute of Nutrition, 1978) conducted a study on subjects with fluorosis, with and without genuvalgum and normal subjects and showed that the urinary hydroxyproline levels were increased in genuvalgum subjects compared to normal subjects. Shivashankara et al. (2000) reported elevated serum alanine transaminase, and normal protein and albumin levels in fluorotic children residing in endemic area of Kheru Nayak thanda of Gulbarga district.
2.15 Hormonal Status Studies as related to fluorosis:

Hormonal status studies revealed that the fluorosis-affected subjects had normal levels of 25(OH)D levels and elevated PTH levels.

Srivastava et al. (1989) reported significantly elevated serum parathyroid hormone and normal serum 25(OH)D in fluorotics. Raghuramulu et al. (1997) reported the low serum 25(OH)D$_3$ levels in skeletal fluorosis subjects, and subjects without bone deformity in endemic area than in subjects from non-endemic area. Sivakumar et al., (1977) reported that the concentration of parathyroid hormone was considerably much higher in fluoroctic subjects compared to normal subjects, but this level was higher in genuvalgum subjects. Gupta et al. (2001) also reported elevated serum parathyroid hormone levels in villages of Rajasthan.

2.16 Nutritional status as related to fluorosis:

Studies reported so far comprised of anthropometric and dietary investigations as related to fluorosis studies. Anthropometric studies showed that the fluorosis-affected subjects had low levels of BMI in the two studies reported so far. Experimental studies on animals also showed growth retardation with excess of fluoride and decrease in body weight.

Sivaparvathi (1999) reported low BMI in fluorotic males and females residing in Varikuntapadu mandal of Nellore district of AP. Thus, the fluorotics were suffering from chronic energy deficiency grades I and III in males and females, respectively. Silpa (2001) reported fluorotic males and females residing in Madanapalli division of Chittoor district of Andhra Pradesh were suffering from low normal BMI.

Chinoy et al., (1993) investigated beneficial effects of ascorbic acid (AA) and calcium on reversal of fluoride toxicity in male rats. The rats treated with NaF for 30 days showed a decrease in body weight compared to the control. The body weights of NaF+AA treated rats were significantly recovered compared to the NaF alone treated ones. The recovery in body weights of NaF+Ca$^{2+}$ treated rats was less (P<0.02) compared to the NaF+AA group. In another study, the excess of fluoride either in diet
or in drinking water retards growth and cause decrease in body weight reported in male mice (Chinoy and Sequeira, 1989).

Dietary survey studies reported that the fluorosis-affected subjects had low intakes of energy, protein, calcium and vitamin C.

The studies conducted in rural areas of Farid Kot district of Punjab (Sangh and Bal, 1998), in Tilai pani and Hirapur villages of Mandla district of central India (Chakma et al., 2000) and in Banasthali village of Rajasthan district (Mukta Agrawal and Purva Johri, 1998) reported that the intakes of energy, protein, calcium and vitamin C were lower compared to the RDA.

2.17 Effect of Nutrients Supplementation on Fluoride Toxicity:

Nutrients supplementation studies showed decreased effect on fluoride toxicity in fluorosis affected subjects. Experimental studies on animals also showed the beneficial effects on reversal of fluoride toxicity.

Gupta et al., (1994) in children in the age groups of 3-12 years residing in two fluorotic areas of Shivadaspura and Vanasthali of Jaipur, showed that the supplementation of 250 mg calcium, 500 mg ascorbic acid and 800 IU vitamin D₃ in tablet form showed significant increase in serum calcium and ascorbic acid levels and at the same time significant decrease in serum alkaline phosphatase, serum and urinary fluoride levels.

Chinoy et al., (1993) investigated on beneficial effects of ascorbic acid and calcium on reversal of fluoride toxicity in male rats. The serum protein level significant recovery observed with ascorbic acid (50 mg) treatment group than the calcium (62.5 mg) treatment group. Serum protein levels showed a significant decline with sodium fluoride (10 mg) treatment group compared to control group.

Chen et al., (1999) reported the relationship between milk consumption and the incidence of dental fluorosis among the suburban and rural children 8 to 14 years residing in Pingxiang district of China. The rate of dental fluorosis of the milk-consuming children (7.2 per cent) was lower than that of non-milk consuming children (37.5 per cent).
Khandare et al., (2004) evaluated the effect of tamarind ingestion over defluoridated water supply to adolescent boys (13 to 14 years) residing in welfare hostels from Nalgonda district of Andhra Pradesh. After 3 weeks of tamarind ingestion, there was significant increase in urinary pH and fluoride excretion and significant decrease in calcium and copper. There was no change in magnesium as compared with controls ingesting defluoridated water without tamarind supplements. Khandare et al., (2000) also studied the effect of tamarind ingestion on fluoride toxicity in dogs and showed the beneficial effect of 10 gms of tamarind increases urinary pH and facilitates increased fluoride excretion and deceased retention in bone.

Medical intervention is not possible for this disease due to non-availability of any specific treatment modality (Arjun et al., 2004).

Due to the strong affinity of fluoride toward calcium, the latter is an antidote for fluoride poisoning (Editorial, 1980).

The administration of calcium compounds is recommended in the treatment of intoxication by fluorine compounds as well as in prophylaxis. In such situations, the simplest procedure is to consume milk, which is rich in calcium and proteins. The CaF_2 which is formed is only slightly soluble in aqueous solution and hence is less toxic (Machoy-Mokrzynska et al., 1993).

The effect of cereals on fluoride retention has been studied in normal humans. Fluoride excretion in urine was significantly high on rice-based diets as compared with a juwar-based diet (Lakshmaiah and Srikantaiah, 1977).

Similar experiments on rats using sorghum, wheat, and rice based diets have shown increased retention of fluoride in femur bone with sorghum-based diets as compared with wheat and rice based diets (Lakshmi and Lakshmaiah, 1999).

Tamarind is used extensively as a souring agent in raw and cooked forms in Indian food preparations, more so in the southern part of India. Previous studies in dogs and humans (Khandare et al., 2000 & 2002) suggested the beneficial effect of tamarind ingestion on fluoride toxicity by way of increased urinary excretion and decreased retention in bone when tamarind was given with fluoridated water.
Defluoridated water alone has a beneficial effect by mobilizing fluoride from the bone, but the process is very slow. Tamarind increases urinary pH and increased urinary pH facilitates increased fluoride excretion. The present study investigated the possibility that tamarind ingestion might provide an additional beneficial effect of mobilizing bone fluoride after providing children with defluoridated water (Arjun et al., 2004). A significant correlation between fractional fluoride clearance and urinary pH was shown by Whitford et al. (1976).

The dependence of fluoride reabsorption on urinary pH is similar to the renal handling of other weak acids. Hydrogen fluoride is a weak acid, and in its undissociated form (HF) appears to be more readily reabsorbable. The pK for the reaction HF → H + F is between 3.45 and 3.23 (Patel et al., 1971), which has been proved by several other studies (Whitford et al., 1982; Whitford and Pashley 1991).

Several studies have reported the renal clearance of fluoride to always be lower with acid urine than with alkaline urine (Whitford et al., 1976; Ekstrand et al., 1982; Ekstrand et al., 1980; Whitford et al., 1977).

Increase in urinary fluoride on tamarind supplementation and defluoridated water suggests that the incremental fluoride excretion was from the bone pool. Tartaric acid is a major component of the tamarind paste (8.4 per cent to 12.4 per cent), which does not get metabolized and is excreted as such through the urine. Inhibition of carbonic anhydrase by tartrate may lead to production of alkaline urine, and there are efforts to determine its mechanism.