CHAPTER-I

Introduction
Fluorosis is an endemic disease prevalent in 17 states in India resulting from long-term exposure to high levels of fluoride (Susheela, 1999; WHO, 1984). Sixty two million people in 150 districts in India are exposed to the risk of fluorosis (Susheela, 1999). The concentration of fluoride in drinking water varies from one geographical region to another. The water fluoride levels range from 0.5 to 25.0 ppm in different parts of the country. World Health Organisation (WHO) defines a water fluoride >1ppm as endemic for fluorosis. Continued ingestion of excess amount of fluoride accumulates in the body and the major site of fluoride accumulation is the bone. Fluoride is a potent toxin, which can alter accretion and resorption of bone tissues. The most prominent features of endemic fluorosis are dental and skeletal abnormalities. The characteristic feature of dental fluorosis is dental mottling and teeth exhibit the first signs of chronic fluoride toxicity. The skeletal changes are caused by accumulation of excessively ingested fluoride, which has been incorporated into the hydroxyl apatite crystals leading to formation of fluoroapatite. The newly formed bone with fluoroapatite structure is poor in crystalline and matrix strength, which leads to a spectrum of changes of skeletal fluorosis namely osteoporosis, osteosclerosis, osteomalacia and fracture of bone (Krishnamachari, 1986; Susheela et al., 1993). These differences may be due to several factors, such as dose, duration of fluoride exposure, environmental factors, dietary habits or their combination (Teotia et al., 1994). The severity of fluorosis increases with age, which is also indicative of age dependent accumulation of fluoride in the body. Males are more vulnerable showing higher severity of dental and skeletal fluorosis compared to females (Kaharna et al., 1997; Choubisia et al., 1997; Ray et al., 1981; Pushpa Bharathi and Meera Rao, 2003). Endemic fluorosis in India with dental and skeletal changes was first reported from surveys in Nellore district by Shortt et al (1937).

Calcium is essential for the maintenance of bone health and plays an integral role in the homeostatic process that maintains a proper balance between calcium levels in blood and bone. The functions of calcium in bone include: bone formation and growth, maintenance of bone density, bone strength and structure, and preventing osteoporosis. Deficiencies in calcium and vitamin D can cause decreased bone density (osteoporosis), weak bones, and can lead to diseases including rickets, stress fractures and osteopenia. Vitamin D is necessary for the calcification of bone.
In the absence of vitamin D, calcium absorption from the gut is diminished. Parathyroid hormone regulates calcium level in blood and calcium metabolism in the bones. It acts directly on the bone releasing calcium from bones and thus maintaining the plasma calcium levels (Swaminathan, 1993).

Fluoride is a potent toxin, which can alter accretion and resorption of bone tissue. It also affects 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, which is characterized by immobilization of joints of the axial skeleton and other major joints of the extremities. A combination of osteosclerosis, osteomalacia, and osteoporosis of varying degrees as well as exostosis formation characterizes the bone lesions. In a proportion of cases secondary hyperparathyroidism is observed with associated, characteristic bone changes. Increased metabolic bone turn over, impaired bone collagen synthesis and increased avidity for calcium are the features in fluoride toxicity. Alterations in hormones concerned with bone mineral metabolism are seen in fluorosis (Krishnamachari, 1986). In vitamin D deficient individuals, cartilage cells of matrix and the osteoid matrix are not calcified when fluoride intoxication occurs (Chatterjee, 1985). Fluorosis has been linked to the combination of excess fluoride, low calcium intake and high PTH levels. The increase in PTH correlated well with excess fluoride ingestion (Dote et al, 2002).

In addition, fluoride has been shown to inhibit the activity of many enzymes such as those involved in the pentose pathway, antioxidant defense systems, and the myosin ATPase path (Park et al., 1999; Vani et al., 2000). Generation of free radicals and enhanced lipid peroxidation has also been considered to play an important role in the pathogenesis of fluorosis (Rzeuski et al., 1998; Sharma et al., 1998; Zhi-Zhong et al., 1989; Shanthakumari et al., 2006).

Fluoride is believed to replace the hydroxyl ion and possibly the bicarbonate ion associated with hydroxyapatite, a mineral phase during formation of bone (Mc Cann et al., 1957) forming hydroxyfluoroapatite, altering the mineral structure of bones (Chachra et al., 1999). Fluoroapatite is less soluble, more compact, and slower to undergo remodeling in bone (Grynpas, 1990). Fluoride is a cumulative poison that increases metabolic turnover of the bone in favour of bone formation. It stimulates
bone cell proliferation by directly inhibiting osteoblastic acid phosphatase activity and by prolonging or enhancing the mitogenic signals of growth factors (Krishnamachari, 1986; Gupta et al., 1993). Therefore, it is necessary to survey bone formation and bone resorption as a result of absorption of fluoride, as well as urinary contents of fluoride, calcium and phosphate (Ando et al. 1998). Recent studies (Topuz et al. 2006; Ando et al. 1998) show that high concentrations of fluoride in water and food lead to the stimulation of bone resorption markers in fluorotic adults. In animal study (Turner et al., 1997), fluoride treatment leads to the stimulation of bone resorption and is coupled to increase in bone formation markers.

Studies on the kinetics of calcium metabolism using radioactive $^{45}$Ca showed that in florotics simultaneous increase in bone formation as well as resorption occurs. While osteosclerosis a main feature of fluorosis, could be a reflection of higher rate of collagen mineralisation (bone formation), increased plasma hyperparathyroid activity often encountered in endemic fluorosis explains the phenomenon of increased bone catabolism (Sriranga reddy et al., 1977).

Srivastava et al. (1989) reported normal calcium, normal 25(OH) D3 and 1,25(OH) 2D3 but elevated PTH and serum alkaline phosphatase levels in fluorotic subjects. Similar results have been reported by other investigators (Sivakumar and Krishnamachari, 1976; Shivashankara et al., 2000; Gupta et al., 2001). Raghuramulu et al. (1997) showed significantly higher levels of serum 25(OH) D in genuvalgum subjects. Involvement of vitamin D, a closely related nutrient in bone metabolism and its conversion from 25(OH) D3 to 1,25(OH) 2D3 metabolite needs to be explored in subjects with endemic genuvalgum (Raghuramulu et al., 1997). Vijayabhaskar et al. (2007) reported elevated levels of MDA but decreased levels of CAT and GST in fluorotic subjects. Other investigators have also reported similar findings (Shanthakumari et al., 2007, Shivarajashankara et al., 2001). Barat (1998) reported normal levels of TSH and T3 but elevated T4 in fluorotic subjects.

Biochemical studies reveal that fluorotic subjects have normal serum calcium, phosphorus but elevated alkaline phosphatase levels (Srikantia and Siddique, 1965; Krishnamachari, 1978; Moudgil et al., 1986; Misra et al., 1992; Chakma et al., 2000; Shivashankara et al., 2000).
Research studies so far carried out on fluorosis are mostly prevalence studies. Very few studies have addressed biochemical and radiological aspects (Krishnamachari and Kamala Krishnaswamy, 1973; Chakma et al., 2000; Shivashankara et al., 2000; Moudgil et al., 1986; Misra et al., 1992; Gupta et al., et al 1993). Srivastava et al. (1989) and Gupta et al. (2001) carried out hormonal and radiological and very few other studies assessed oxidative stress in fluorotic subjects (Shantha kumnri et al., 2006; Shivarajashankara et al., 2001). Studies involving oxidative, biochemical and hormonal assessment with a comprehensive approach are lacking and in India, few data are available on changes in biochemical markers of bone metabolism such as serum bone specific alkaline phosphatase (BAP), tartrate-resistant acid phosphatase-5b (TRAP-5b) and urinary excretion measurement of C-terminal telopeptides of type-I collagen (CTX). This investigation has been undertaken with a view to examine such aspects in South Indian fluorotic adults of both sexes, who had ingested water with fluoride content of >5-8.2 ppm.

Objectives of the study:

The investigation was planned with the following objectives:

1. To compute the prevalence and severity of dental and skeletal fluorosis in the selected areas.

2. To assess fluoride status, bone mineral status and biochemical markers of bone metabolism status of fluorotic and their non-fluorotic counterparts.

3. To assess calcitropic hormonal status, thyroid status, oxidative stress and antioxidant enzyme status of fluorotics and their non-fluorotics counterparts.

4. To study the differences between fluorotic and non-fluorotic counter parts for various parameters.

5. To study the associations between fluoride status and other parameters-bone mineral status, biochemical markers of bone metabolism status, calcitropic hormonal status, thyroid status, oxidative stress and antioxidant enzyme status indices.