"ENVIRONMENTAL HEALTH PERSPECTIVE

OF

FLUOROSIS IN CHILDREN"

A THESIS SUBMITTED
UNIVERSITY OF RAJASTHAN
FOR THE DEGREE
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DOCTOR OF PHILOSOPHY
(ENVIRONMENTAL SCIENCE)

By

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INDIA

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IN LOVING MEMORY
OF
MY BELOVED MOTHER

LATE SMT. KRISHNA KUMARI GUPTA

Phone : { 510134 / 511071 / 511175 { Ex. : 248 (DIRECT)

इन्दिरा गांधी मानव पारिस्थितिकी, पर्यावरण एवं जनसंख्या अध्ययन केन्द्र
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CERTIFICATE

This is to certify that the thesis entitled "ENVIRONMENTAL HEALTH PERSPECTIVE OF FLUOROSIS IN CHILDREN" submitted by *SUNIL KUMAR GUPTA* for the degree of Doctor of Philosophy (Environmental Science) of the University of Rajasthan is the result of bonafide research work carried out by him under my supervision and guidance. He worked for more than 100 days each year during his research period. This thesis has not previously formed the basis of the award of any degree, diploma, fellowship or any other similar title or recognition.

(Dr. T.I Khan)

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(SUNIL KUMAR GUPTA)

PREFACE

Fluorosis is a common problem in most developing countries. In Rajasthan alone all 32 districts have been identified as fluorosis prone areas. While the WHO standards permit only 1.5 mg/l as a safe limit for human consumption people in several districts of Rajasthan are bound to consume water with fluoride concentrations even up to 28 mg/l. This causes permanent deformities, severe joint pains, general debility and misery in human being. Fluorosis is known to take the three forms: clinical, skeletal and dental. Considerable work has been done all over the world on all these aspects. Unfortunately the results appeared to indicate that the manifestations in fluorosis are irreversible.

The severity of the problem prompted the Department of Science and Technology, Government of Rajasthan (DST) to sponsor an investigation on fluorosis affected children. The study showed that fluorosis could be reversed at least in children. This was a unique finding and created much interest in the scientific community. In view of the great promise of this finding another investigation was carried out on a larger sample with double blind control in areas with different fluoride concentrations in drinking water. This study confirmed that fluorosis is indeed reversible at least in children.

A more detailed investigation was then taken up to evaluate the effect of high fluoride ingestion on human health and to examine the mechanism of the reversal of fluorosis through Clinical and Biochemical examination. This study explained the physiological and biochemical processes of fluorosis in children. This thesis embodies the results of all these field studies and laboratory experiments conducted on the environmental health aspects of fluorosis in children.

It is expected that appropriate State and Non Government agencies will be using these findings to control and cure the fluorosis cases in the country.

SUMMARY

This thesis embodies the results of the field studies and laboratory experiments conducted on the environmental health aspects of fluorosis in children, at the environmental science laboratory of Indira Gandhi Center, and, laboratory of the department of physiology, SMS medical college, University of Rajasthan, Jaipur.

Fluorosis is a common problem in most developing countries. In Rajasthan alone all 32 districts have been identified as fluorosis prone areas. While the WHO standards permit only 1.5 mg/l as a safe limit for human consumption people in several districts in Rajasthan are consuming water with fluoride concentrations even up to 44 mg/l. This causes permanent deformities, severe joint pains, general debility and misery in human being.

Fluorosis is known to take the three forms: clinical, skeletal and dental. Considerable work has been done all over the world on all these aspects. Unfortunately the results appeared to indicate that the manifestations in fluorosis are irreversible.

In view of the gravity of the problem this study was designed with the following objectives to find out the solution of the problem at treatment level and at preventive level.

1. To study the environmental and dietary factors, viz. fluoride intake through drinking water and eatables, dietary calcium, protein and vitamin C, responsible for fluoride toxicity,
2. To evaluate the effect of high fluoride ingestion on human health through Clinical and Biochemical examination.

3. Critical analysis based on available literature to evolve a comprehensive health management strategy to overcome the problem of fluorosis in children.

Four target areas were then selected. The villages representing target areas were Rampura (drinking water fluoride 4.6 mg/l), Ram Sagar Ki Dhani (drinking water fluoride 2.4 mg/l), Shivdaspura (drinking water fluoride 5.6 mg/l) and Raipuria (drinking water fluoride 13.6 mg/l).

50 Fluorosis affected children aged up to 12 years from a cross section of each of the target area (village) comprising different socio-economic groups were chosen.

These children were evaluated for nutritional status especially for vitamin C, fluoride and calcium in diet. The severity of fluorosis was evaluated by dental, clinical, skeletal staging and biochemical estimations.

All types of presentations of clinical, dental and skeletal fluorosis were observed in these areas.

The presentation of Dental fluorosis ranged from grade 0 to 4, Clinical fluorosis from grade 1 to 2 and Skeletal fluorosis from grade 1 to 3. The severity of presentation was maximum in Raipuria, the area with highest fluoride concentration in drinking water (13.6 mg/l). The severe forms of presentations observed in this area were enamel hypoplasia in dental fluorosis, rarefaction of the long and flat bone in skeletal fluorosis and severe form of clinical presentations in clinical fluorosis.

The dietary factors relating to fluoride indicated that water fluoride concentration is one of the important parameters for the toxicity and clinical presentations of the disease. Apart form this, the lack of dietary protein intake,

and, low dietary calcium and ascorbic acid were other factors found to be associated with increased toxicity of fluoride intake.

The dietary fluoride intake was almost equal in all areas. The major difference in fluoride intake was mainly related to the water fluoride intake. The clinical presentations could not be related to dietary protein, calcium and vitamin C intake in this study, as dietary intake of protein, calcium and vitamin C was almost equal in all four areas.

Serum calcium levels in all the four target areas were in low to normal range (7.6 to 10.2 mg/dl). Considering the pathophysiology of fluorosis and the compensatory Parathyroid (PTH) mechanism, the observed findings are well with in the expectations.

The serum alkaline phosphatase (SAP) activity was observed to be high (20-52 KA Units). The observed analysis of the values indicates that there is no correlation between SAP activity and fluoride levels in drinking water.

The normal range of Serum inorganic phosphorus (SIP) is 3.7 - 5.6 mg/dl in the age group of 4-12 years. The SIP levels were observed (5.08 – 5.58 mg/dl) to be within normal range before starting the treatment.

The serum ascorbic acid was observed to be in the normal range (0.3-1.8 mg/dl) in all the target areas except one (group B). The leukocyte ascorbic acid levels (3 - 43 $\mu g/10^8$ WBCs) were well below the normal range in all areas

N - Acetyl neuraminic acid (Sialic acid), a component of glycoprotein, is an important parameter in detection of fluoride toxicity. The normal value ranges from 59-64 mg/dl in human being. Lower values (23.5 - 57.2) of sialic acid were observed.

The glucosaminoglycans (GAG) are components of glycoproteins. The normal values of GAG are 9-11 mg/dl. Elevated values of GAG (15-43 mg/dl) have been observed in all target areas.

High blood fluoride (0.5 -1.9 mg/l) and serum fluoride (0.5-1.5 mg/l) levels were observed in all areas.

Urinary fluoride level has been used to estimate the amount of fluoride absorbed and is recognized as one of the best indices of fluoride intake. Increased urinary fluoride levels (4-30 mg/l) in children of all groups were observed.

Parathyroid hormone levels (PTH) were high in all groups. The PTH levels were higher (30 – 260 pmol/l) in areas consuming more fluoride in comparison to areas consuming less fluoride in drinking water.

Ingestion of fluoride leads to a decrease in serum calcium levels (hypocalcemia) and increases in the serum PTH. The system attempts to correct the hypocalcemia by leaching out calcium from the body reserve mainly in the bones. This calcium is bound to the ground substance (collagen matrix). An increase in the PTH action degrades the ground substance by increasing the sulphation of chondroitin sulphate, not only in the bones, but all over the body. This in turn greatly enhances the leaching of calcium from bones (bone resorption) and therefore increases the levels of hyaluronic acid in bone and other tissues. Increased degradation and excretion of hydroxyproline, glucosaminoglycans, phosphates and pyrophosphates have also been observed with increased PTH. An increase in the PTH action thus impairs the laying down of collagen fiber. It has been observed that in fluoride toxicity there is a decrease in hydroxyproline (effects the solubility of collagen), decrease in lysine (decreases the collagen cross linkage and increases the solubility of collagen

protein) and an increase in proline residue (due to impairment in hydroxylation process). It has been hypothesised that all these effects are mediated through hyperparathyroidism secondary to increased fluoride ingestion.

In teeth the major changes associated with fluorosis are appearance of dermatan sulphate with decrease in molecular size of the hydroxyappetite crystal, which may be due to some catabolic events leading to degradation of large parent molecule and inhibition of the biosynthetic assembly of such molecule.

Depending upon the above observations the possible approaches indicated are treatment of the disease and prevention.

* *Treatment of the disease*

Calcium, Vitamin C and Vitamin D supplementation

The presence of calcium in gut directly effects the absorption of fluoride ions and will also improve serum calcium levels. Vitamin D3 in low doses enhances calcium absorption and retention without causing hypercalcemia and thus directly effects the absorption of fluoride ions. It also inhibits the excessive release of parathyroid hormone thereby preventing excessive activation of osteoblasts thus preventing hyperosteoidosis and osteopenia. Ascorbic acid controls collagen formation, maintains the teeth structure and is also essential for bone formation. These structures are adversely effected by higher fluoride intake.

* *Prevention*

Reduce the intake of fluoride by reducing the fluoride intake through water, by using simple defluoridation process.

Another strategy to avoid the reemergence of the disease once treated is to change the dietary habits without disturbing the available resources of food and the customs, so as to get protein, calcium and vitamin C rich diet.

THIS STUDY EXPLAINS THE PHYSIOLOGICAL AND BIOCHEMICAL PROCESSES OF FLUOROSIS IN CHILDREN, ALONG WITH PREVENTION AND TREATMENT OF FLUOROSIS.

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INTRODUCTION

INTRODUCTION

FLUORIDE is a natural beneficial nutrient found in varying concentrations in air, water and soil. When consumed in optimal amounts, it improves dental health. All of the major medical and dental organizations endorse the local use of fluorides and ingestion of fluoridated water as the most effective dental public health measure in existence. If the drinking water contains fluorides in a range of 0.6 to 0.7 ppm, no additional fluoride need be taken. WHO standards permit 1.5 mg/l as a safe limit for human consumption

Too much fluoride ingestion during childhood, however, can lead to fluorosis. The American Dental Association and the American Academy of Pediatrics have recently changed their recommendations for optimal fluoride exposure in children in order to prevent fluorosis but maintain dental protection.

Fluorosis is a disease that affects people of all age groups. There is ample evidence to prove that even newborn babies have also been its victims. It not only affects the body of a person but also renders them socially and culturally crippled. The form of F and the route and duration of consumption as well as individual susceptibilities, have varied the toxic effects to such an extent that a comparison of the data obtained is scarcely warranted. Since the degree of dental fluorosis may in part be related to the fluoride content of the water supply, the influence of regional climate conditions on water consumption and therefore on total fluoride ingestion should be recognized.

A number of biological effects have been ascribed to fluorides. They include the effects on bone, teeth, kidney, thyroid, neurological functions and growth in general.

General effects of high fluoride ingestion related to the doses of fluoride are indicated below:

Concentration or dose of fluoride	Medium	Effect
0.0022 ppm	Air	Injury to vegetation
1 ppm	Food or Water	Dental caries protection
2 ppm or more	Food or Water	Mottled teeth enamel
8 ppm	Food or Water	10% osteosclerosis
50 ppm	Food or water	Thyroid changes
100 ppm	Food or water	Growth retardation
125 ppm or more	Food or water	Kidney changes
20-80 mg/day or more	Food or Water	Crippling fluorosis
2.5-5.0 g	Acute dose	Death

EXTENT OF THE PROBLEM

Fluorosis, though a common endemic problem in almost all developing countries, and India, is more wide spread and acute in the state of Rajasthan where all the 32 districts have been declared as fluorosis prone areas. A sizable percentage of the population including all age groups is severely affected by fluorosis in fluoride rich areas. People in several districts in Rajasthan are consuming water with fluoride concentrations of up to 44 mg/l. It has led to permanent deformities, severe joint pains, general debility and misery to a large population. The status in Rajasthan and India is indicated below.

- In Rajasthan all the 32 districts have been declared as fluorosis prone areas. A few of the worst affected districts are Nagore, Jaipur, Sikar, Jodhpur, Badmer, Ajmer, Sirohi, Jhunjhunu, Churu, Bikaner, and Ganganagar.
- In India the problem has reached alarming proportions affecting at least 15 states. They are:

(I) States where 50-100% districts are affected:

Andhra Pradesh, Tamil Nadu, Uttar Pradesh, Gujrat, Rajasthan

(II) States where 30-50% districts are affected:

Bihar, Harayana, Karnataka, Maharashtra, Madhya Pradesh, Punjab.

(III) States where less than 30 % districts are affected:

J & K, Delhi, Orissa, Kerala

The need to develop a well thought out strategy to attack this problem, therefore, can not be over emphasized. It requires an urgent attention of medical as well as of social workers. Despite the crying need there seems to be no orchestrated research and developmental effort to tackle fluoride toxicity in the country. International community is not interested in pursuing research in this direction because they are not facing any acute problem. Thus Indian researchers must gear themselves to handle this malaise. This will go a long way in improving the lot of afflicted population especially in Rajasthan.

There is a need to plan a multipronged attack on the problem. Whatever strategy is designed, it has to cover the medical, social as well as environmental aspects. The slackness on either front will not yield the desired results. Therefore the areas needing

urgent attention are treatment of the disease and prevention through treatment of the water and changing dietary habits.

- *Treatment of the disease*: Literature reviewed indicated that fluorosis can not be reversed.
- *Prevention*: Reduce the intake of fluoride by (a) reducing the fluoride intake through water, by using effective defluoridation process (b) changing the dietary habits without disturbing the available resources of food and the customs, so as to get protein, calcium and vitamin C rich diet.

This study was envisaged to

1. study the environmental and dietary factors,
2. evaluate the effect of high fluoride ingestion on human health,
3. evaluate the methods of defluoridation, and to
4. evolve a health management strategy to overcome the problem of fluorosis in general and specifically in children.

OBJECTIVES

The objectives of this study were as follows:

1. To study the environmental and dietary factors, viz. fluoride intake through drinking water and eatables, dietary calcium, protein and vitamin C, responsible for fluoride toxicity,
2. To evaluate the effect of high fluoride ingestion on human health through Clinical and Biochemical examination.
3. Critical analysis based on available literature to evolve a comprehensive health management strategy to overcome the problem of fluorosis in children.

Review of literature relating to sources and metabolism of fluoride

A. SOURCES

The fluoride content of surface and underground waters depends on a wide variety of factors, the major being the availability and solubility of the parent fluoride minerals with which these waters are in contact. (WHO, 1970).

Fluorides in drinking water of Rajasthan have been found to originate from indigenous rocks, which extend from Delhi to Gujarat. The geological distribution of rocks in Rajasthan reveals that fluorotic ores occupy large areas of eastern and south-east parts of this state, in constricted synclinal bands in the central region of Aravali synchronium. Secondly, around the mica mines, ground water is rich in fluorides and Rajasthan is a rich source of mica. (Shiv Chandra, 1983).

The sources of fluoride in surface or ground waters are

1. The sea
2. The atmosphere
3. The earth's crust
   (1) Rock forming minerals
   (2) Rocks
   (3) Commercial ores
   (4) Soils

Rocks, soil, water, air, plants and animals all contain fluoride in widely varying concentrations. Fluoride enters the body by ingestion or by inhalation. Part of it is absorbed and parts excreted. High fluoride foods such as tea or some fish may increase intake significantly. The use of fluorides in industry leads to occupational exposure.

Major sources of fluoride for human exposure are Water, Food, Air, Medicament, and Cosmetics (RGDWM, 1993)

1. a. *Water*

Fluorine accounts for about 0.3 gm/kg of the earth crust. In 1987 IARC classified inorganic fluoride in-group 3. Usually the surface waters are not contaminated with high fluoride, whereas ground water may contain high fluoride because the usual source of fluoride is fluoride rich rocks. When water percolates through these rocks it leaches out fluoride from these resulting in building up of fluoride concentration. The rocks rich in fluoride are:

Flurospar CaF_2 (Sedimentary rocks, lime stones, sand stones)

Cryolite Na_3AlFPO_6 (Igneous, Granite)

Fluoroapetite $Ca_3(PO)_2$ $Ca(FCl)_2$

The average concentration of fluoride in different rocks are given below:

Rocks	**Average fluoride element content (ppm)**
Granites	870
Slates and clays	800
Basalts	360
Phosphorites	31000
Sandstone	180
Limestone	220

Although there are several sources of fluoride intake, it is roughly estimated that 60% of the total intake is through drinking water. (RGDWM, 1993)

b. *Food*

The fluoride of food items depends upon the fluoride contents of the soil and water used for irrigation, therefore the fluoride content of the food items may vary from place to place. The available data indicate that in general the fluoride content of the various food items is as follows (given in decreasing amount of fluoride)

Cereals > leafy vegetables> pulses> fish Meat> Fruits

Few examples of fluoride rich food items are given below

Water Tea Fluoridated toothpaste Pan Supari Tobacco

Green garlic Onion Cabbage Soyabean Carrot Corn Potato

Baking powder egg Cows liver & kidney

Plants and vegetables grown in soil and water rich in fluoride.

Infants who ingest high amounts of fluoride can be at risk of dental fluorosis (Heilman-JR *et al.*, 1997). The authors analyzed the fluoride concentration of 238 commercially available infant foods. Fluoride concentrations ranged from 0.01 to 8.38 micrograms of fluoride per gram, with the highest fluoride concentrations found in infant foods containing chicken. Therefore Infant foods, especially those containing chicken, should be considered when determining total fluoride intake.

The possible association of risk factors between mild-to-moderate enamel fluorosis and exposure during early childhood to infant formula, fluoride toothpaste,

and/or fluoride supplements was also shown (Levy-SM, 1994; Pendrys-DG *et al.*, 1994; Levy-SM *et al.* 1991). Analysis revealed that mild-to-moderate enamel fluorosis on early forming enamel surfaces was strongly associated with both milk-based and soy-based infant formula use, as well as with frequent brushing.

An exhaustive study, which focuses on the fluoride content of crops and other items grown/available in Anantpur district in A.P. was conducted (Venkateswaralu Rao and Mahajan, 1991). The highlights of the publication are: -

1. 98 food items commonly used in 41 villages of Anatanpur district Andhra Pradesh, were investigated for fluoride content.
2. 32 locally grown agricultural crops are known to have fluoride ranging from 0.2 to 11.0 mg/kg with the exception of coconut water where even traces of fluoride was not detected.
3. The intake of Fluoride from water and food ranges from 2.2-7.3 mg. (0.05-0.32 mg/kg-1 BW).
4. The 6 brands of tea analysed for Fluoride have shown the fluoride content ranges from 60 mg/kg to 112 mg/kg.

The results of the studies conducted by Sengupta and Pal (1971), Lakdawala and Puneker (1973), Chari *et al.* (1975) and Rajyalaxmi (1982) have been given in Annexure1.

c. *Drugs*

Prolonged use of certain drugs has been associated with the chronic adverse effects of fluoride e.g. sodium fluoride for treatment of osteoporosis, Niflumic acid for the treatment of rheumatoid arthritis, use of fluoride mouth rinse (Proflo) to render the tooth stronger (RGDWM, 1993).

d. *Air*

The use of fluorides in industry leads to occupational exposure e.g. inorganic fluoride compounds is used in the production of aluminum. Fluorides are also released during the manufacture and the use of phosphate fertilizers (RGDWM, 1993).

e. *Cosmetics viz. Toothpaste's & Mouth Rinses*

Highly significant associations were found between estimated fluoride ingestion from toothpaste and fluorosis (Rock *et al.*, 1997; Levy-SM *et al.*, 1997). The mean DMF score of the fluorosis group was half that of the fluorosis-free children. The results of the study suggest that toothpaste swallowing may be a factor in the production of fluorosis. A need was expressed (Holt-RD & Murray-JJ, 1997) to clarify the most appropriate fluoride concentration in toothpaste's used for children; ensure that other sources of fluoride do not increase the risk of dental fluorosis: investigate the effectiveness of fluoride toothpaste in inhibiting root surface caries in adults. Manufacturers should: continue to improve the performance of fluoride toothpaste; ensure that all pastes maximize fluoride bioavailability; develop active agents to help reduce oral disease;

label products clearly with ppm F; review the delivery systems so as to reduce the risk of dental fluorosis.

Thirty Brands of Toothpastes (which are labeled as `Fluoridated' and those which are not labeled as Fluoridated but both manufactured by the same firm) have been analyzed for fluoride content by laboratories practicing quality control procedures. It has been evident that there is no brand of toothpaste so far analyzed that is free of fluoride. The fluoride content arising from the raw material used for the manufacturing of paste viz. calcium carbonate, talc and chalk have high fluoride arising as a contaminant from raw materials, can be as high as 800-1000 ppm. In the fluoridated brands, there is a deliberate addition of fluoride, which may range form 1000-4000 ppm. (RGDWM, 1993)

The mouth rinses that are sold with special brand names, are nothing but fluoridated water of a very high content of fluoride. (RGDWM, 1993)

The contention has been that if fluoridated tooth paste or mouth rinse when used (the reason(s) for its use will be dealt in diseases of the teeth), it is only a topical application and when the oral cavity is rinsed with water, the chemical is washed away. These views no longer hold good as the blood vessels in the oral mucosa and the sublingual blood vessel (the one below the tongue) absorb fluoride within minutes. The sub-lingual blood vessel is used as a route of drug delivery, especially for cardiac patients. Indian studies have also shown that absorption of fluoride within minutes after brushing the teeth with fluoridated toothpaste (Rajan, *et al.* 1987; 1988, 1989). The Saliva obtained from the oral cavity when analyzed for Fluoride; an hour after brushing the teeth has also revealed high fluoride content.

In view of these evidences, the Drugs and Cosmetics Act of 1945, which had no specific stipulation(s) in the addition of toxic chemicals to tooth paste, was amended during 1991. The Draft Gazette notification had brought out 3 stipulations for the manufacturing toothpaste.

e1. The tooth paste, when manufactured, should not contain more than 1000 ppm of Fluoride (the 1000 ppm is not for deliberate addition) but was providing room for the natural contaminant fluoride, as it was considered unfair to the manufacturer that the raw material should be defluoridated and then manufacture the paste.

e2. Children below 7 years should not use Fluoride containing toothpaste, and this should be inscribed on every tube and carton.

e3. Expiry and manufacturing date should be inscribed on the carton as some brands of paste are known to decompose due to addition of sodium monoflurophosphate.

The final Gazette Notification was published in 1992, almost a year after the publication of the Draft Gazette Notification (60 days are normally granted for eliciting the view of the public) and strange enough the 2nd stipulation that children below the age of 7 year should not use Fluoride containing toothpaste, and the warning to be inscribed on the carton and tube `vanished' from the notification.

f. Other

f1. Inorganic fluoride compounds are used in the production of aluminum and use of phosphate fertilizers. (RGDWM, 1993)

f2. Apart from the available drinking water supply the bottled mineral water may also be a source of excessive fluoride ingestion. Villena-RS *et al.* (1998) in their study reported that specific bottled waters contained: 1) Significant concentrations of fluoride not reported by the producer; 2) Fluoride concentrations of no preventive effect, although the producer had advertised the water as a Fluoridated Mineral Water; 3) Fluoride concentrations high enough to cause dental fluorosis, although the producer did not alert the consumer to this fact. It is therefore concluded, that a sanitary regulatory system for the control of the level of fluoride in the bottled mineral waters marketed is necessary.

CHEMOBIOKINETICS AND METABOLISM

A large proportion of the ingested and inhaled fluoride is rapidly absorbed through gastrointestinal tract and lungs. Absorbed fluoride is carried by blood and is excreted via the renal system or gets accumulated in calcified tissues. Adults and children over 3 years of age readily excrete about 90% of the fluoride they ingest. Children under three years of age excrete only about 50% of the ingested fluoride. Approximately 90% of the fluoride retained in the body are deposited in the skeleton and teeth.

Most of the fluoride bound in the skeleton and teeth has a biological half-life of several years. No significant accumulation occurs in soft tissues. Renal excretion appears to be based on glomerular filtration followed by a variable tubular re-absorption, which is higher at low pH and low urinary flow rates. Fluoride passes through the placenta and also appears in low concentrations in saliva, sweat, and milk.

Unless the ingested fluoride is in an insoluble form it is quickly absorbed (Ericsson, 1958; Weddle and Muhler, 1954;Hudge, 1965) and although excretion via the kidney is very efficient, up to **half the absorbed fluoride is incorporated into the skeleton**, where it accumulates with time (Largent & Heyroth, 1949).

The plasma concentration of fluoride does not correspond to the cells of the mineralizing tissues (Weatherell, 1969). When fluoride is absorbed, there will be a temporary increase in fluoride gradient from plasma to the bone cells forming appetite crystallites of the mineralizing tissues. During this time, the small, highly hydrated crystallites in areas of active mineralisation and possibly also the inter-crystalline protein

will trap relatively large amounts of fluoride. A short time after ingestion, however, the fluoride concentration in the plasma will fall, the concentration gradient will be reversed and the fluoride acquired at the mineralizing front will tend to diffuse back from the crystallites across the cell into the plasma (Weatherell, 1969; Costeas *et al.*., 1970). In this way, the small crystallites and perhaps also the protein and water in the areas of active mineralisation will tend to trap and retain fluoride near the membrane of the mineralizing tissue cells at a level which is high relative to the fluoride concentration in the blood.

Normal plasma F- levels of individuals in non- - fluoridated areas vary between 0.007 and 0.02 ppm. Following low F- intakes of 1 or 2 mg, the peaks are reached after 30 min, followed by a rapid elimination over 4 to 6 h (Henschler D *et al.*, 1975).

24-h Patterns of Free Plasma Fluoride

Fluoride (F) in urine has long been known to reflect intestinal F absorption as well as F liberation from a fluoride-rich skeleton, but blood and plasma F reflects a limited absorption process of gradual F depletion of an F-rich skeleton. Some of the variations of plasma inorganic F may have been obscured by analytical methods, which have comprised the fraction of organically bound plasma F also. While the ionizable F (F-) of a young person's fasting plasma generally is as low as 0.01 – 0.02 ppm, or 0.5 – 1 uM/1, the organically bound F may be 5 to 10 times higher. The nature of organic F bonding in plasma is not yet understood, but it is evident that the ionized or easily ionizable plasma F is the physiologically active fraction. (Ericsson Y , 1975)

PERMISSIBLE LIMITS OF FLUORIDES IN POTABLE WATERS

The permissible limit of fluoride in drinking is 1.0-1.5 mg/l as F (Manual on Water Supply and Treatment, 1991). U.S. Public Health Service Drinking Water Standards (1962) allow a fluoride concentration in drinking water from 0.8 to 1.7 mg/l, dependent on the annual average of maximum daily air temperature of the area concerned. **(Table 1)**

Table – 1: Recommended Limits of Fluoride Concentration
(USPHS Drinking Water Standards, 1962)

Annual Average of maximum daily air temperatures ^{O}C	Recommended control limits mg F/l		
	Lower	Optimum	Upper
10.0-12.1	0.9	1.2	1.7
12.1-14.6	0.8	1.1	1.5
14.7-17.7	0.8	1.0	1.3
17.8-21.4	0.7	0.9	1.2
21.5-26.2	0.7	0.8	1.0
26.3-32.5	0.6	0.7	0.8

The fluoride temperature relationship is based on the premise that children drink more water in warm climates, and therefore the fluoride content in the water supply should be reduced to prevent excessive total fluoride consumption **(Choi *et al.*, 1979).**

World Health Organisation guidelines allows up to 1.5 mg/l of fluorides in drinking water. (WHO, 1984)

MAGNITUDE OF THE PROBLEM (RGDWM, 1993)

Fluorosis continues to be an endemic problem. More and more areas are being discovered where people are forced to ingest fluoride rich water, as there is no alternative. There is also a growing concern about the effect of fluoride on the fetus especially on the formation of primary teeth. Defluoridation of water, though a simple process has not been put to successful use in most areas.

1. *International Status*

The problem of high fluorides exists in many countries like, Pakistan, Bangladesh, Argentina, United States of America, Morocco, Middle-east countries, Japan, South African Countries, New Zealand, Thailand etc.

2 National Status

The problem has reached alarming proportions affecting at least 15 states of India. They are further categorized as follows:

2.1 States in which 50-100% districts are affected;

Andhra Pradesh, Tamil Nadu, Uttar Pradesh, Gujrat, Rajasthan

2.2 States in which 30-50% districts are affected;

Bihar, Harayana, Karnataka, Maharashtra, Madhya Pradesh, Punjab.

2.3 States in which less than 30 % districts are affected;

J & K, Delhi, Orissa, Kerala

3. *Status in Rajasthan*

All the 32 districts in Rajasthan have been declared as fluorosis prone areas. The worst affected districts are Nagaur, Jaipur, Sikar, Jodhpur, Badmer, Ajmer, Sirohi, Jhunjhunu, Churu, Bikaner, Ganganagar etc.

As per a survey carried out by Public Health Engineering Department, Rajasthan in year 1991-93 , for status of water supply in villages / habitations, nearly 16560 (about 20%) villages/habitations were found to be affected by excess fluoride (more than 1.5 ppm), out of which 5461 villages /habitations had fluoride more than 3 ppm **(Table 2).**

Table 2: Fluoride Affected Villages /Habitation

(PHED survey 1991-93)

S. NO.	DISTRICT	TOTAL			FLUORIDE >1.5mg/l			FLUORIDE >3.0mg/l		
		Vill.	Habit.	Total	Vill.	Habit.	Total	Vill.	Habit.	Total
1	AJMER	985	952	1937	654	371	1025	352	232	584
2	ALWAR	1946	2449	4395	537	342	879	155	68	223
3	BANSWARA	1431	3175	4606	293	551	844	35	60	95
4	BHARATPUR	1345	549	1894	529	81	610	152	11	163
5	BARMER	1623	2780	4403	597	221	818	181	68	249
6	BHILWARA	1566	968	2534	678	318	996	392	227	619
7	BIKANER	580	366	946	84	2	86	7	0	7
8	BUNDI	826	332	1158	42	9	51	3	0	3
9	CHITTORGARH	2173	904	3077	115	48	163	14	9	23
10	CHURU	926	199	1125	240	8	248	27	1	28
11	DHOLPUR	551	983	1534	142	157	299	22	18	40
12	DUNGARPUR	846	681	1527	127	235	362	30	55	85
13	GANGANAGAR	4437	4190	8627	426	418	844	149	129	278
14	JAIPUR	3140	7618	10758	1187	1985	3172	491	739	1230
15	JAISALMER	518	1172	1690	300	184	484	96	65	161
16	JALORE	666	823	1489	369	107	476	115	45	160
17	JHALAWAR	1448	124	1572	42	5	47	15	3	18
18	JHUNJHUNU	824	208	1032	96	3	99	15	1	16
19	JODHPUR	860	2801	3661	314	99	413	59	8	67
20	KOTA	1881	288	2169	44	0	44	17	0	17
21	NAGAUR	1374	1972	3346	778	147	925	322	42	364
22	PALI	904	651	1555	242	88	330	69	34	103
23	S. MADHOPUR	1464	2191	3655	452	268	720	121	69	190
24	SIKAR	931	2401	3332	331	461	792	125	144	269
25	SIROHI	446	92	538	176	5	181	43	1	44
26	TONK	1019	881	1900	515	209	724	199	71	270
27	UDAIPUR	3179	5561	8740	431	497	928	74	81	155
	TOTAL	37889	45311	83200	9741	6819	16560	3280	2181	5461

REVIEW OF LITERATURE RELATING TO FLUORINE AND HUMAN HEALTH

Fluorine is the most electronegative of all elements. It has not only notable chemical qualities but also has physiological properties of great importance for human health. The discovery of a relation between excessive contents of fluorides in water and endemic mottling of the enamel (circa 1930) was perhaps the starting point of research into fluorides and hard tissue physiology and pathology.

TOXIC EFFECTS OF LARGER DOSES OF FLUORIDE

Investigations of toxic effects of fluoride in humans have evoked a lively interest throughout the world because public health programs of fluoridation for the prevention of dental caries have always been considered to involve the risk of remote cumulative intoxication. However, the indices of early intoxication are poorly defined and this has resulted in an element of speculation and confusion about the toxic potentialities of the fluoride ion. At the very onset, a clear distinction must be made between acute toxic effects, which result from a single massive dose, and the chronic toxic effect of large doses spread over a number of years. The latter may be confined to a minor physiological alteration or may produce a major crippling disease.

Acute Fluoride Intoxication

Current knowledge about the doses of fluoride that produce acute intoxication is derived principally from suicidal or accidental poisoning (Lidbeck, Hill & Beeman, 1943; Sharkey & Simpson, 1933). With the more widespread use of fluoride in industry, in agriculture and in the home, there is need for additional evaluation of acute toxic effects.

The acute lethal dose of fluoride for man is probably about 5 g as NaF (Goodman & Gilman, 1965). Although the precise doses of different fluorides are not known, the probable range is 2-10 g for soluble compounds such as hydrofluoric acid, hydrofluosilicic acid, potassium fluoride, sodium fluoride, sodium fluosilicate, and ammonium fluoride. The form of F used and the method and length of administration, as well as individual susceptibilities, have varied the toxic effects to such an extent that a comparison of the data obtained is scarcely warranted. However, there is evidence that fluosilicates are more toxic than either NaF or CaF_2 and that NaF is more toxic than CaF_2 . The minimum fatal dose is determined by the carrying vehicle, by the promptness and completeness of vomiting and by the speed of initiation of therapy.

Acute fluoride intoxication, whether caused by ingestion or inhalation of relatively large amounts of fluoride-containing compounds, has not been so well described as chronic fluoride intoxication. This is due, in part at least, to the fact that acute fluoride intoxication is rare. In Roholm's (1937) worldwide survey of the published literature on the subject, 112 cases were recorded. Greenwood (1940) extended this survey and recorded 18 additional cases. From 1939 to 1957, 305 additional cases were recorded in the medical literature. The latter figure suggests a wider prevalence than really exists, because 263 cases of poisoning occurred in a single episode at the Oregon

State Hospital (Lidbeck, Hill & Beeman, 1943). Thus, in the medical reports covering a period of 85 years, there were only 132 scattered cases of acute fluoride poisoning, with 303 additional cases related to two epidemic-type accidents.

The acute effects of the ingestion of massive doses of fluoride are first those of an irritant poison, and later become apparent in enzyme systems such as those engaged in metabolism, energetics, and cellular respiration and in endocrine functions. However, no system of the body can be considered exempt. Thus, in cases of acute poisoning, early involvement of the alimentary, cardiovascular, respiratory and central nervous systems, with corresponding symptoms, is a characteristic feature and such cases commonly have a fatal outcome in two to three days.

The frequency of the symptoms reported in connection with 34 fatal cases of acute fluoride poisoning as described by Roholm (1937) are shown in Table 1. The best available description of massive, non-fatal intoxication by NaF is the case given by Peters (1948).

The acute toxicity of fluoride manifests itself chiefly by local corrosive action, besides action due to absorption. After ingestion of fluorine compounds in high doses, there is diffuse abdominal pain, diarrhea and vomiting. There is excessive salivation, with thirst, perspiration and painful spasms in the limbs.

It is obvious that rapid measures to empty the stomach and reduce fluoride absorption are most effective for preventing death or damage from massive fluoride ingestion. Provoked vomiting, followed by the ingestion of a large volume of milk, will generally be the immediately available emergency treatment. The same precautionary

measures may be taken should a child ingest large quantities of caries-preventive fluoride tablets or fluoride toothpaste, regardless of the fact that the risks are very small in such cases according to calculations and limited experience.

Robinowitch (1945) described an interesting variant in his patient, who apparently took a large amount of NaF and died of the effects of altered calcium metabolism but without the appearance of severe gastroenteritis, which has been mentioned in almost all the other reports. Tetanic spasms due to lowered serum calcium have been described in some of the cases of acute fluoride poisoning.

Similarly, the inhalation of gaseous fluorine leads first to irritation of the mucous membrane of the eyes and air passage and subsequently to symptoms due to absorption.

The irritant effects of fluoride, sometimes referred to as local effects, chiefly concern occupational injuries to the skin. More specifically, these local effects concern the corrosive action of :

a) Solutions of fluoride-containing acids on the skin;

b) Fluoride-containing acid vapors or gases on the eyes, nasal mucosa and face;

c) Such vapors or gases on the respiratory tract.

The pathological changes in acute intoxication are haemorrhagic gastroenteritis with a tendency to necrosis, acute toxic nephritis and varying degrees of parenchymatous damage in other organs- for example, liver and heart muscle. Roholm (1937) reported 32 fatal cases of acute poisoning along with the gross pathological changes in various tissues.

Acute Experimental Fluoride Intoxication

No description of acute fluoride intoxication would be complete without a reference to the experimental work, which has been more closely studied and whose results are better appreciated than the scantily studied clinical cases of intoxication. Tappeiner (1989) used dogs, rabbits, guineapigs and cats as experimental animals. As much as 0.5 g NaF per 100 g of body-weight was given internally and 0.15 g was given by injection subcutaneously and intravenously. The following characteristic symptoms were observed :

(5) A condition of drowsiness and weakness resulting from paralysis of the vasomotor centers;

(6) Cramps which attacked a single organ or the entire body and were epileptic in character;

(7) Paralysis of the vasomotor centers;

(8) Acceleration and deepening of the breathing with paralysis;

(9) Vomiting;

(10) Secretion of salivary and tear glands which was not controlled by atropine;

(11) Early rigor mortis following death.

Leone, Geever & Moran (1956), in their experimental work on dogs and mice on the acute and subacute toxicity of sodium fluoride, concluded that the mean acute lethal dose of sodium fluoride in unanesthetized dogs infused to death by continuous intravenous infusion at the rate of 5.4 mg of fluoride ion per minute was 36.0 + \ - 0.5 mg/kg. The principal effects were progressive depression of blood pressure, heart rate and central nervous system with vomiting and defecation, all occurring with administration of approximately 20 mg/kg. At a mean dose of 30.6 mg/kg, there was a depression of the respiratory rate and a conversion to atrioventricular, nodal or ventricular rhythm with terminal ventricular fibrillation or asystole.

In a group of dogs infused intravenously with selected fractions of the acute lethal dose, an approximate LD_{50} was estimated to be 20 mg/kg. The major effects observed in this group was vomiting, defecation and central-nervous-system depression. In dogs given fluoride by mouth, single doses up to 3100 mg/kg produced only vomiting and diarrhea and transient moderate depression. A slight drop in serum calcium followed the infusion of fluoride in another group of dogs in which serum Ca was determined. The pathological findings were chiefly those of generalized hyperaemia and acute focal haemorrhages.

The gross pathological changes in acute poisoning provide evidence of the potential toxic properties of fluoride and are an indication of the possible hazards and dangers inherent in a single exposure to a large concentration of F. But it must be emphasized that the response of tissues to the relatively minute concentration derived from natural sources or absorbed from industrial contamination over long periods of time is quite different and does not simulate acute toxic effects.

Chronic Fluoride intoxication

A number of biological effects have been ascribed to fluorides. Although many reports of such effects are unsubstantiated, several have been studied sufficiently to deserve careful summarization, including the effects of bones, teeth, kidney, thyroid, neurological functions and growth in general. Smith & Hodge (1959) have related the concentrations or doses of fluoride to the biological effects indicated in the tabulation below :

Concentration or dose of fluoride	***Medium***	***Effect***
0.0022 ppm	Air	Injury to vegetation
1 ppm	Food or Water	Dental protection
2 ppm or more	Food or Water	Mottled teeth enamel
8 ppm	Food or Water	10% osteosclerosis
50 ppm	Food or water	Thyroid changes
100 ppm	Food or water	Growth retardation
125 ppm or more	Food or water	Kidney changes
20-80 mg/day or more	Food or Water	Crippling fluorosis
2.5-5.0 g	Acute dose	Death

Effect of chronic fluoride intoxication on Human Health and Biochemical Changes

Excess fluoride damages (RGDWM, 1993)

Teeth

Bones and Joints

In final stages it causes

Premature aging

And may cause:

- Skeletal Fluorosis,
- Dental Fluorosis,
- Non Skeletal manifestation, or
- any combination of the above.

Effect on teeth

Discoloration	Delayed eruption
Chipping of edges	Pitting

Effect on Bones and Joints

Heel pain	Painful and restricted joint movements
Deformities in limbs	Hunch back

In extreme cases

Paralysis	Muscular wasting
premature aging	

Affect on other systems due to high fluoride ingestion

Central nervous system

Depression	Nervousness
Tingling in hands and feet	Polyuria
Polydipsia	Headache

Muscles

Weakness	Spasm
Myalgia	Paralysis

Gastrointestinal system

Pain abdomen	Dysentery
Constipation and Diarrhea	Nausea
Dyspepsia	Dryness in mouth

Urinary system

Oligurea	Hematuria
Itching	Various other kidney disorders

Skin

Painful (Red or Blue) ecchymotic patches, disappearing itself within 10 days.

Extensive work has been done regarding fluorine and human health by many researchers.

Dental Fluorosis:

Dean and Co-workers (Dean & Elvove 1935,1937;Dean, 1942) related the appearance and severity of dental fluorosis to different fluoride levels in the drinking water with the aid of a special classification and weighing of the severity of the lesions.

As the fluoride content of drinking water increases the community index increases. Dean (1942) reported the following community indices.

Very mild	2-3mg F/l
Mild	4 mg F/l
Moderate	5-6 mg F/l

Thergaonkar and Bhargava (1974) studied water quality and incidence of fluorosis in Jhunjhunu district of Rajasthan and observed that the incidence of mottled teeth and fluorosis was 65 percent. The permissive and excessive values of fluoride in drinking water are 1.0 to 2.0 mg F/l. But according to this survey wide spread incidence of mottled teeth were observed even with range of 0.7-1.5 mg F/l. The incidence of fluorosis is probably reduced by calcium beyond 30 mg/l.

The minimal daily fluoride intake in infants that may cause very mild or mild fluorosis in human beings was estimated about 0.1 mg per kg body weight (Forsman, 1977). This figure was derived from examination of 1094 children from areas with water fluoride concentrations of 0.2-2.75-mg/ l.

Myers (1978) concluded that fluorosis is of a very mild or mild character in areas with drinking water naturally containing fluoride levels of upto 1.5-2 mg/l.

Mathur and Mathur (1996) studied 40 patients of fluorosis in three villages of Jalore district where fluoride level of water is more than 5 ppm and found 100% mottling of teeth.

Choubisa et *al.* (1996) were conducted a survey in fifteen villages of Dungarpur district of Rajasthan for the prevalence of dental and skeletal fluorosis in villagers and their domestic animals. Fluoride concentration (mean) in drinking waters of these villages varied between 1.7 to 6.1 mg/l. An overall 73.0 and 82.9 percent prevalence of dental fluorosis was observed in children (below 18-year age) and adults respectively. 100% prevalence of dental fluorosis in children and adults was observed at 5.2 and 3.8 mg/l fluoride concentration respectively. The prevalence of skeletal fluorosis in adults was 32.5 percent and the highest prevalence (60.8%) was observed at 6.1-mg/l-fluoride concentration. Male subjects relatively showed higher prevalence of skeletal fluorosis 66.2% cattle and 67.5% buffaloes showed the evidence of dental fluorosis. Cent percent prevalence of dental fluorosis in calves of both animal species was observed at the above 2.8-mg/l-fluoride concentration. The highest prevalence of skeletal fluorosis, 61.6 percent in cattle and 66.6 percent in buffaloes has been observed at 6.0-mg/l-fluoride concentration.

Evans *et al.* (1991) concluded (1) that human maxillary central incisors are most susceptible to fluorosis during a critical period of as little as four month duration, commencing at 22 months of age; and (2) that for these incisors, fluoride exposure during the months prior to this period carries less risk than continued exposure for up to 36 months beyond this critical time. Ishii-T *et al.* (1991) reported two 'at-risk' periods for the production of moderate or severe fluorosis were evident. One started at birth and ended early in tooth development, while the other started later and ended at eruption. The duration of F exposure, although determining the initial degree of fluorosis, did not influence the rate of post-eruptive enamel loss.

It have been observed that the curves presenting the intraoral distribution of the severity of dental fluorosis corresponded with the curve presenting the completion time of primary enamel formation of the various tooth types, with the exception of the first molars in high fluorosis communities. The similarity of the curves suggests that the later in life enamel is completed, the higher is the severity of dental fluorosis.

This relation seems to be explained by the prevailing feeding and dietary habits, which result in minimal intake of fluoride in the first 18 months of life during breast-feeding, followed by increasing fluoride ingestion in the following years through consumption of tea, sea -fish and F-containing magadi salt.

A study by van-Palenstein-Helderman-WH *et al.* (1997) reported In nine Tanzanian low fluorosis communities with a prevalence of pitting fluorosis of less than 2% and in five moderate fluorosis communities with a prevalence of pitting fluorosis of 16-59%, **incisors and first molars were the least affected teeth.**

In four high fluorosis communities with a prevalence of pitting fluorosis of 86-97%, maxillary incisors exhibited lower Thylstrup-Fejerskov Index values than the maxillary canines, premolars and molars. The mandibular teeth exhibited increasing Thylstrup-Fejerskov Index values from the anterior to the posterior region.

Skeletal Fluorosis:

A significant increase in the levels of haptoglobin ($p < 0.01$) and C-reactive protein ($p < 0.01$) as well as a raised erythrocyte sedimentation rate were seen in patients of skeletal fluorosis as compared to both types of controls (Susheela *et al.*, 1994). The present study suggests the possibility of a subclinical inflammatory reaction occurring in patients with skeletal fluorosis.

Endemic skeletal fluorosis can have a wide variety of radiographic appearances (Wang-Y *et al.*, 1994), including calcification and/or ossification of the attachments of soft-tissue structures to bone, osteosclerosis, osteopenia, growth lines, and metaphyseal osteomalacic zones.

The radiographic findings were classified as osteosclerosis, osteopenia, intermittent growth lines, and diaphyseal widening or soft-tissue ossification.

Two different osteopenic patterns were defined: an osteoporotic pattern with overall decreased bone density and an osteomalacic pattern that combines the features of osteoporosis with bone deformity.

Soft-tissue ossification included involvement of ligaments, tendons, and interosseous membranes.

Gupta-SK *et al.* (1993) revealed a picture similar to metabolic 'superscan' on skeletal scintigraphy in all subjects, i.e. increased tracer uptake in axial and appendicular skeleton, reduced soft tissue uptake, poor or absent renal images, prominent costochondral junction and 'tie' sign in sternum. Increased uptake was present in all subjects irrespective of age, water fluoride content, serum alkaline phosphatase level and radiological abnormalities. Our findings suggest the presence of a high bone turnover state in endemic skeletal fluorosis irrespective of other variables.

Radiological spectrum of endemic fluorosis and its relationship with calcium intake was evaluated by Mithal-A *et al.* in 1993. They observed a coarse trabecular pattern, axial osteosclerosis with distal osteopenia and diffuse osteopenia. Subjects with osteopenic changes had a significantly lower dietary intake of calcium than those groups having normal radiological findings, predominant osteosclerosis or coarse trabecular pattern. This suggests the role of calcium intake in determining the skeletal changes in endemic fluorosis.

Other effects of high fluoride ingestion

Fluoride and mental efficiency

Li-Y *et al.*(1994) investigated 157 children, aged 12-13, born and grew up in a coal burning pattern endemic fluorosis area and an experiment on excessive fluoride intake in rat. The results showed: (1) Excessive fluoride intake since early childhood would reduce mental work capacity (MWC) and hair zinc content: (2) The effect on zinc metabolism was a mechanism of influence on MWC by excessive fluoride intake; (3) Excessive fluoride intake decreased 5-hydroxy indole acetic acid and increased norepinephrine in rat brain; whether this is also a mechanism of the influence on MWC awaits confirmation.

*Fluoride and thyroid (*Yang-Y *et al., 1994)*

Fluoride has inhibitory effect on iodine uptake. It have been observed that in high iodine and high fluorine areas, the thyroid enlargement prevalence rate among inhabitants and that among children were 3.8% and 29.8%, respectively. The dental fluorosis prevalence rate among inhabitants and that among children was 35.48% and 72.9%, respectively. The pupils' average intelligence quotient (IQ) was 76.67 +/- 7.75, slightly lower than the control point. The thyroid iodine-131 (131I) uptake rate was markedly lower than the control point. The serum TSH was obviously higher than the control point.

Fluoride and Alkaline phosphatase activity

Fluoride at micromolar concentrations significantly and dose-dependently stimulated [3H] thymidine incorporation into DNA in DP-1, DP-2 (normal human dental pulp cells) and TE-85 cells (human osteoblastic osteosarcoma cell line). Fluoride significantly increased the enzyme's activity in DP-1 and TE-85 by 177 +/- 12% and 144 +/- 12.3%.

Fluoride and Cancer (Liu-YQ, 1993)

The results suggest that sodium fluoride promoted the growth of precancerous lesions of the liver induced by DEN in rats, and this has provided some data to the understanding of the relationship between fluorosis and neoplasms.

*fluoride and diabetes (*Trivedi-N *et al.* ,1993)

The study showed that chronic fluoride toxicity in humans could result in significant abnormalities in glucose tolerance which are reversible upon removal of the excess fluoride.

Fluoride and proteoglycan (Waddington-RJ *et al.*, 1993)

Differential effects of fluoride were observed in both metabolism and biochemical characterization of proteoglycans following incubation at the two concentrations. Fluoride decreased uptake and led to an accumulation of glycosaminoglycan within the proteoglycan of the matrix. Chondroitin sulfate was the predominant glycosaminoglycan identified, with the additional presence of dermatan sulfate and heparan sulfate

identified. Dermatan sulfate levels increased in 3 mm-treated teeth. Fluoride-treated proteoglycans had a reduced molecular weight (200-90K to 180-79K); this reduction is primarily a result of smaller glycosaminoglycan chains, with limited reduction in the size of the core protein of 6 mm-treated teeth occurring. Such alterations in the biochemical metabolism and hence structure and function of proteoglycan may be implicated in the hypomineralization seen in fluorosis.

*Fluorosis and lactation (*Yuan-SD *et al.*, 1991)

The effect of fluorosis on lactation, lactotroph function and ultrastructure were studied in lactating rats. The results were as follows: 1) Inhibition of lactation in lactating rats with chronic fluorosis was assessed by stunting growth of pups and decrease in the amount of milk suckled by pups in 30 min. Metoclopramide, a blocker of dopamine receptor, could improve lactation in these rats. 2) During chronic fluorosis serum PRL level was decreased, however, PRL content in pituitary was increased. Electronmicroscopic examination showed accumulation of large mature secretary granules and appearance of extremely large abnormal secretary granules in lactotroph cytoplasma. These findings indicate that hormone release of pituitary lactotrophs is obstructed in lactating rats with fluorosis, and the toxic effect of fluoride is mediated by an enhanced function of dopaminergic system in hypothalamus.

DIAGNOSIS:

The following criteria should be taken into consideration before diagnosing a case of fluorosis:

- ♠ High fluoride contents of the drinking water
- ♠ Endemicity of the fluorosis in the area
- ♠ Clinical manifestations of fluorosis in the population: Dental, Clinical and Skeletal.

  Thorough clinical examination relating to dental staining of the teeth (needs to be differentiated from other stains of the teeth) is required to diagnose dental fluorosis. The diagnosis of skeletal fluorosis needs evaluation of early warning signs viz. Severe pain and stiffness in the neck and back bone Severe pain and stiffness in the joints, Severe pain and rigidity in the hip region (pelvic girdle). The clinical fluorosis can be evaluated by three simple diagnostic tests. (Susheela AK, 1989)

  a. The individual is made to bend and touch the toes without bending the knees. If there is pain or stiffness in the backbone, hip and joints, this exercise will not be possible.
  b. The individual is made to touch the chest with the chin. If there is pain or stiffness in the neck, this exercise will not be possible.
  c. The individual is made to stretch the arms sideways, fold the arm and try to touch the back of the head. If there is pain or stiffness in the shoulder joint and backbone, this exercise will not be possible

- ♠ Biochemical evaluation :

The commonly required biochemical evaluations are serum calcium, serum alkaline phosphatase, fluoride levels in whole blood, serum and urine, Ascorbic acid levels in serum and leucocyte, Serum Parathyroid estimation, Serum Sialic Acid and Serum Glucosaminoglycan.

- ♠ Radiological evaluation

REVIEW OF LITERATURE RELATING TO PATHOPHYSIOLOGY OF FLUOROSIS

The fluoride toxicity in human being is affected mainly by two factors:

1. Fluoride ingestion through its sources: Although there are several sources of fluoride intake, It is roughly estimated that 60% of the total intake is through drinking water (RGDWM, 1993).
2. Intake of preventive factors (Calcium, Ascorbic acid, Vitamin D and Protein) from dietary or other sources.

The pathological changes observed in fluorosis in tissues are as follows:

(Pandit CG, 1940; Pandit CG *et al.*, 1940; Wadhawani TK, 1954; Venkateswarlu P *et al.*, 1957; Narayana Rao D, 1942; Ekstrand J *et al.*, 1979; Teotia SPS *et al.*, 1985; Weatherell JA *et al.*, 1975; Jowsey I, 1975; Teotia SPS *et al.*, 1975;ICMR, 1979; Burkhart JM, 1968)

The exact pathophysiology of skeletal, clinical and dental fluorosis is still not well understood, probable pathological changes observed are;

a. Formation of hydroxyfluoroapatite
b. Activation of osteoblasts leading to hypocalcemia by interfering with absorption and metabolism of calcium. Consequently hyperosteoidosis and osteopenia caused by hyperparathyroidism
c. Fluoride disturbs the collagen formation of bones and teeth by interfering with Vitamin C synthesis and utilization.

Study reported by Rugg-Gunn-AJ (1997) prevalence of developmental defects of dental enamel in permanent teeth was highest in the region with the highest water fluoride concentration, in rural areas and in malnourished subjects. Maxillary incisor teeth were the most affected teeth in all regions. The findings have implications for those in public health who determines optimum fluoride levels in drinking water in Saudi Arabia and beyond.

The fluoride ion may exert direct action on enzymes but more frequently act indirectly by complexing with ions of metals. Farley *et al.* (1983) observed increased serum alkaline phosphatase activity.

Fluoride affects calcium metabolism, which directly as well as by feed back mechanisms changes PTH levels in blood (Teotia SPS, 1985; Teotia SPS, 1972) and hyperplasia of parathyroid glands (Faccini JM , 1969).

The elevated content of glucosaminoglycans (GAG) in bone and its reflection in serum is considered as an index to assess fluoride toxicity at an early stage. Till recently, the only reliable criterion for assessing fluoride toxicity was radiographs, which are helpful in diagnosing disease at late stages (Shusheela AK *et al.*, 1982).

The ratio of sialic acid upon GAG has been found sensitive to detect fluoride toxicity at an early stage. This ratio reduces by 30-50% in human sera in fluoride poisoning (Jha *et al.*, 1983).

The exact pathophysiology of skeletal and dental fluorosis is still not well understood. Various workers have put forward the possible pathophysiology relating to the dental, skeletal and clinical fluorosis.

Dental fluorosis:

The mechanism by which fluoride produces the effects is still not clear. The most favored current explanation (Teotia SPS,1972) is that fluoride replaces the hydroxyl group of the appetite lattice in bone and tooth mineral to produce a more stable crystallite, less easily dissolved by acid of the dental plaque and less easily resorbed by osteoclast. While such a change in solubility might feasibly explain a reduction in dental caries, it does not so convincingly account for the more dramatic histological changes of skeletal and dental fluorosis often manifest in cellular changes and in alteration to the calcifying matrix.

Teeth:

Local transfer of fluoride from one part of a bone to another has been reported from animal experiments (Likins *et al.*., 1959). In the case of deciduous dentine, some of this fluoride lost by resorption from the pulpal surface might be taken up by the still mineralizing surface enamel of the underlying permanent teeth. Such fluoride transfer could make a significant contribution to the relatively high fluoride concentration of permanent enamel and the higher incidence of dental fluorosis found in permanent teeth, most of which are situated directly beneath a deciduous precursor and all of which, during eruption, come into close proximity with the overlying resorbing bone. (Weatherell *et al.*, 1975)

Dental fluorosis appears to be acquired during enamel development and, in the fully mineralized tissue, fluoride uptake is very small. Once the tooth is erupted, some of the fluoride acquired during formation is removed by abrasion whereas at other sites,

demineralization brought about by a fall in pH at the tooth surface, increases fluoride uptake (Weatherell *et al.*, 1973).

There was a relatively high fluoride/phosphorus ratio F/P ratio in the late-forming or early-maturing region of enamel and, as the tissue mineralized, this decreased. Preliminary measurements in which an attempt has been made to relate fluoride to tissue volume, have suggested that a considerable amount of the fluoride present during this early phase of mineralization was lost as the mineral content of the tissue increased.

Fluoride seems to have less effect on dentine than on bone or enamel. This could be explained by the inter-position of the non-mineralized predentine zone which, according to the above hypothesis, would provide a protective barrier between the crystallites at the mineralizing front of the tissue and the odontoblasts.

Wright-JT *et al.* in 1997 reported that the development of human enamel involves a complex series of events including the secretion and degradation of a unique extracellular matrix. Ameloblasts progress through a succession of cellular phenotypes executing specialized secretary and regulatory functions. When performing optimally, ameloblasts produce a highly structured and mineralized tissue. Given the elaborate developmental events required for normal enamel formation, it is not surprising that a variety of enamel malformations arise from defects in matrix synthesis, secretion and extracellular processing.

Normal matrix secretion and post-secretary processing by ameloblasts can be affected by a variety of hereditary and environmental conditions. These disturbances

can result in an abnormal amount and/or composition of matrix proteins, and subsequently, an altered enamel structure and/or mineral content. For example, abnormal matrix removal during enamel maturation apparently contributes to hypomineralization associated with dental fluorosis.

Enamel maturation is characterized by massive crystal growth in both width and thickness, resulting in the most highly mineralized of all mammalian skeletal tissues (Robinson-C *et al.*, 1997). The control of this process is mediated via a carefully orchestrated series of events that are temporally and spatially regulated, and it requires the coordinated degradation and removal of the endogenous enamel matrix. This is affected by both neutral metalloproteases and serine proteases, which are developmentally restricted and may be further modulated by changes in the chemistry of the enamel crystals themselves. Failure of these mechanisms, or the adventitious entry of mineral-binding proteins during the later stages of maturation, may result in the incomplete maturation of the enamel crystals and the eruption of dysplastic tissue.

Determinants and mechanisms of enamel fluorosis (Whitford GM, 1997)

Enamel fluorosis occurs when

1. Fluoride concentrations in or in the vicinity of the forming enamel are excessive during its pre-eruptive development.
2. Fluoride concentrations in plasma, enamel and other tissues reflect the difference between intake and excretion, i.e. fluoride balance.
3. Thus, fluoride balance and tissue concentrations and the risk of fluorosis are increased by factors such as high protein diets, residence at high altitude, and certain metabolic and respiratory disorders that decrease pH.

4. Factors that increase urinary pH and decrease the balance of fluoride include vegetarian diets, certain drugs and some other medical conditions.
5. In addition to the diet, modern sources of ingested fluoride include a variety of dental products, some of which have been identified as risk factors for fluorosis.
6. Fluoride absorption is inversely related to dietary calcium which, at high concentrations, may cause net fluoride secretion into the gastrointestinal tract.
7. The excretion of absorbed fluoride occurs almost exclusively via the kidneys, a process which is directly related to urinary pH.
8. Although several other fluoride-induced effects might be involved in the aetiology of fluorosis, it now appears that inhibition of enzymatic degradation of amelogenins, which may delay their removal from the developing enamel and impair crystal growth, may be of critical importance.

In addition to the effects of fluoride, disturbances in enamel formation that can be confused with fluorosis are caused by chronic acidosis and hypoxia independently of the level of fluoride exposure.

Structural relationships in orthophosphate $(PO_4)^{-3}$, carbonate $(CO_3)^{-2}$, and hydroxyl $(OH)^{-}$ groups were studied in 2 teeth with grave fluorosis manifestations, removed for orthodontic indications, by infrared spectroscopy (Nikolishin-AK *et al.*, 1991). Two intact teeth were examined for control. The findings evidence is elevated protein level in fluorosis-involved dental enamel and impaired enamel microstructure; this may be explained by specific features of fluorosis clinical manifestation and by increased enamel permeability in grave fluorosis.

Aoba T (1997) reported that fluoride participates in many aspects of calcium phosphate formation in vivo and has enormous effects on the process and on the nature and properties of formed mineral.

The most well documented protective effect (Aoba T, 1997) of fluoride in therapeutic dosages is that

1. This ion substitutes for a column hydroxyl in the appetite structure, giving rise to a reduction of crystal volume and a concomitant increase in structural stability.
2. In the process of enamel mineralization during amelogenesis (a unique model for the cell-mediated formation of well-crystallized carbonatoapatite), free fluoride ions in the fluid phase are supposed to accelerate the hydrolysis of acidic precursor(s) and increase the driving force for the growth of apatitic mineral. Once fluoride is incorporated into the enamel mineral, the ion likely affects the subsequent mineralization process by reducing the solubility of the mineral and thereby modulating the ionic composition in the fluid surrounding the mineral, and enhancing the matrix protein-mineral interaction.

Excessive ingestion of fluoride will cause:

Excess fluoride leads to anomalous enamel formation by retarding tissue maturation. It is worth noting that enameloid/enamel minerals found in vertebrate teeth have a wide range of CO_3 and fluoride substitutions. In the evolutionary process from elasmobranch through enameloid to mammalian enamel, the biosystems appear to develop regulatory functions for limiting the fluoridation of the formed mineral, but this development is accompanied by an increase of carbonate substitution or defects in the mineral. In research on the cariostatic effect of fluoride, considerable emphasis is placed on the roles of free fluoride ions (i.e., preventing the dissolution and accelerating the kinetics of remineralization) in the oral fluid bathing tooth mineral.

Fejerskov-O *et al.* (1994) elaborated the detailed changes in dental tissue. They established that a linear relationship exists between fluoride dose and enamel fluorosis in human populations. With increasing severity,

1. The subsurface enamel all along the tooth becomes increasingly porous (hypomineralized), and the lesion extends toward the inner enamel.
2. In dentin, hypomineralization results in an enhancement of the incremental lines. After eruption, the more severe forms are subject to extensive mechanical breakdown of the surface.
3. The continuum of fluoride-induced changes can best be classified by the TF index, which reflects, on an ordinal scale, the histopathological features and increases in enamel fluoride concentrations.
4. Human and animal studies have shown that it is possible to develop dental fluorosis by exposure during enamel maturation alone. It is less apparent

whether an effect of fluoride on the stage of enamel matrix secretion, alone, is able to produce changes in enamel similar to those described as dental fluorosis in humans.

5. The clinical concept of post-eruptive maturation of erupting sound human enamel, resulting in fluoride uptake, most likely reflects subclinical caries. Incorporation of fluoride into enamel is principally possible only as a result of concomitant enamel dissolution (caries lesion development).

6. At higher fluoride concentrations, calcium-fluoride-like material may form, although the formation, identification, and dissolution of this compound are far from resolved.

7. It is concluded that dental fluorosis is a sensitive way of recording past fluoride exposure because, so far, no other agent or condition in man is known to create changes within the dentition similar to those induced by fluoride.

Since the predominant cariostatic effect of fluoride is not due to its uptake by the enamel during tooth development, it is possible to obtain extensive caries reductions without a concomitant risk of dental fluorosis.

The mechanism by which fluoride produces the effects is still not clear. The most favored current explanation (Weatherell JA *et al.*, 1975) is that fluoride replaces the hydroxyl group of the appetite lattice in bone and tooth mineral to produce a more stable crystallite, less easily dissolved by acid of the dental plaque and less easily resorbed by osteoclast. While such a change in solubility might feasibly explain a reduction in dental caries, it does not so convincingly account for the more dramatic histological changes of

skeletal and dental fluorosis often manifest in cellular changes and in alteration to the calcifying matrix.

Milan AM *et al.* in 1999 reported that the fluoride-induced alterations to the biochemical structure of dentine phosphoproteins would appear to influence the phosphorylation of these macromolecules only, possibly affecting posttranslational events. Such alterations may play a role in disrupting the patterns of mineralization seen during fluorosis.

Skeletal Fluorosis:

The exact mechanism of bony fluorosis is not fully known yet. Probable hypotheses indicated by some authors (Teotia SPS *et al.*, 1972; Jowsey I *et al.*, 1975), that are given in Fig. 1.Fluorosis affects the function of parathyroid by altering serum calcium. Any stimulation, which causes decreased level of circulating calcium, would induce parathyroid release.

Fluorosis affects the function of parathyroid by altering serum calcium. Any stimulation which causes decreased level of circulating calcium would induce parathyroid release.

At molecular level (ICMR, 1979) the fluoride intoxicated nascent collagen of bone indicated only 12 high molecular weight protein band in comparison to normal nascent collagen of bone with 23 bands.

Fluoride ingestion ⟹ increase in osteoblast ⟹ increase in matrix formation

⇩

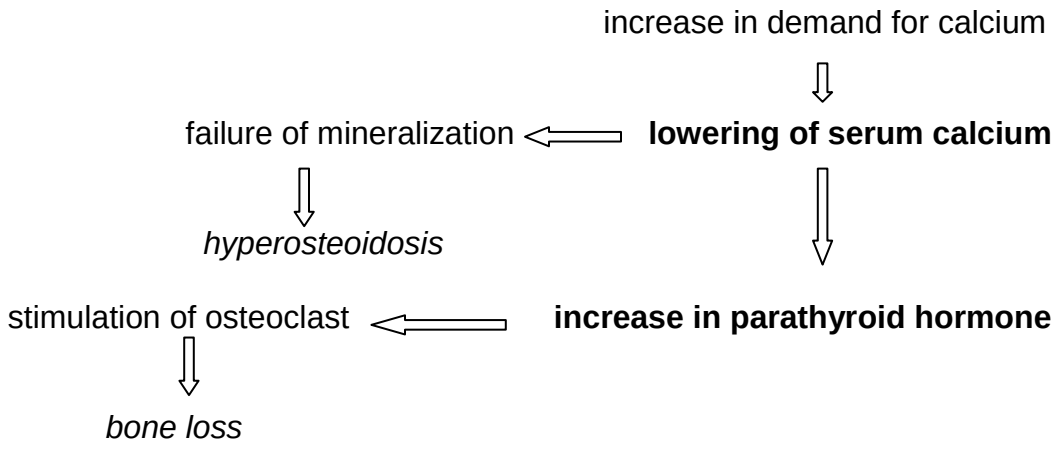


Fig. 1

The fluoride concentration is not the same in all parts of the skeleton and in general is highest at bone surfaces. This is perhaps the principal reason why cancellous bone, which has a very high surface/mass ratio contains 2-3 times as much fluoride as compact cortical bone (Weidmann and Weatherell, 1959; Singer & Armstrong, 1962). In the compact cortical bone itself, the surfaces usually contain the highest fluoride concentration and, in the human femur, the periosteal surface usually contains more fluoride than the endosteal surface (Weatherell, 1969). This is probably because the amount of fluoride present in the surface region depends partly on the amount of bone formation at that surface and upon the amount of resorption taking place.

Although strong, hard bone has undoubtedly been demonstrated in cases of chronic human skeletal fluorosis (Roholm, 1937; Franke *et al.*.,1972) increases in radiodensity seem sometimes to have been interpreted as increases in physical density which, in fact, they merely reflected the production of more, but perhaps abnormal and

weaker, poorly mineralized bone (Johnson, 1952; Weatherell, 1969; Nordin, 1973). Fluorotic bone is not always hard. In experimental studies it has often proved to be porous and poorly mineralized with wide osteoid seams.

The detection of dermatan sulphate, a sulphated isomer of glycosaminoglycan, in large quantities in cancellous bone in highly significant. Accumulation of dermatan sulphate in cancellous bone leads to cartilage formation. This is an abnormal circumstances where dermatan sulphate is formed in bone. The bio-chemical pathway(s) by which it is formed is worth exploring to understand the mechanism of `new bone' formation and ectopic calcification in fluoride toxicity. It has also been found that dermatan sulphate disappears gradually from the ligaments, leading to its calcification. This avenue, if perused, is likely to provide information on the mechanism of `new bone' formation and ectopic calcification in fluorosis and fluoride toxicity. (Jha *et al.*, 1983).

Fluor osteopathy, as Razumov-VV *et al.* (1997) supposes, is a morphologic repetition of phylogenesis early stages in osteogenesis. Thus, osteosclerosis and osteoporosis demonstrated by X-ray should be considered as manifestation of bone fluorosis. Fluor-induced changes of bone tissue could not be adequately termed as "osteoporosis" and "osteosclerosis", so is defined as "fluor osteopathy".

Clinical fluorosis:

Generalized muscle fiber atrophy leads to a damage of muscle nuclei mitochondria myofilaments and raises serum creatinine phosphokinase activity as observed in patients of fluorosis (ICMR, 1979). This provided evidence of primary involvement of skeletal muscle in fluoride intoxication and negated the earlier view that muscle involvement was secondary to skeletal and neural factors. Ingestion of excessive fluoride causes formation of deficient collagen fibers with abnormal biochemical sites, which provide an impetus for pathological calcification - an important event occurring during fluoride intoxication.

Considering the pathological changes due to hypocalcemia (e.g. fluoride ingestion) leading to secondary hyperparathyroidism and the observed changes in the fluorosis, it is obviously clear that both situations are similar.

REVIEW OF LITERATURE RELATING TO TREATMENT OF FLUOROSIS

PREVENTION AND TREATMENT

Fluorosis is not a disease affecting older persons only. There is ample evidence to prove that even newborn babies and kids of younger age have also been its victims. It not only affects the body of a person but also renders them socially and culturally crippled. The need to develop a well thought out strategy to attack this problem, therefore, can not be over emphasized. It requires an urgent attention of both medical as well as of social workers.

Despite the crying need there seems to be no orchestrated research and developmental effort to tackle fluoride toxicity in the country. International community is not interested in pursuing research in this direction because they are not facing any acute problem. Thus Indian researchers must gear themselves to handle this malaise. This will go a long way in improving the lot of afflicted population especially in Rajasthan.

Fluorosis is a preventable crippling disease. No effective therapeutic agent is available which can cure fluorosis. (Krishnamachari, 1996)

Even in areas where defluoridation was adopted a large population had already developed toxic effects considered irreversible. Vitamins C and D, and, salts of Calcium, Magnesium, Glutamic acid or Aluminum are prescribed in an attempt to reverse these effects. The results were, however, inconclusive and largely negative (Reddy GS , 1971; Ming HO YU, 1988; Kartreider NL,1972; Pantchek MB,1975; Pandit CG, 1940; Pandit

CG, 1940a; Wadhawani TK,1954; Venkateswarlu P, 1957; Narayana Rao D, 1942; Ekstrand J, 1979; Shangguan C, 1995; Grekhova TD, 1994).

Pandit *et al.* (1940) reported the coexistence of severe forms of human fluorosis and vitamin C deficiency. Later Pandit and Narayan Rao (1940) demonstrated the ameliorative effect of vitamin C rich foods (not vitamin C alone) on fluorosis in monkeys. However, the beneficial effect of other factors present in the vitamin C rich foods was not reported. Wadhwani (1954) reported improvement in radiological picture after administration of heavy doses of ascorbic acid. But he did not assess vitamin C status before instituting the therapy and his findings were questioned by many. Later Wadhwani (1954) and Pandit *et al.* (1957) stated that the aggravated condition of fluorosis with vitamin C deficiency as encountered in endemic fluorosis might very well be a complex superimposition of the signs and symptoms of scurvy on those of fluorosis. The coexisting condition of scurvy responds to vitamin C therapy, but it may not affect the basic condition of fluorosis. Venketeshwarlu and Narayana Rao (1942) reported that fluoride ingestion did not enhance the body need for ascorbic acid. It also did not increase utilization or destruction of vitamin C in the body tissues.

Narayana Rao (1942) observed that dietary Calcium protected the organisms against fluoride toxicity by reducing its absorption. Jenkins *et al.*. (1970) Studied the reversal of these effects through vitamins C and D and salt of calcium, magnesium or aluminium but their results were inconclusive. Ekstrand and Ehrnebo (1979) observed that simultaneous presence of strongly fluoride binding ions, especially calcium ions, reduced the absorption of fluoride.

ICMR Bulletin (1979) reported considerable reduction in cellular ascorbic acid content indicating that fluoride ions interfered with vitamin synthesis pathway of the gland or alternatively with utilization of the vitamin. Ascorbic acid controls collagen formation, maintains the teeth structure and is also essential for bone formation. These structures are adversely effected by higher fluoride intake.

Narayana Rao (1942) observed that dietary Calcium protected the organisms against fluoride toxicity by reducing its absorption. Ekstrand and Ehrnebo (1979) observed that simultaneous presence of strongly fluoride binding ions, specially calcium ions, reduced the absorption of fluoride and also improved serum calcium levels as observed by Teotia *et al.* (1985).

Burkhardt and Joswey (1968) indicated that administering added amounts of calcium as an oral supplement could prevent secondary Hyperparathyroidism and hyperosteoidosis. Further Joswey *et al.* (1975) indicated that vitamin D can be added to produce an increase in the absorption of calcium and thus directly affected the absorption of fluoride ions.

Preliminary studies (Joswey *et al.*, 1975) suggested that an ingestion of 25 mg of fluoride per day could be counteracted by an oral supplement of one gram of calcium per day along with 50,000 IU of vitamin D given twice a week.

Gupta et *al.* (1994) tried experiments for reversal of clinical and dental fluorosis and observed significant improvements in 29 children by controlled calcium, vitamin D3 and ascorbic acid supplementation well below the toxic dosage.

Gupta et *al.* (1996) conducted a **double blind control trial** to examine the effect of a combination of calcium, vitamin D3 and ascorbic acid supplementation in fluorosis affected children. In this study 25 children were selected from an area consuming water containing 4.5 ppm of fluoride. All the children were in an age group of 6 to 12 years and weighed 18 to 30 kg. They were graded for clinical, radiological and dental fluorosis and relevant biochemical parameters. Grade I skeletal fluorosis and all grades of manifestations for dental and clinical fluorosis was observed. These children were given ascorbic acid, calcium and vitamin D_3 well below the toxic dosages in a double blind manner using lactose as placebo. Follow up revealed a significant improvement in dental, clinical and skeletal fluorosis and relevant biochemical parameters in these children. Thus, this study indicated that Fluorosis ***could be reversed, at least in children***, by a therapeutic regimen, which is fairly cheap, simple and easily available, and without any side effects.

Efficiency of Glutamic acid for therapy (Grekhova-TD *et al.*, 1994) of early signs of occupational fluorosis was studied in workers engaged into cryolite production. The study proved that use of Glutamic acid in occupational conditions prevents progressing of metabolic disorders. The results encourage recommendations to include glutamate into therapeutic and prophylactic nutrition of workers exposed to fluor compounds, into nutritive additions according to special recipe.

REVIEW OF LITERATURE RELATING TO DEFLUORIDATION TECHNIQUES

METHODS OF DEFLUORIDATION OF WATER

When the concentration of fluoride in water source exceeds the permissible level of 1.5 mg/l consistently, it is essential to consider some remedial measures to prevent the incidence of fluorosis. First approach is to check the aquifers from different depths around the same location for the possible water source having fluoride level within the permissible levels. Another option is to consider a different water source altogether. If these approaches fail, then the defluoridation of water has to be practised.

Several methods have been suggested from time to time for removing excessive fluorides. These methods can be classified into four categories based on the principle of fluoride removal (Killedar *et al.*. 1988)

- Adsorption
- Ion Exchange Methods
- Precipitation Methods
- Miscellaneous Methods.

The materials used in these methods that have been shown significant defluoridation capacity can be summarized as given in Table1. All these methods suffer from one or more of the drawbacks of high initial cost, lack of selectivity for fluorides,

poor fluoride removal capacity, separation problems, and complicated or expensive regeneration.

Fluoride removal capacity of material, working pH, interference, merits and demerits of different existing defluoridation methods are presented in table2.

Table 1

Existing Methods and Materials

(D.J.Killedar *et al..,* 1988)

Adsorption	**Ion Exchange**	***Precipitation***
Activated carbon	1. Fluoride exchangers	1. Lime slurry
I Raw materials for A.C.	(i) Degreased and alkali treated bones	2. Alum treatment (Nalgonda)
(i) Wood	(ii) Bone charcoal	(i) Fill & draw Method
(ii) Lignite	(iii) Inorganic Ion Exchanger	(ii) Continuous flow method
(iii) Coal	(iv) Tricalcium phosphate	(iii)Package treatment plant for HP installation
(iv) Bone	(v) Florex	(iv) Alum floc blanket technique
(v) Petroleum residues	2. Anion Exchangers	
(vi) Nut shells	Weak base/strong base anion exchanger resin	
II Other materials	3. Cation exchangers	
(i) Rice husk	(i) Saw dust carbon	
(ii) Saw dust	(ii) Defluron-1	
(iii) Cotton waste	(iii) Polystyrene cation exg. Resin	
	(iv) Carbion	
	(v) Defluron-2	
	4. Activated Alumina	

Table - 2

Treatment options *for Defluoridation*

(Solsona, 1985)

Method	**Capacity/ dose**	**Working pH**	**Interference**	**Advantages**	**Disadvantages**
Aluminium sulphate	150 mg/mg F	Ambient	--	Well known process	Sludge produced low pH of treated water
Lime Softening	30 mg/mg F	Ambient	--	Well known process	Sludge produced high pH of treated water
Alum & Lime(Nalgonda Technique)	(150 mg alum +7mg lime)/mg F	Ambient	--	Low techno logy	Sludge produced High chemical dose
Bone	900 g F/ cum	Ambient	Arsenic	Locally Available	Loss of material, Taste problems
Bone char	1000 g F/ cum	Ambient	--	Locally Available	Control of raw water pH
Activated Carbon	Variable	<3.0	Many	--	pH. changes before and after treatment
Carbon prepared agricultural waste like rice husk, coffee waste etc.	300 mg/kg	7	--	Locally Available	Required treatment with KOH
Defluoron-2	350 g F/ cum	7	Alkalinity	--	Steep decline in capacity with alkalinity
Activated Alumina	2000-4000 g F/cum	5	Alkalinity	Effective, simple in application	
Electro dialysis	High	Ambient	Turbidity	Can remove other ions used with high salinity	Requires skilled operators, costly, not much used
Reverse osmosis	High	Ambient	Turbidity	-"-	-"-

DETAILS OF FLUORIDE REMOVAL METHODS

Extensive work has been done for the removal of fluoride from water by many researchers. Several defluoridation technologies are available, however only a few are suitable for field application. Ideally the defluoriadation technology of choice should use indigenously available inexpensive material having an excellent potential for fluoride removal and at the same time simple for field application.

Adsorption Methods

Mckee and Johnston (1934) studied powdered activated carbon for fluoride removal and found it to possess an excellent removal capacity. The process was found to be pH dependent, and good removal was reported at a pH of 3.0 or less. At a pH of 7.0, only little fluoride removal could be achieved.

Srinivasan (1959) prepared carbon from paddy husk by its digestion in one percent KOH and its over night soaking in two- percent alum solution. The material removed about 320 mg of F per Kg and showed maximum removal efficiency at a pH of 7. Soaking the spent material in a two- percent alum solution for 12-14 hours regenerated the carbon.

Ion Exchange Methods

Studies by Smith and Smith (1937) on a degreased, caustic and acid treated bone material showed that it could reduce fluoride concentration very effectively from 3.5 to less than 0.2 mg/l. The removal mechanism suggested was an exchange of the carbon radical with the fluoride. Because of the high costs of the bone, it was not used widely.

Maier (1953) studied the removal of fluoride using "Bone Char". The principle of the method is the exchange of carbonate ion of the bone char with fluoride ion present in water. Bone char could be regenerated with NaOH to remove bound fluoride after exhaustion and can be reused.

Bellack (1971) investigated that bone char would not be very practical for fluoride rich waters that contain arsenic because arsenic competes with fluoride and the normal caustic- regeneration process cannot strip it off. As a result, the fluoride exchange capacity of the bone char was reduced.

Bulusu et. al . (1979) studied green and yellow varieties of serpentine using jar tests as well as column tests. The yellow variety showed better fluoride removal capacity than the green variety. The medium could not be regenerated and had to be discarded after use.

Thomson and Mc Garvey (1953) reported on the use of strong base anion exchange resins for fluoride removal when these resins are used in their chloride form. Because other anions in the raw water will also be removed, the fluoride exchange capacity depends upon the fluoride to total anions ratio.

Bhakuni (1970) studied defluoron -2, a cation exchanger that was developed from sulphonated coal. This was initially treated with aluminium and used for defluoridation. Fluoride levels of less than 1 mg/l were achieved when the raw water F concentration varied between 3-10 mg/l. Capacity of defluoron - 2 varied with alkalinity and fluoride concentration. After exhaustion, defluoron- 2 was required to be regenerated with alum.

Precipitation Methods

Culp and Stoltenberg (1958) investigated alum for use at a municipal plant in Lacrosse, Kansas to treat a soft, highly mineralised water supply containing 3.6 mg/l of fluoride. Good fluoride removal was achieved in the pH range corresponding to minimum solubility of $Al(OH)_3$, and at an alum dosage of 225 mg/l. An alum dose of 315 mg/l was required to reduce the fluoride to 1.0 mg/l.

When alum is added to water, it reacts with the alkalinity in the water to produce insoluble $Al(OH)_3$. Rabosky and Miller (1974) suggested that fluoride ions be removed from solution by adsorption onto the $Al(OH)_3$ particles. The $Al(OH)_3$ and adsorbed fluorides can then be separated from water by sedimentation.

Excess fluoride can be removed from water containing high magnesium hardness by the addition of lime. (Scott *et al.*., 1937). This method is economical only when removal of both hardness and fluoride are desired. Even then, this is effective only for waters having fluoride concentration in the range of 3-4 mg/l. Empirically the amount of fluoride removed is $0.07\ F\ (Mg)^{1/2}$, where F represents initial fluoride present (mg) and Mg is the magnesium removed in the forms of flocs. High pH of the treated water as well as requirement of high magnesium hardness limits its application for defluoridation of drinking water.

FIELD METHODS OF DEFLUORIDATION

Activated Alumina Method

Boruff (1934) was first to study activated alumina for fluoride removal and shortly there after Fink and Lindsay (1936) and Swope and Hess (1937) successfully demonstrated its use on small scale.

Studies by Savinelli and Black (1958) showed that the capacity is a function of the fluoride concentration, pH of the treated water, and the amount of regenerant used. Higher initial fluoride concentration, low pH and higher amount of regenerant resulted in increased F removal capacity. They also found that the capacity was not affected by the sulphate or chloride concentration (upto 1000 mg/l of each) of the water. The medium was regenerated by caustic solution first followed by an acid rinse.

Rubel Woosley (1979) concluded that to reduce the fluoride concentration to a low level, activated alumina is the most popular and most effective because of its ease of application and cost effectiveness.

Wu and Nitya (1979) Studied water defluoridation with activated alumina and reported an optimum pH of 5 for the removal of fluoride in water. At the optimum pH for fluoride removal, the rate of adsorption of fluoride ion in water is a function of the ratio of the initial fluoride concentration to the activated alumina dose. The Langmuir isotherm can be used to model fluoride adsorption on activated alumina. From the Langmuir model the total adsorptive capacity of activated alumina for fluoride removal was found to be 12 mg/g.

Chio and Chen (1979) studied the removal of fluoride from waters by adsorption and reported maximum removal of fluoride by activated alumina at pH 5.0-8.0. The efficiency of fluoride removal generally increases when the initial concentration of fluorides in solution is decreased.

Hao and Huang (1986) studied adsorption characteristics on hydrous alumina and observed that the solution pH and the surface loading govern the fluoride removal by activated alumina. The optimal pH was found to be 5. However, at pH of less than 6.0, the aluminium- III dissolved from activated alumina reacts with fluoride ions and forms alumino-fluoro complexes. These complexes are unstable in neutral or alkaline pH regions. As a result, the role of pH value in minimising alumina dissolution should be considered. As the surface loading increases, the adsorption density of fluoride increases, but the percentage of fluoride removal decreases.

Bulusu and Nawlakhe (1988) studied the effect of controlling factors such as pH, the contact time, the ratio of adsorbate (Fluoride ion) to adsorbant (activated alumina) on the rate of fluoride removal in batch operations. Important conclusions were:

(i) Initial rates of adsorption of fluoride on AA of 105-88 micron size decreases progressively after the initial 30 minutes and give rather slow approach to equilibrium, which are not attained completely even after 144 hours.

(ii) Nearly linear variation of the amount adsorbed with square root of the time of reaction has been found to obtain for the initial fraction of the adsorption reaction studies at pH 9 to 4.

(iii) The rate of adsorption of fluoride increases with the decreasing pH of the solution.

Bulusu and Nawlakhe (1990) studied the defluoridation of water with activated alumina in continuous contact system. The fluoride removal capacity at 1 mg F/l break through were 727, 587 and 308 mg F per litre of AA medium at 4,8 and 16 meq/l basicity respectively and the corresponding values at 2 mg F/l break through were 1292,880 and 578 mg F per litre AA medium. The hydrochloric acid regenerant requirements for treating 1 cum water containing 5.2-5.6 mg F/l for 1 mg\l break through value were 268,332, and 632 gm respectively at 4,8 and 16 meq/l basicity values, the corresponding requirement to 2 mg F /l break through was 151,222 and 339 gm.

Venkobachar and Iyengar (1996) reported the fluoride removal capacity of activated alumina grade G-87 and AD-101 a 1900 and 1750 mg F/kg respectively. AA G-87 exhibited a decrease in the binding capacity as pH varied from 3 to 8 with no optimum pH. An increase in alkalinity from 400 to 600 and 800 mg/l, decreases the fluoride uptake capacity of 1900 mg/kg to 1642 mg/kg and 1337 mg/kg respectively. Hardness even upto 800 mg/l as $CaCO_3$ did not affect fluoride-binding capacity of AA G-87. Capacity of AA increased as initial fluoride concentration increased.

Limitation of Use of Activated Alumina:

(i) Capacity of activated alumina depends upon the alkalinity of water and decreases considerably with increasing alkalinity.

(ii) The output decreases significantly with progress of cycles. Within 40 cycles of operation, the capacity of activated alumina has been reported to reduce 50 to 80% of the original depending upon basicity and fluoride level of influent water and required fluoride in effluent. (Water quality and defluoridation techniques, 1993 & Bulusu *et al.*., 1983)

(iii). Higher cost than Nalgonda technique.

(iv). Requires a cumbersome process of alkali/acid regeneration.

NALGONDA TECHNIQUE

Nawlakhe *et al.*. (1975) studied the usefulness of alum in removing fluorides from drinking waters and brought out the scope of its application in water supplies. The method is known as Nalgonda Technique and involves the addition of lime, alum and bleaching powder (optional) to the raw water.

Flocculation, sedimentation and filtration follow the addition of these chemicals. Main conclusions were: -

- ♠ The alum dose required depends upon the concentration of fluorides, alkalinity and total dissolved solids in raw water.
- ♠ The lime dose required in the process is 1/20 to 1/25 that of alum.

- ♠ Adequate alkalinity is necessary in raw water to achieve 1 mg F/l in treated water.
- ♠ The proportion of fluoride removal per unit of alum increases with test water fluorides.

Selvapathy *et al.*. (1995) studied the influence of fluoride and alkalinity on the residual aluminium concentrations during the treatment of water using alum and lime/ bleaching powder. With increase in alum dose, the fluoride concentration in the treated water decreased while the residual aluminium registered a significant increase. Percent fluoride removal increased with initial fluoride concentration for a given alkalinity but decreased, with increase in alkalinity for a given fluoride concentration. Aluminium is now regarded as a neuro toxin and far from innocuous. Hence, WHO has recommended a guideline limit of 0.2 mg/l in drinking water. (WHO, 1984) Limits of aluminium in drinking water may severely restrict the choice of treatment strategies for defluoridation.

Limitations of Nalgonda Technique.

(i). Water sample can be defluoridated provided the total dissolved solids (TDS) are below 1500mg/l. Desalination may be necessary when the total dissolved solids exceed 1500 mg/l. Most of the ground water samples in fluoride affected area have a high TDS.

(ii). Defluoridation is possible if total hardness of water is below 250 mg/l.

(iii). With increase in alum dose, due to more initial fluoride concentration and alkalinity, the fluoride concentration in the treated water decreases while the residual aluminium increases. Percent fluoride removal increases with initial fluoride concentration for a given alkalinity but decreases with increase in alkalinity for a given fluoride concentration. (Selvapathy *et al.*. 1995).

(iv). Water samples require to be analysed for water quality. Fluoride content, alkalinity, TDS and hardness should be known for assessing and fixing the dosage of alum and lime.

(v). Daily operations need to be monitored to ensure that fluoride is reduced and there is no residual aluminium in water.

(vi). Accidental extra addition of alum would reduce pH below optimum range for flocculation resulting in high aluminium in treated water and low F removal efficiency.

Keeping in view these limitations of Nalgonda technique and Activated Alumina method, and fluorosis problem it becomes necessary to develop a simple and low cost method for defluoridation of water. (Gupta SK, 1998)

KRASS DEFLUORIDATION PROCESS (Gupta SK, 1998)

A safe, efficient, free from residual aluminum in treated water, and cost effective defluoridation technique / process is not available and needs to be developed in order to prevent the occurrence of fluorosis. The performance of the filter based on KRASS defluoridation process indicated that the process differs from the known processes in its

simplicity, cost effectiveness and results in traces of residual aluminum in treated water. Fluoride could be removed effectively for influent concentration upto 26 mg/l. Variation in raw water parameters like, pH, alkalinity, and total dissolved solids of input water did not affect this process. The taste of treated water appears to be the same as that of the raw water. No toxicity on 96 hours fish test was found in the treated water.

METHODOLOGY

The work was initiated under the following three main heads:

1. Selection of area, and evaluation of patients
2. Providing treatment to the selected group
3. Formulations of comprehensive health management strategy to overcome the problem of fluorosis.

SELECTION OF AREA, AND EVALUATION OF PATIENTS

1 Identification and survey

In the areas having high fluoride content in drinking water, to up date information on fluorosis and selection of areas to be studied.

a. **Based upon the data available with the Public Health Engineering Department of Rajasthan and the Investigators, 20 villages were selected for the fluoride analysis in drinking water.**

b. **Four target areas were then selected. The villages representing target areas were Rampura (drinking water fluoride 4.6 mg/l) , Ram Sagar Ki Dhani (drinking water fluoride 2.4 mg/l) , Shivdaspura (drinking water fluoride 5.6 mg/l) and Raipuria (drinking water fluoride 13.6 mg/l).**

2. *Selection of children below 12 years of age for treatment*

50 Fluorosis affected children aged up to 12 years from a cross section of each of the target area (village) comprising different socio-economic groups were chosen. The willingness of the parents to adhere to the prescribed regimen was one of the important primary considerations.

3. *Evaluation of selected children*

Approach adopted for the evaluation is indicated below.

- ♠ Nutritional Status – As per specifications of the Indian institute of Nutrition in their publication entitled nutritive value of Indian Foods.
- ♠ Fluorosis staging – For Dental, Clinical and Skeletal fluorosis
- ♠ General Clinical examination to exclude other diseased conditions.
- ♠ Biochemical parameters

  Chemical estimations were carried out for serum calcium, serum inorganic phosphorus, serum alkaline phosphatase, and, fluoride levels in whole blood, serum and urine, Ascorbic acid levels in serum and leucocyte were also measured. Serum Parathyroid estimation, Serum Sialic Acid and Serum Glucosaminoglycan were carried out.

4. Dietary evaluation:

Identification of available and routinely used eatable food items and calculation of their available fluoride contents using the table published by WHO narrating food items and their fluoride contents.

5. *Statistical evaluation of the results*

Clinical, nutritional and biochemical results were correlated in all groups and standard statistical methods were used for analyzing results. Data

collected was statistically analyzed. Apart from computing the mean and standard deviations, the level of significance determined in terms of 'p' value.

TREATMENT

Treatment trial was also given based on the previous studies conducted by Gupta et al (1994, 1996). The methodology adopted was as follows:

- Providing the treatment to this selected group of children follow up
- This sample of 30 children in each target area was given the proposed therapy in a double blind control manner, after matching (Barker DJP, 1991) the two groups.
    - Medication group, i.e. children likely to be receiving medicine, and
    - Placebo group, i.e. children likely to be without medication that is, receiving placebo.

    Calcium, ascorbic acid and vitamin D3 were used as medicine and lactose as placebo. The dosage of medicines was kept as follows in all the four target areas. These dosages were decided on the basis of earlier studies carried out by the team.

    a. Ascorbic acid 500 mg twice daily
    b. Calcium 1000 mg as available elemental calcium once daily
    c. Vitamin D3 60,000 I.U. once in three days (because of long half-life of cholicalciferol)
    d. Lactose as placebo was used to match all the above medications.

All the three medicines were provided in three different colored capsules, with each medicine and placebo in capsules of the same color. Medicines and placebo was supplied to the target group in double blind control fashion. Detailed instructions were given for their administration. The medication was fully supervised by a field worker attached to the project.

The compliance of medication was kept under strict control. No changes were made in food or water consumption.

HEALTH MANAGEMENT STRATEGY

Literatures were reviewed to formulate a strategy on comprehensive health management of fluorosis. The strategy was formulated to cover two fronts (1) Treatment of the disease (2) Prevention of the disease by (a) Domestic defluoridation (b) Dietary changes.

ESTIMATION OF BIOCHEMICAL PARAMETERS

SERUM CALCIUM (Connerty VH, 1966)

Chemical Principles of Test

O-Cresolphthalein complexon (OCPC) combines with alkaline earth metals to assume a purplish red color. The 8 hydroxyquinoline in the color reagent affords color development of calcium specifically.

Calcium content of the sample can be determined by measuring the absorbance at 570 nm. The density of the purplish red color produced by OCPC is proportional to the calcium content.

Details of Procedure

1. Bring all reagents, specimen, and standard/control sera to room temperature, 15 degree to 30 degree Celsius prior to use
2. NOTE : Because the color produced by the reaction of calcium with o-cresolphthalein complexon changes reversibly with temperature, the “Standard” (Std) should be prepared fresh for each test run.
3. Label a series of test tubes with “Specimen”(S), “Standard” (Std) and “Blank” (Bl).
4. Accurately pipette 0.05 ml of serum or urine into “Specimen” test tube 0.05 ml Standard Solution into “Standard” test tube and 0.05 ml of distilled water into “Black” test tube.
5. Accurately pipette 5.0 ml Buffer Solution into all tests tubes.
6. Mix well.
7. Accurately pipette 0.05 Ml. Color Reagent into all test tubes.

8. Mix well and allow to stand for 5 minutes.
9. Within 2 hours, using an accurately calibrated spectrophotometer or colorimeter, measure the absorbance of the “Specimen” and the “Standard” at 570 nm against the “Blank” tube to determine net absorbance.

Results

Calculations

The calcium values are obtained from the calibration curve or calculation.

1. From the calibration curve.

   The calcium content corresponding to the measured absorbance can be read directly from the previously prepared calibration curve.

2. By calculation

   The calcium content is calculated from the following equation:

$$\frac{A\ \text{Sample}}{A\ \text{Standard}} \times C\ \text{Standard (mg/dl)} = C\ \text{Sample (mg/dl)}$$

Where: A = Absorabance at 570 nm

C = Calcium Concentration (mg/dl)

SERUM ALKALINE PHOSPHATASE ***(VARLEY H, 1975)***

The following methodology was adopted to estimate Serum Alkaline Phosphatase (SAP)

Principle :

Serum ALP hydrolyzes phenyl phosphate into phenol and disodium hydrogen phosphate at pH 10.0. The phenol so formed reacts with 4-Aminoantipyrine in alkaline

medium in presence of oxidizing agent Potassium ferricyanide to form a red colored complex whose abosrbance is proportional to the enzyme activity.

Disodium Phenyl Phosphate + H_2O $\xrightarrow[\text{pH 10.0}]{\text{ALP}}$ Phenol + Disodium Hydrogen Phosphate

Phenol + 4 Amino-antipyrine + Potassium ferricyanide $\xrightarrow{\text{Alkaline medium}}$ Red colored complex

Procedure :

Pipette into the test tubes labeled Blank (B), Standard (S), Control (C), and Test (T) as follows :

	B	S	C	T
Working Buffered Substrate	1.0 ml	1.0 ml	1.0 ml	1.0 ml
Deionized Water	3.1 ml	3.0 ml	3.0 ml	3.0 ml
Incubate for 3 minutes at 37 degree Celsius				
Serum	-	-	-	0.1 ml
Phenol Standard(3)	-	0.1 ml	-	-
Incubate for 15 minutes at 37 degree Celsius				
Color Reagent (2)	2.0 ml	2.0 ml	2.0 ml	2.0 ml
Serum	-	-	-	0.1 ml

Mix well after each addition of reagent and measure absorbance (A) for blank, Standard (S), Control (C) and Test (T) against deionised water on photocolorimeter using a green filter at 510 nm.

Calculations :

$$\text{Serum ALP in KA Units} = \frac{\text{A of (T)} - \text{A of (C)}}{\text{A of (S)} - \text{A of (B)}} \times 10$$

Expected Normal values :

3.0 – 13.0 KA Units. Higher values are found in children.

SERUM INORGANIC PHOSPHORUS (Gomorri, 1942)

Principle :

Ammonium molybdate under acidic conditions reacts with phosphorus to form phosphomolybdate complex which is reduced to blue colored complex by metol. The absorbance of color developed is proportional to the inorganic phosphorus concentration.

Procedure :

Pipette into clean, dry test tubes labeled Blank (B), Standard (S) and Test (T).

	(B)	(S)	(T)
Catalyst Reagent (1)	1.0 ml	1.0 ml	1.0 ml
Molybdate Reagent (2)	1.0 ml	1.0 ml	1.0 ml
Deionized Water	0.1 ml	--	--
Standard (4)	**--**	**0.1 ml**	**--**
Serum/Diluted Urine	--	--	0.1 ml
Metol Reagent (3)	1.0 ml	1.0 ml	1.0 ml

Calculations

$$\text{Serum Phosphorus in mg\%} = \frac{\text{A of (T)}}{\text{a of (S)}} \times 5$$

SERUM ASCORBIC ACID (Natelson, 1971- Dintrophenyl hydrazine method)

Reagents

1. Trichloroacetic acid (10%) : 10 gm analytical reagent to 100 ml. with water.
2. Dinitrophenyl hydrazine reagent : 2 gm. 2.4- dinitrophenyl hydrazine; 250 mg. thiourea and 30 mg of $CuSO_4$. 5 H_2O made up to 100 ml. with 9N H_2SO_4 (50 ml. concentrated H_2SO_4 added to 150ml. water, cool before using). Kept in refrigerator. Used supernatant or filtered through glass wool, stable for one week.
3. Sulfuric acid (65%). Added 70 ml. concentrated H_2SO_4 (specific gravity 1.84) to 30 ml. distilled water, kept in refrigerator and used cold.

Procedure

1. Into 0.2 ml. of serum, 0.8 ml. of 10% Trichloroacetic acid was blown rapidly. Mixed well. Allowed to stand for 5 minutes and centrifuged at 2000 rpm.
2. Transferred a 0.5 ml. aliquot to a small test tube and added 0.2ml of dinitrophenyl hydrazine reagent, stoppered and incubated at 37 degree Celsius for 3 hours. Chilled in ice bath and added 0.8 ml of cold 65% H_2SO_4. Mixed well.
3. Allowed to stand for 30 minutes at room temperature.
4. Read transmission on spectronic 20 at 520nm and noted the corresponding optical density from the chart.
5. Blank comprised 0.5ml trichloroacetic acid treated as for the serum.
6. Standard comprised 0.2 ml. of the 1 mg/dl ascorbic acid standard treated as for the unknown.

Calculations

Optical density of unknown

$$\frac{\text{Optical density of unknown}}{\text{Optical density of standard}} \times 1 = \text{mg of AA/100 ml.}$$

Procedure notes

1. If centrifugates were cloudy, added 0.5 ml. of carbontetrachloride to all tubes, shook and centrifuged taking 0.5 ml. aliquot of supernatant as above.
2. 0.01 ml. of sample could be assayed.

LEUCOCYTE ASCORBID ACID (Denson and Bowers, 1961).

Principle

W.B.C. ascorbic acid : The method of Bessey, Lowry and Brock (1947) was modified to permit of easier separation of the white cells. Counting of the cells was adopted as an alternative to the tedium and inaccuracy of weighing of small sample with adherent fluid and red cells, or to an assessment of weight indirectly by an additional biochemical estimation of a cell constituent, for example acid insoluble phosphorus.

Reagents

1. Diluent for blood

   200 ml. physiological saline,

   50 ml. 6% Dextran,

   1.0 ml. 10% sequestrene.

   This was prepared in bulk and distributed in 12.5 ml. amounts.

2. Reagent :

   100 vols. 2.2% 2,4 – dinitrophenyl hydrazine in 10 N H_2SO_4

5 vols. 5% thiourea.

5 vols. 0.6% $CuSO_4$ 5 H_2O.

3. 0.5% W/V trichloracetic acid A.R.

4. 0.65% H_2SO_4.

Methods

1. Approximately 3 ml. of blood was delivered into 12.4 ml. diluent and allowed to stand for 30 minutes during which time majority of the red cells sedimented by gravity.
2. The supernatant fluid contraining the white cells and platelets was removed, thoroughly mixed.
3. An aliquot of 0.2 ml. removed and white cell count was performed on this with the electronic cell counter. (cell – Dyn 800).
4. A measured 10-12 ml. volume of the leucocyte containing fluid was centrifuged at 3000 r.p.m. for 15 minutes and supernatant discarded by inverting the tube and allowing it to drain for 30 seconds.
5. To the compact button of white cells and platelets, 1.3 ml. of 5% trichlor-acetic acid was added and the deposit thoroughly homogenized.
6. The tubes were left at this stage at 2- 4 degree Celsius and the estimations performed in batches with in 3-4 days.
7. The homogenized deposit was centrifuged and 1.0 ml. of the T.C.A. supernatant removed to a 3" x ½" test tube.
8. 0.3 ml. of reagent was added and the tubes incubated at 37 degree Celsius for 4 hours.

9. They were then cooled in ice water and 1.5 ml. of 65% H_2SO_4 added.
10. The transmission was read at 520 mμ against a reagent blank.
11. A standard consisting of 0.4, 0.8, 1.6, 4.0 and 10 μg of ascorbic acid in 1 ml. vol of 5% T.C.A. were similarly treated.

Calculation

The Ascorbic acid content of W.B.C. expressed as $\mu g/10^8$ W.B.C's and calculated as follows :-

$$\frac{\text{Optical density of unknown}}{\text{Optical density of standard}} \times 1.3 \times \frac{\text{Strength of standard}}{\text{Number of W.B.C. in Aliquot.}} = \mu g/10^8 \text{ W.B.C.}$$

SERUM SIALIC ACID By tryptophne perchloric scid reaction (Seibert Pfaff and Seibert, 1948).

Principle

Elevated serum polysaccharide in a disease might be in part due to the presence of neuraminic acid. A red brown color is produced when serum is treated with tryptophane in presence of perchloric acid.

Application of this procedure to crystalline sialic acid gave an absorption spectrum and the serum component of "Sialic Acid" gives the tryphtophane acid reaction.

Reagents

1.	Saline	0.9%
2.	Perchloric acid (C.P.)	60%
3.	Tryptophane	0.25%

4. Standard Sialic Acid 0.15 mg. per ml of sialic acid

Procedure

1. To 0.25 ml. of standard and to 0.25 ml. of serum add 0.75 ml. of 0.9% saline, 2.0 ml. of 0.25% tryptophane and 3.0 ml. of 60% perchloric acid. A control containing 1 ml. of saline, 2.0 ml. of 0.25% of tryptophane reagent and 3 ml. of perchloric acid reagent was also prepared.
2. Capped the tube with glass marbles and immersed in a boiling water bath for exactly 10 minutes.
3. Cooled in tap water, allowed to stand for 40 minutes with occasional shaking and filtered through whatman No.42 paper. Took readings at 500 millimicron.
4. **Calculations**

$$\frac{\text{OD Test} - \text{OD Blank}}{\text{OD St.} - \text{OD Blank}} \times 0.15 \times 0.25 \times 100 = \text{mg/100 ml}$$

PROTEIN BOUND HEXOSE (Weimer and Mohsin, 1952 Modified)

Principle

Carbohydrate in the proteins precipitated from serum by alcohol was determined colorimetrically by its reaction with concentrated sulphuric acid and orcinol.

Reagents

1. Ethanol : 95%
2. Orcinol – H_2SO_4 reagent : 7.5 volumes of reagent (A) mixed with one volume of reagent (B).

   Reagent A : 60 ml. of concentrated. H_2SO_4 and 50 ml. of H_2O.

   Reagent B : 1.6 gm of Orcinol in 100 ml of H_2O.

3. Glactose-mannose standard : 0.1 mg/ml. Each of galactose and mannose in distilled water.
4. 0.1 N NaOH.

Procedure

1. To 0.1 ml. of serum in test tube, added 5 ml. of 95% ethanol and mixed.
2. Centrifuged for 15 minutes and decanted. Suspended the precipitated in 5 ml. of 95% ethanol, centrifuged and decanted.
3. Dissolved the precipitated proteins in 1 ml. of 0.1 N NaOH.
4. Prepared a blank (1 ml. of H_2O) and a standard (1 ml. of galactose-mannose standard).
5. Added 8.5 ml. of orcinol- H_2SO_4 reagent to all the test tubes. Mixed well by inversion.
6. Caped the tubes by glass marbles to minimise evaporation and placed the tubes in water baths at 80 degree Celsius for exactly 15 minutes.
7. Cooled the tubes in tap water and readings taken in a systronic type 103 spectrophotometer at 540 millimicrons.

Calculations

$$\frac{OD_t - OD_{bl}}{OD_{st} - OD_{bl}} \text{ X } 0.2 \text{ x } 100 \text{ mgm Hexose/100 ml.}$$

PARATHYROID HORMONE

Method Description

The PTH-MM II RIA is a disequilibrium procedure using delayed tracer addition to increase sensitivity. Antiserum is directed to only the mid-region of human parathyroid hormone. Iodination is done by conventional methods utilizing synthetic hPTH (Tyr43) 44-68. In this RIA, sample and PTH-MM antiserum are combined and incubated for 15 minutes at room temperature. Tracer is then added, followed by a second incubation for 2 hours at 4 degree Celsius. Phase separation is done in 15 minutes with a pre-precipitated complex of second antibody, carrier, and PEG added in a single pipetting step. Standards are expressed as picomoles/ liter of mid-molecule fragment.

Assay Procedure

1. Reconstitute the PTH-MM-II Control Level 1 and level 2 and allow liquid reagents to equilibrate to room temperature. Mix all reagents gently before using.
2. Set up labeled 12x75 mm glass tubes in duplicate according to the protocol in TABLE-I.
3. Add reagents as follows :-
    a. Total count tubes

       Set aside until step number 5
    b. Nonspecific binding (NSB)

       100 μL of 0 stasndard
    c. 0 standard

       100 μL of 0 standard
    d. 200 μL of PTH-MM-II antiserum

PTH-MM II standard (A-F)

100 μL of PTH-MM II standard

200 μL of PTH-MM II antiserum

e. Quality control and unknown sera

100 μL of serum

200 μL of PTH-MM II antiserum

4. Vortex the tubes gently without foaming and incubate for 15 (+/- 5) minutes at 20-25 degree Celsius.
5. Add 200 μL of 125I PTH-MM II to all tubes.
6. Vortex the tubes gently without foaming and incubate for 2 hours (+/- 15) minutes at 2-8 degree Celsius (refrigerator or crushed ice bath).
7. Vigorously mix the DAG-PPT; add 500 uL to all the tubes except the total count tubes.
8. Vortex the tubes gently without foaming and incubate for 15 (+/- 5) minutes at 20 degree Celsius.
9. Centrifuge the tubes at minimum of 760 x g. for 20 minutes at 20-25 degree Celsius.
10. Immediately decant the supernatant from all the tubes except the total count tubes by inverting them for a maximum of 2 minutes. Blot the tubes with an absorbent paper to remove any drop of supernatant that may be remaining on the rims before turning the tubes upright.
11. Using a gamma scintillation counter, count the precipitate of each tube and the total count tubes for a sufficient time to achieve statistical accuracy.

Procedural Safe guards

1. Assay all samples in duplicate to ensure confidence in values obtained.
2. Reagents must be used within the proper dating.
3. Add each aliquot of reagent to the lower third of the assay tube to ensure complete mixture of reagents.
4. Some manufacturers' disposable borosilicate glass tubes yield elevated nonspecific bindings.
5. If you choose to aspirate the supernatant from the precipitate, be careful not to disturb the precipitate.
6. Final centrifugation of assay tubes must be between 20-25 degree Celsius.
7. To completely monitor the consistent performance of an RIA there are additional factors which may be checked. INCSTAR suggests a check of the following parameters to assure consistent kit performance.
    a. Total counts
    b. Maximum Binding

       Average counts per minute (CPM) of 0 standard Tube/Average CPM of Total Count Tubes.
    c. Nonspecific Binding

       Average CPM of NSB Tube/Average CPM of Total Count Tubes.
    d. Slope of Standard Curve

       For example, monitor the concentrations at 80, 50 and 20% suppression points of the standard line.

Results

Procedure for calculating Values of Unknowns

There are many methods in existence for calculating results of RIAs. Each is based on obtaining a calibration curve by plotting the extent of binding against stated concentrations of the standards. This graph may be either a linear or logarithmic scale. Each of these methods gives essentially the same values for controls and samples, although certain assays may “fit” better into one particular method versus another. The calculation method of choice for INCSTAR laboratories is %B/Bo versus log concentration.

1. Calculate the average CPM for each standard, control and unknown sample.
2. Subtract the average CPM of the NSB tubes from all counts.
3. Divide the corrected CPM of each standard, control, or unknown sample by the corrected CPM of the 0 standard.

$$B/B0\ (\%) = \frac{\text{CPM of standard or Unknown Sample} - \text{CPM of NSB}}{\text{CPM of 0 standard} - \text{CPM of NSB}}$$

4. Using three-cycle semi-log or log-log graph paper, plot percent B/B0 for the PTH standards (Vertical axis) versus the concentration (horizontal axis).
5. Draw a best-fit line through the points.
6. Interpolate the levels of PTH in the samples from the plot.
7. If any unknown sample reads greater than the highest standard, it should be diluted appropriately with 0 standard and assayed again.
8. If an unknown sample has been diluted, correct for appropriate dilution factor.

9. Calculate the maximum binding by dividing CPM of 0 standard by the average total counts obtained in the total count tubes.

Expected Values

Each laboratory should establish its own normal range. Serum Human parathyroid (hPTH) levels were measured in 99 normal males and females using the PTH-MM II kit. The average hPTH was found to be 48.1 +/- 11.9 pmol/L (1 S.D.) Individual values ranged from 28.5 (minimum) to 90.7 (maxiumum) pmo/L. The average serum hPTH level in females.

There are several ways to express this data :

	pmol/L	ng/ml hPTH(Tyr 43) 44.68	ng/ml hPTH 1-84
	---	---	---
Normal Value	48.1 +/- 11.9	0.14 +/- 0.04	0.45 +/- 0.11
Range (2 S.D.)	24.3 – 71.9	0.07 – 0.22	0.23 – 0.68

FLUORIDE ESTIMATION IN WATER, SERUM, BLOOD AND URINE

(Ion Selective Electrode Method through the ORION Model 94-09 fluoride - APHA,1989)

Principle

The fluoride electrode is an ion selective sensor. The key element in the fluoride electrode is the laser-type doped lanthanium fluoride crystal across which a potential is established by fluoride solution of different concentrations. The crystal contacts the sample solution at one face and an internal reference solution at the other.

The fluoride electrode measures the ion activity of fluoride in solution rather than concentration. Fluoride ion activity depends on the total ionic strength of the solution and pH, and on fluoride complexing species. Adding an appropriate buffer provides a

nearly uniform ionic strength background, adjusts pH, and breaks up complexes so that, in effect, the electrode measures concentration.

(B) *Apparatus*

- Ion- meter , ORION make
- Reference electrode
- Magnetic stirrer
- Fluoride electrode ORION Model 94-09

(C) *Reagents*

(i) Stock Fluoride Solution

221.0 mg anhydrous Sodium Fluoride dissolved in distilled water and diluted to 1000 ml. 1.00 ml of this solution had 100 ppm fluoride.

(ii) Standard Fluoride Solution

100 ml stock fluoride solution diluted upto 1000 ml with distilled water. 100 ml of this solution contained 10 ppm fluoride.

(iii) Fluoride Buffer: Total Ionic Strength Adjuster (TISAB)

TISAB was prepared by taking 2500 ml distilled water and 285 ml glacial acetic acid, 290gm NaCl and 60 gm di-sodium citrate (in place of 1,2 cyclohexylencdiamine tetra acetic acid CDTA) were added to this water and stirred for dissolving. Beaker was placed in a cool water bath and slowly 6 N NaoH (625 ml) was added with slow continuously stirring until pH was between 5.3 and 5.5. Now distilled water added to make it upto 5 litre .

(D) *Procedure*

(i) Instrument calibration

* Preparation of fluoride standards.

Standards equivalent to 1.0 and 10 mg F/l were required for calibration. Standards of 1.0 mg F/l was prepared by diluting 10.0 ml of standard fluoride solution with distilled water to 100 ml.

* Calibration

Instrument was calibrated with the help of above standards before measurements of fluoride concentration by the following procedure:

- 25 ml of 1.0 mg F/l standard was measured and mixed with 25 ml TISAB in a beaker.
- After rising, electrode was placed into beaker and the value of standard concentration entered at ready mode of instrument.
- 25 ml of 10.0 mg F/l standard was measured and mixed with 25 ml TISAB in a beaker.
- Electrode was rinsed with distilled water and placed in this beaker. The value of 2^{nd} standard was entered at ready mode of instrument.
- Slope of instrument was checked for the desired range 54-60 mV.

Measurement of Fluoride Concentration of Samples

25 ml of sample and 25 ml of TISAB were mixed in a beaker. Electrode was rinsed with distilled water and placed into beaker. Concentration was noted when “Rdy” was displayed on the instrument.

SELECTION OF TARGET AREAS AND FLUOROSIS GRADING

TARGET AREA:

Regions having high fluoride content in drinking water near Jaipur. These were divided into three groups in four target areas.

Group 1. Fluoride content of drinking water 1 to 4 mg/l

Group 2. Fluoride content of drinking water 5 to 8 mg/l

Group 3. Fluoride content of drinking water more than 8 mg/l

The rationales being that usually skeletal fluorosis is not noticed in people consuming water containing up to 4 mg/l of fluoride. Severe types of fluoride toxicity are often found in people consuming water containing over 8 mg/l of fluoride. In the 4 to 8 mg/l F ranges a variety of dental, skeletal, and clinical fluorosis has been observed. The 8 to 12 mg/l F range is associated with very severe manifestations. Hardly any data is available for higher ranges.

Target group:

Children below 12 years of age suffering from fluorosis.

Economics Status:

Children were selected from groups of socio-economic status representing a cross section of the community.

CRITERIA USED FOR FLUOROSIS GRADING

Clinical Fluorosis (Teotia Sps Et Al,1985)

(I) Mild - Generalized bone and joint pain

(II) Moderate - Generalized bone and joint pain, stiffness and rigidity, restricted movements at spine and joints

(III) Severe - Symptoms of moderate grading with deformities of spine and limbs, knock knees, crippled or bedridden state

Diagnosis of clinical fluorosis

Clinical fluorosis was diagnosed considering the well-known fact that the population was consuming water containing high levels of fluoride. Other causes of joint pains were excluded clinically. Standard clinical test to assess muscle spasm and decreased joint movement (finger toe test, neck bending test and hand stretch test) were positive.

Skeletal Fluorosis : (Radiological Examination -Teotia Sps Et Al,1985)

(I) Mild - Osteosclerosis only

(II) Moderate - Osteosclerosis, periosteal bone formation, calcification of membrane, ligaments, capsules, muscular attachments, tendons

(III) Severe - Findings as in moderate with exostoses, osteophytosis and associated metabolic bone disease

Dental Fluorosis (Deans, 1934)

Type	weight	Description
Normal Enamel	0	The enamel presents the usual translucent semi-vitriform type of structure. The surface is smooth, glossy, and usually of a pale, creamy-white color.
Questionable fluorosis	0.5	Slight aberrations from the translucency of normal enamel seen, ranging from a few white flecks to occasional white spots. This classification is used in instances where a definite diagnosis of the mildest form of fluorosis is not warranted and a Classification of "Normal" not justified.
Very mild fluorosis	1	Small opaque, paper-white areas scattered irregularly over the tooth but not involving as much as approximately 25% of the tooth surface. Frequently included in this classification are teeth showing no more than about 1-2mm of white opacity at the tip of the summit of the cusps of the bicuspids or second molars.
Mild fluorosis	2	The white opaque areas in the enamel of the teeth are more extensive, but do not involve as much as 50% of the tooth.
Moderate fluorosis	3	All enamel surface of the teeth are affected and surfaces subject to attrition show marked wear. Brown stain is frequently a disfiguring feature.
Severe fluorosis	4	All enamel surface are affected and hypoplasia is so marked that the general form of tooth may be affected. The major diagnosis of this classification is the discrete or confluent pitting. Brown stains are widespread, and teeth often present a corroded like appearance.

OBSERVATIONS

Observations have been recorded in three parts

1. Selection of area,
2. Evaluation of patients for –
    2a. Clinical, dental and skeletal fluorosis
    2b. Dietary evaluations, and
    2c. Biochemical evaluations.
3. Changes in clinical, dental and skeletal fluorosis after treatment in the selected children of different groups. The therapy proposed was kept same in all four areas.

AREA SELECTION

Out of the 20 villages selected for initial survey, following four villages were selected for detailed study. The drinking water fluoride concentration in the selected villages were as follows

Group A	Ramsagar ki Dhani	2.4 ppm
Group B	Rampura	4.6 ppm
Group C	Shivdaspura	5.6 ppm
Group D	Raipuria	13.6 ppm

EVALUATION OF PATIENTS FOR FLUOROSIS (CLINICAL, DENTAL AND SKELETAL)

(Different grades of dental, skeletal and clinical fluorosis are presented in photographs 1-18)

EVALUATION OF CLINICAL, DENTAL AND SKELETAL FLUOROSIS AT RAM SAGAR KI DHANI*: Table 1 indicates the grading of clinical, dental and skeletal fluorosis in children at RAM SAGAR KI DHANI.* Grading of clinical, dental and skeletal fluorosis before the treatment indicated presentations of varying severity. The presentation of dental fluorosis ranged from grade 0 to 4, clinical fluorosis was of grade 1 and skeletal grade 1.

Table 1

Cases	Dental	Clinical	Skeletal
1	4	1	2
2	3	1	1
3	3	0	0
4	2	1	0
5	3	1	1,2
6	4	1	0
7	2	1	0
8	3	1	1
9	3	1	1
10	0.5	1	0
11	3	1	1
12	3,4	1	1
13	4	1	2
14	1	1	0
15	2	1	1
16	3	1	0
17	3	1	1
18	3	1	1
19	2	1	1
20	3	1	0
21	3	1	0
22	0.5	1	0
23	4	1	0
24	2	1	0
25	0.5	1	0

Cases	Dental	Clinical	Skeletal
26	3	1	0
27	3	1	1
28	0	1	0
29	1	1	0
30	2	1	1
31	3,4	1	1
32	4	1	3
33	1	1	2
34	2	1	1
35	3	1	0
36	3	1	2
37	3	1	1
38	2	1	1
39	3	1	0
40	0	1	0
41	3	2	1,2
42	4	1	0
43	3	1	1
44	3	1	2
45	0.5	1	0
46	3	1	1
47	3,4	1	1
48	4	1	3
49	1	1	2
50	2	1	1

EVALUATION OF CLINICAL, DENTAL AND SKELETAL FLUOROSIS AT RAMPURA: *Table 2 indicates the grading of clinical, dental and skeletal fluorosis in children at RAMPURA. Grading of clinical, dental and skeletal fluorosis before the treatment indicated presentations of varying severity. The presentation of dental fluorosis ranged from grade 0 to 4, clinical fluorosis was of grade 1 and skeletal grade 1.*

Table 2

Cases	Dental	Clinical	Skeletal
1	1	1	0
2	3	1	1
3	0	1	0
4	1	1	1
5	2	1	0
6	0	1	1
7	2	1	0
8	0.5	1	1
9	0	1	2
10	3	1	1
11	2	1	1
12	2	1	0
13	1	1	2
14	2	1	1
15	3	1	0
16	2	1	0
17	1	1	2
18	3	1	2
19	0	1	0
20	3	1	1
21	2	1	0
22	0.5	1	1
23	2	1	0
24	3	1	0
25	0	1	1
26	1	1	1
27	2	1	0
28	1	1	0
29	0	1	1
30	2	1	1
31	2	1	0
32	0	1	1
33	2	1	0
34	0.5	1	1
35	0	1	0
36	3	1	1
37	2	1	1
38	2	1	0
39	1	1	2
40	2	1	1
41	3	1	0
42	2	1	0
43	1	1	0
44	3	1	0
45	0	1	0
46	3	1	0
47	2	1	0
48	0.5	1	1
49	2	1	0
50	3	1	0

EVALUATION OF CLINICAL, DENTAL AND SKELETAL FLUOROSIS AT SHIVDASPURA: Table 3 indicates the grading of clinical, dental and skeletal fluorosis in children at SHIVDASPURA. Grading of clinical, dental and skeletal fluorosis before the treatment indicated presentations of varying severity. The presentation of dental fluorosis ranged from grade 0 to 4, clinical fluorosis was of grade 1 and skeletal grade 1.

Table 3

Cases	Dental	Clinical	Skeletal
1	2	1	2
2	3	1	1
3	4	1	0
4	3	1	0
5	2	1	1,2
6	3	1	0
7	4	1	1
8	3	1	2
9	1,2	1	0
10	2	1	1
11	2,3	1	1
12	2	1	3
13	3	1	2
14	2,3	1	1
15	2	1	0
16	2	1	0
17	3	1	0
18	0	1	0
19	3	1	1
20	0.5	1	0
21	4	1	0
22	2	1	0
23	0.5	1	0
24	3	1	1
25	3	1	0
Cases	**Dental**	**Clinical**	**Skeletal**
26	0	1	0
27	1	1	1
28	2	1	0
29	2	1	0
30	1	1	0
31	3	1	0
32	4	1	1
33	3	1	2
34	1,2	1	0
35	2	1	1
36	2,3	1	1
37	2	1	3
38	3	1	2
39	2,3	1	1
40	2	1	0
41	0	1	0
42	1	1	1
43	2	1	0
44	2	1	0
45	1	1	0
46	3	1	0
47	4	1	1
48	3	1	2
49	1,2	1	0
50	2	1	1

EVALUATION OF CLINICAL, DENTAL AND SKELETAL FLUOROSIS AT RAIPURIA

Table 4 indicates the grading of clinical, dental and skeletal fluorosis in children at RAIPURIA. Grading of clinical, dental and skeletal fluorosis before the treatment indicated presentations of varying severity. The presentation of dental fluorosis ranged from grade 0 to 4, clinical fluorosis from grade 1 to 2 and skeletal fluorosis from grade 1 to 3. A special and one of the severe presentations of dental fluorosis was observed in this area. About 5 children showed enamel hypoplasia. Apart from the other presentations rarefaction of long and flat bones was one of the important observations of the skeletal fluorosis in this area.

Table 4

Cases	Dental	Clinical	Skeletal
1	3	1	0
2	4	2	1
3	3,4	2	1
4	3	2	2
5	4	1	3
6	4	1	0
7	2,3	2	1,2
8	1,2	1	1
9	3,4	2	1
10	4	1	2
11	4	2	3
12	3,4	2	1
13	4	1	0
14	3	1	1
15	4	1	0
16	3	1	1
17	2	2	0
18	3	1	3
19	3	2	3
20	3,4	2	0
21	4	1	0
22	4	1	0
23	0,1	1	2
24	2,3	1	0
25	4	2	0

Cases	Dental	Clinical	Skeletal
26	2,3	2	0
27	4	2	0
28	1,2	1	0
29	1	1	1
30	4	2	0
31	4	1	2
32	4	2	3
33	3,4	2	1
34	4	1	0
35	3	1	0
36	4	1	0
37	3,4	2	0
38	4	1	0
39	4	1	0
40	0,1	1	2
41	2,3	1	0
42	4	2	0
43	4	1	3
44	4	1	0
45	2,3	2	1,2
46	1,2	1	1
47	3,4	2	0
48	4	1	2
49	4	2	0
50	1,2	1	0

TABLES 5 – 9: DAILY FLUORIDE INTAKE

(THROUGH WATER AND FOOD IN EACH AREA).

Table 5

Total fluoride intake in Ram Sagar Ki Dhani ranged from 5-11 mg / day, out of which about 25-30% was through food. Dental grades were ranging from 0.5 to 4 (Average 2.71), skeletal grade ranges from 0-2 (average 0.68) and clinical grade ranges from 0-1 (average 0.95).

Table 6

Total fluoride intake in Rampura ranged from 9 -14 mg / day, out of which about 15 -25% was through food. Dental grades were ranging from 0.5 to 3 (Average 1.73), skeletal grade ranges from 0 -2 (average 0.5) and clinical grade ranges from 1-1 (average 1.0).

Table 7

Total fluoride intake in Shivdaspura ranged from 9 - 20 mg / day, out of which about 15 -20% was through food. Dental grades were ranging from 0 to 4 (Average 2.44), skeletal grade ranges from 0 -3 (average 0.79) and clinical grade ranges from 1-1 (average 1.0).

Table 8

Total fluoride intake in Raipuria ranged from 23 - 64 mg / day, out of which about 7- 10% was through food. Dental grades were ranging from 1 to 4 (Average 3.43), skeletal grade ranges from 0 -2 (average 0.95) and clinical grade ranges from 1-2 (average 1.5).

DENTAL AND SKELETON GRADE WITH AVERAGE FLUORIDE INTAKE PER DAY

S.No.	Age	Weight	Average Total waterIntake per day	Average Fluoride Intake per day			Grading		
				Fluoride through water	Fluoride through food	Total Fluoride through food and water	Dental Grade	Skeletal Grade	Clinical Grade
		kg	ml	mg	mg	mg			
Table 6 - Ram Sagar Ki Dhani									
1	10	22.50	1687.50	4.05	1.23	5.28	4	2	1
2	9	23.13	2313.00	5.55	2.16	7.71	3	1	1
3	12	28.62	2146.50	5.15	1.67	6.83	3	0	0
4	10	24.53	1839.38	4.41	1.02	5.43	2	0	1
5	9	36.14	3613.50	8.67	1.97	10.64	3	1,2	1
6	7	21.15	2115.00	5.08	1.88	6.95	4	0	1
7	10	24.53	1839.38	4.41	1.02	5.43	2	0	1
8	7	17.55	1755.00	4.21	1.30	5.52	3	1	1
9	10	22.37	1677.38	4.03	3.63	7.66	3	1	1
10	7	18.41	1840.50	4.42	3.33	7.74	0.5	0	1
11	5	13.82	1726.88	4.14	3.53	7.67	3	1	1
12	8	21.38	2671.88	6.41	3.72	10.14	3,4	1	1
13	6	13.91	1738.13	4.17	7.28	11.46	4	2	1
14	6	17.64	2205.00	5.29	3.06	8.35	1	0	1
15	12	26.87	2014.88	4.84	1.06	5.89	2	1	1
16	8	17.33	1732.50	4.16	2.21	6.37	3	0	1
17	15	30.87	2315.25	5.56	1.36	6.91	3	1	1
18	14	30.78	2308.50	5.54	1.59	7.13	3	1	1
19	10	26.78	2008.13	4.82	2.09	6.91	2	1	1
20	8	18.90	1890.00	4.54	2.51	7.05	3	0	1
SD				1.09	1.47	1.72	1.09	0.67	0.22
Mean				4.97	2.38	7.35	2.71	0.68	0.95

DENTAL AND SKELETON GRADE WITH AVERAGE FLUORIDE INTAKE PER DAY

S.No.	Age	Weight	Average Total waterIntake per day	Average Fluoride Intake per day			Grading		
				Fluoride through water	Fluoride through food	Total Fluoride through food and water	Dental Grade	Skeletal Grade	Clinical Grade
		kg	ml	mg	mg	mg			
Table 7 - Rampura									
1	13	29.25	2193.75	10.09	1.61	11.70	1	0	1
2	10	28.80	2160.00	9.94	2.29	12.23	3	1	1
3	11	29.80	2150.00	9.94	2.29	12.23	3	1	1
4	8	20.25	2025.00	9.33	2.88	12.31	0	0	1
5	10	20.79	1559.25	7.17	2.10	9.27	1	1	1
6	3	9.72	1458.00	6.71	3.12	9.83	2	0	1
7	11	23.18	1738.13	8.00	1.56	9.56	0	1	1
8	7	17.37	1737.00	7.99	3.26	11.25	2	0	1
9	15	39.60	2970.00	13.66	1.28	14.94	3	0	1
10	4	14.04	1755.00	8.07	4.01	12.08	0.5	1	1
11	15	36.09	2706.75	12.45	1.20	13.65	0	0	1
12	11	23.13	1734.75	7.98	1.10	9.08	3	1	1
13	13	27.05	2028.38	9.33	1.13	10.46	2	1	1
14	14	35.37	2652.75	12.20	1.56	13.76	2	0	1
15	12	26.55	1991.25	9.16	1.46	10.62	1	2	1
16	10	20.70	2070.00	9.52	2.79	12.31	2	1	1
17	15	39.60	2970.00	13.66	1.28	14.94	3	0	1
18	13	38.93	2919.38	13.43	1.09	14.52	2	0	1
19	5	12.69	1586.25	7.30	3.89	11.19	1	0	1
20	15	36.27	2720.25	12.51	1.05	13.56	3	0	1
SD				2.29	0.97	1.84	1.09	0.61	0.00
Mean				9.92	2.05	11.97	1.73	0.50	1.00

DENTAL AND SKELETON GRADE WITH AVERAGE FLUORIDE INTAKE PER DAY

S.No.	Age	Weight	Average Total waterIntake per day	Average Fluoride Intake per day			Grading		
				Fluoride through water	Fluoride through food	Total Fluoride through food and water	Dental Grade	Skeletal Grade	Clinical Grade
		kg	ml	mg	mg	mg			
Table 8 - Shivdas Pura									
1	12	26.00	1950.00	8.78	1.67	10.45	2	2	1
2	8	24.00	3000.00	13.50	3.12	16.62	3	1	1
3	8	31.00	3875.00	17.44	3.01	20.45	4	0	1
4	11	33.00	2475.00	11.14	3.60	14.74	3	0	1
5	8	30.00	3750.00	16.88	3.00	19.88	2	1,2	1
6	10	23.00	1725.00	7.76	1.25	9.01	3	0	1
7	10	23.00	1725.00	7.76	1.26	9.02	4	1	1
8	9	26.00	2600.00	11.70	2.16	13.86	3	2	1
9	8	20.00	2500.00	11.25	3.00	14.25	1,2	0	1
10	8	23.00	2875.00	12.94	2.57	15.51	2	1	1
11	9	24.00	2400.00	10.80	2.13	12.93	2,3	1	1
12	8	28.00	3500.00	15.75	2.50	18.25	2	3	1
13	8	24.00	3000.00	13.50	2.36	15.86	3	2	1
14	8	19.00	2375.00	10.69	2.69	13.38	2,3	1	1
15	9	21.00	2100.00	9.45	3.01	12.46	2	0	1
16	9	30.00	3000.00	13.50	1.98	15.48	2	0	1
17	10	29.00	2175.00	9.79	1.59	11.38	3	0	1
18	8	26.00	3250.00	14.63	2.56	17.19	0	0	1
19	8	23.00	2875.00	12.94	2.67	15.61	3	1	1
20	7	19.00	2375.00	10.69	2.00	12.69	0.5	0	1
SD				2.78	0.65	3.19	1.32	0.91	0.00
MEAN				12.04	2.41	14.45	2.44	0.79	1.00

DENTAL AND SKELETON GRADE WITH AVERAGE FLUORIDE INTAKE PER DAY

S.No.	Age	Weight	Average Total waterIntake per day	Average Fluoride Intake per day			Grading		
				Fluoride through water	Fluoride through food	Total Fluoride through food and water	Dental Grade	Skeletal Grade	Clinical Grade
		kg	ml	mg	mg	mg			
Table 9 - Raipuria									
1	6	13.95	1743.75	23.54	3.13	26.67	3	0	1
2	11	21.00	1575.00	21.26	2.10	23.36	4	1	2
3	11	27.45	2058.75	27.79	1.29	29.08	3,4	1	2
4	9	17.55	1755.00	23.69	2.20	25.89	3	2	2
5	14	35.73	2679.75	36.18	1.23	37.41	4	3	1
6	12	26.24	1967.63	26.56	1.62	28.18	4	0	1
7	13	39.78	2983.50	40.28	1.90	42.18	2,3	1,2	2
8	10	26.19	1964.25	26.52	2.61	29.13	1,2	1	1
9	7	18.90	1890.00	25.52	3.96	29.48	3,4	0	2
10	7	27.09	2709.00	36.57	3.56	40.13	4	2	1
11	6	17.19	2148.75	29.01	3.16	32.17	4	3	2
12	12	35.64	2673.00	36.09	1.56	37.65	3,4	1	2
13	10	20.07	1505.25	20.32	2.21	22.53	4	0	1
14	13	26.55	1991.25	26.88	1.45	28.33	3	0	1
15	9	18.45	1845.00	24.91	2.36	27.27	4	0	1
16	6	13.50	1687.50	22.78	3.69	26.47	3	1	1
17	8	19.22	2401.88	32.43	2.24	34.67	2	0	2
18	13	35.73	2679.75	36.18	1.56	37.74	3	3	1
19	15	61.65	4623.75	62.42	1.89	64.31	3	0	2
20	9	19.49	1948.50	26.30	2.25	28.55	3,4	0	2
SD				9.52	0.82	9.33	1.70	1.12	0.51
MEAN				30.26	2.30	32.56	3.43	0.95	1.50

DIETARY EVALUATION OF THE SELECTED GROUP OF CHILDREN

Table 9 is representing the summary of the daily intake of fluoride through water and food in all four areas.

Table 9

Village	Average Fluoride Intake per day			Grading		
	Fluoride through water	**Fluoride through food**	**Total Fluoride through food and water**	**Dental Fluorosis**	**Skeleton Fluorosis**	**Clinical Fluorosis**
	Mean (SD)	*Mean (SD)*	*Mean (SD)*	*Mean (SD)*	*Mean (SD)*	*Mean (SD)*
Ram Sagar Ki Dhani	5.00 (1.11)	2.45 (1.47)	7.45 (1.70)	2.71 (1.09)	0.68 (0.67)	0.95 (0.22)
Rampura	9.71 (2.23)	2.07 (1.00)	11.79 (1.80)	1.73 (1.09)	0.50 (0.61)	1.00 (0.00)
Shivdas Pura	12.04 (2.78)	2.41 (0.65)	14.45 (3.19)	2.44 (1.32)	0.79 (0.91)	1.00 (0.00)
Raipuria	30.26 (9.52)	2.30 (0.82)	32.56 (9.33)	3.43 (1.70)	0.95 (1.12)	1.50 (0.51)

It was observed that

- Daily protein intake in these children was less then the required amount. The average protein intake was 0.5 – 0.76 gm per day.
- Daily calcium (average 230 – 430 mg/ day) and vitamin C (less then 100 mg/day) intake was less then the desired amount, in all the four areas.
- There was no significant difference in total daily intake of protein, Calcium and vitamin C intake among all the four areas.
- Fluoride intake through food was more or less same in all the four villages in comparison to fluoride intake through water. The fluoride through food was about 30% of the total daily fluoride intake in low fluoride areas (Ram Sagar Ki Dhani –

water fluoride content 2.4 ppm), where as in high fluoride areas (Raipuria- 13,6 ppm) the food was contributing only 7% of daily fluoride intake.

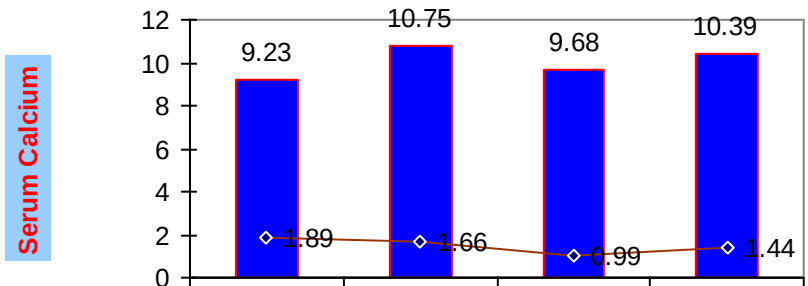
- Clinical presentations vary with the total fluoride intake.
- The severity of dental fluorosis is almost same in three areas (Ram sagar ki dhani – 2.71, Rampura – 1.73 and Shivdaspura – 2.44) except in Ramsagar ki dhani, with drinking water fluoride concentration 2.4 to 5.6 ppm. Daily total fluoride intake ranged from 7 – 14 mg. in these three areas.
- There is abrupt increase in severity of dental fluorosis in area (Raipuria) with drinking water fluoride concentration 13.6 ppm, and total average daily fluoride intake is about 30.00 mg. These observations have indicated that the presentation of dental fluorosis varies with the total fluoride intake.
- The severity of clinical and skeletal fluorosis starts rising as the total fluoride intake starts rising more than 12 mg per day (Fluoride from food – 14%), but as the total daily intake of fluoride increases more than 25 mg (Fluoride from food – 7%), the severity of clinical and skeletal fluorosis increases abruptly.

BIOCHEMICAL EVALUATION

SERUM CALCIUM

Table 10 presents the values of serum calcium in selected four target areas. Figure 10 is a graphical presentation of table 10.

Table 10 & Fig. 10 : SERUM CALCIUM



	Ramsagar ki Dhani	Rampura	Shivdaspura	Raipuria
S.Calcium (Average in mg/dl)	9.23	10.75	9.68	10.39
S.Calcium (SD)	1.89	1.66	0.99	1.44

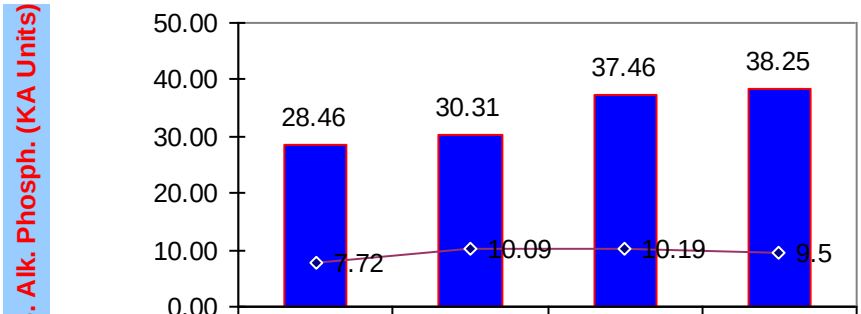
Serum calcium levels in these children were well within normal limits.

Another observation indicated that even the calcium levels have been decreased in group C and D, that is the areas with high fluoride concentrations. This finding is of interest since radiological examination (discussed later on) showed calcification in the bones along with increased Parathyroid hormone levels, indicating increased utilization of the calcium for mineralisation of the bones.

SERUM ALKALINE PHOSPHATASE(SAP)

The SAP activity indicates the osteoblastic bone activity. Table 11 presents the values of serum alkaline phosphatase in selected four target areas. Figure 11 is a graphical presentation of table 11.

Table 11 & Fig. 11: SERUM ALKALINE PHOSPHATASE



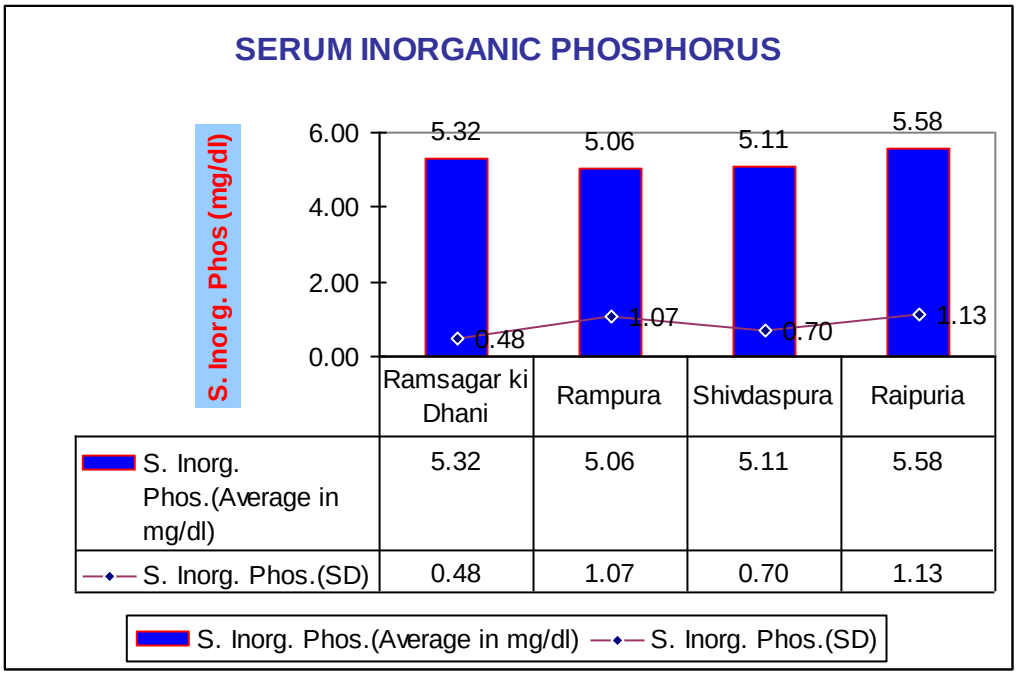
	Ramsagar ki Dhani	Rampura	Shivdaspura	Raipuria
S. Alk. Phos (Average in KA units/dl)	28.46	30.31	37.46	38.25
S. Alk. Phos (SD)	7.72	10.09	10.19	9.5

The serum alkaline phosphatase (SAP) activity was observed to be high.

SERUM INORGANIC PHOSPHORUS

Serum inorganic phosphorus is one of the important parameter, playing vital role in controlling parathyroid hormone secretion and laying down of bone.

Table 12 presents the values of serum inorganic phosphorus in selected four target areas. Figure 12 is a graphical presentation of table 12.

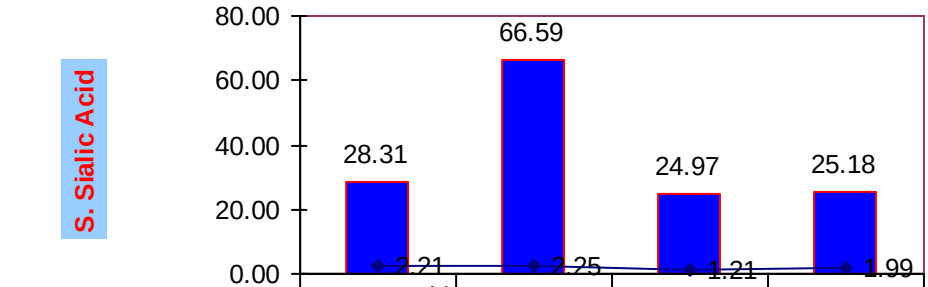


The normal range of Serum inorganic phosphorus (SIP) is 3.7 - 5.6 mg/dl in the age group of 4-12 years. The SIP levels were observed to be well within normal range (5.06 – 5.58 mg/dl).

N - ACETYL NEURAMINIC ACID (SIALIC ACID)

N - acetyl neuraminic acid (Sialic acid), a component of glycoprotein, is an important parameter in detection of fluoride toxicity. The normal value ranges from 59-64 mg/dl in human being. Table 13 presents the values of N - Acetyl neuraminic acid (Sialic acid) in four target areas. Figure 13 is a graphical presentation of table 13.

Table 13 & Fig. 13 : SERUM SIALIC ACID



	Ramsagar ki Dhani	Rampura	Shivdaspura	Raipuria
S. Sialic Acid (Average in mg/dl)	28.31	66.59	24.97	25.18
S. Sialic Acid (SD)	2.21	2.25	1.21	1.99

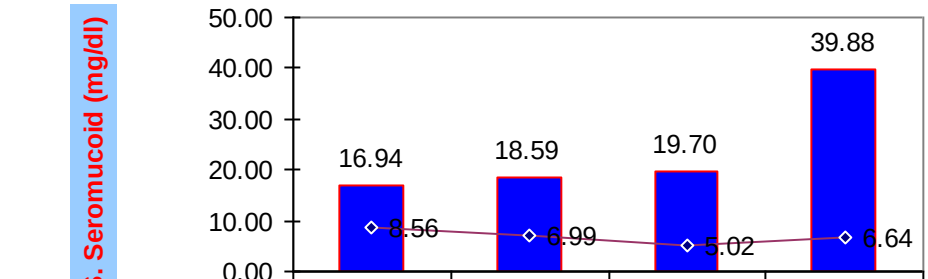
Lower values of sialic acid were observed in this study in all areas except in group B.

It is important to mention that although sialic acid is minimally altered in bone as a result of fluoride ingestion, its levels are decreased in serum as a result of F-toxicity both in animal and man.

SERUM GLUCOSAMINEGLYCANS (SEROMUCOID)

The glucosamineglycans (GAG) are glycoproteins. The normal values of GAG are 9-11 mg/dl. Table 14 presents the values of glucosamineglycans (seromucoid) in selected four target areas. Figure 14 is a graphical presentation of table 14.

Table 14 & Fig. 14 : SERUM SEROMUCOID



	Ramsagar ki Dhani	Rampura	Shivdaspura	Raipuria
Seromucoid (Average in mg/dl)	16.94	18.59	19.70	39.88
Seromucoid (SD)	8.56	6.99	5.02	6.64

Seromucoid (Average in mg/dl) — Seromucoid (SD)

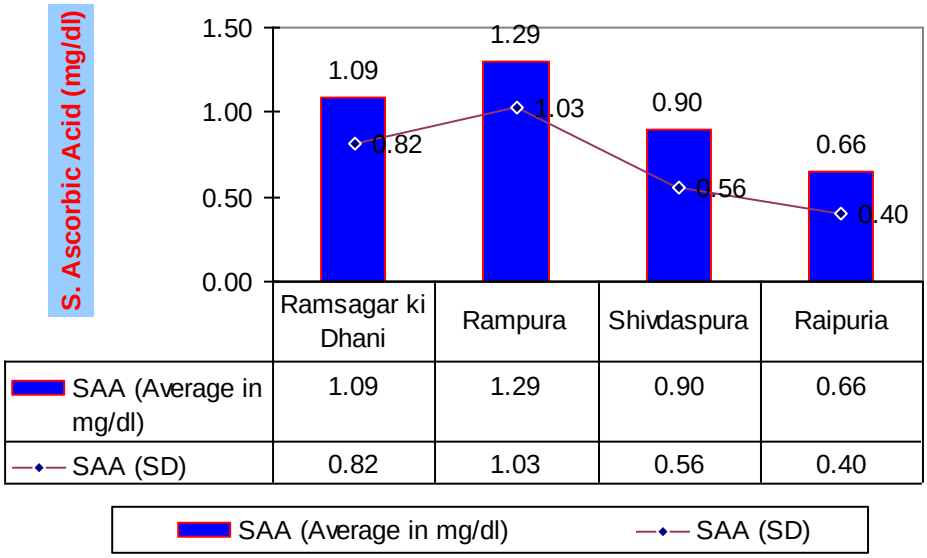
Elevated values of GAG have been observed in all target areas. The maximum elevations have been observed in areas with highest fluoride concentration in drinking water i.e. group D.

Elevated content of GAG in bone and its reflection in serum is considered as an index to assess fluoride toxicity and fluorosis at very early stages. Until recent time, the only reliable criterion for assessing fluoride toxicity was radiographs. However, radiographs are only helpful to diagnose the disease at late stages when the ligaments are calcified and bones become denser. The ratio of sialic acid to GAG has been found to be a sensitive index to detect fluoride toxicity at very early stages both in human and animal models. The ratio of SA/GAG revealed a 30-50% reduction in human sera in fluoride poisoning.

SERUM ASCORBIC ACID

Table 15 presents the values of serum ascorbic acid in selected four target areas. Figure 15 is a graphical presentation of table 15.

Table 15 & Fig. 15 : SERUM ASCORBIC ACID



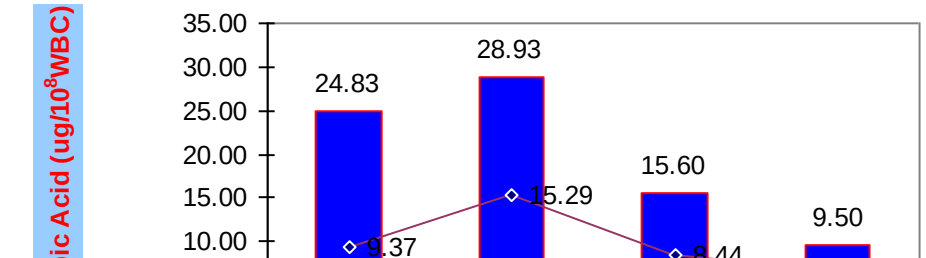
	Ramsagar ki Dhani	Rampura	Shivdaspura	Raipuria
SAA (Average in mg/dl)	1.09	1.29	0.90	0.66
SAA (SD)	0.82	1.03	0.56	0.40

The serum ascorbic acid was observed to be within in normal range in all the target areas except group B.

LEUCOCYTE ASCORBIC ACID (LAA)

The low levels of LAA indicate the long-standing depletion in ascorbic acid and may be due to decreased dietary intake or excessive utilization. Table 16 presents the values of leucocyte ascorbic acid (LAA) in selected four target areas. Figure 16 is a graphical presentation of table 16.

Table 16 & Fig. 16 : LEUCOCYTE ASCORBIC ACID



	Ramsagar ki Dhani	Rampura	Shivdaspura	Raipuria
LAA (Average in ug/108 WBC)	24.83	28.93	15.60	9.50
LAA (SD)	9.37	15.29	8.44	6.66

LAA (Average in ug/108 WBC) — LAA (SD)

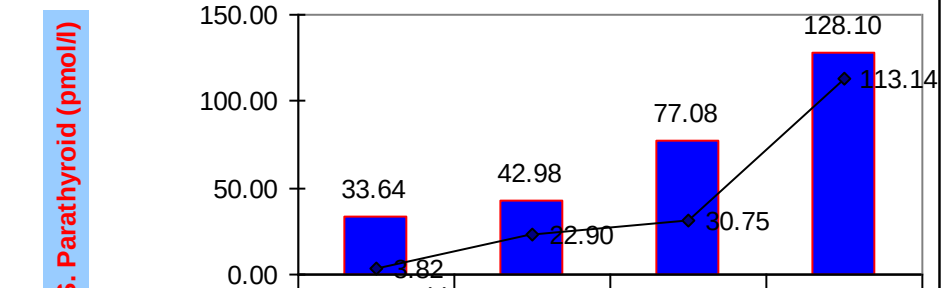
The leucocyte ascorbic acid levels were below the normal range in all areas. The lowest values have been observed in the group D, the area of highest fluoride concentration, which correlates well with the observed low values of SAA in the same group.

SERUM PARATHYROID HORMONE LEVELS (S. PTH):

Serum PTH plays vital role in serum calcium regulation, which is one of the important ions, affected by fluoride. As the fluoride intake increases the PTH secretion also increases to maintain the serum calcium levels.

Table 17 presents the values of serum parathyroid hormone (S. PTH) in selected four target areas. Figure 17 is a graphical presentation of table 17.

Table 17 & Fig. 17: SERUM PARATHYROID



	Ramsagar ki Dhani	Rampura	Shivdaspura	Raipuria
S.PTH-MM II (Average in pmol/l)	33.64	42.98	77.08	128.10
S.PTH-MM II (SD)	3.82	22.90	30.75	113.14

S.PTH-MM II (Average in pmol/l) — S.PTH-MM II (SD)

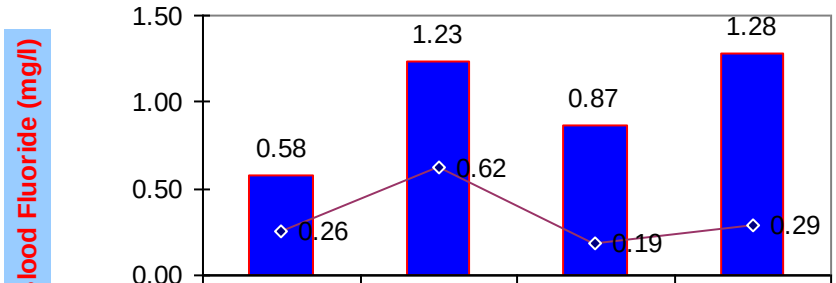
The PTH levels were with in upper range of normal in groups A & B (Areas of moderately high fluoride levels) whereas in group C & D (Areas of very high fluoride levels) Serum PTH levels are high.

BLOOD AND SERUM FLUORIDE

Blood plasma F has been believed only to show a limited absorption process of gradual F depletion of an F-rich skeleton. Some of the variations of plasma inorganic F may have been obscured by analytical methods, which have comprised the fraction of organically bound plasma F also. While the ionizable F (F-) of a young person's fasting plasma generally is as low as 0.01 - 0.02 ppm, or 0.5 - 1 uM/1, the organically bound F may be 5 to 10 times higher. The nature of organic F bonding in plasma is not yet understood, but it is evident that the ionized or easily ionizable plasma F is the

physiologically active fraction. Table 18 & 19 presents the values of Blood fluoride and Serum fluoride in selected four target areas. Figures 18 & 19 are a graphical presentation of tables 18 & 19. High blood and serum fluoride levels have been observed in all areas.

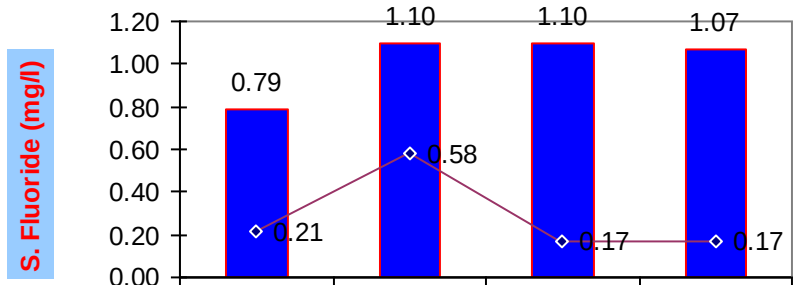
Table 18 & Fig. 18 : BLOOD FLUORIDE



	Ramsagar ki Dhani	Rampura	Shivdaspura	Raipuria
Blood Fluoride (Average in mg/l)	0.58	1.23	0.87	1.28
Blood Fluoride (SD)	0.26	0.62	0.19	0.29

Blood Fluoride (Average in mg/l) — Blood Fluoride (SD)

Table 19 & Fig. 19 : SERUM FLUORIDE



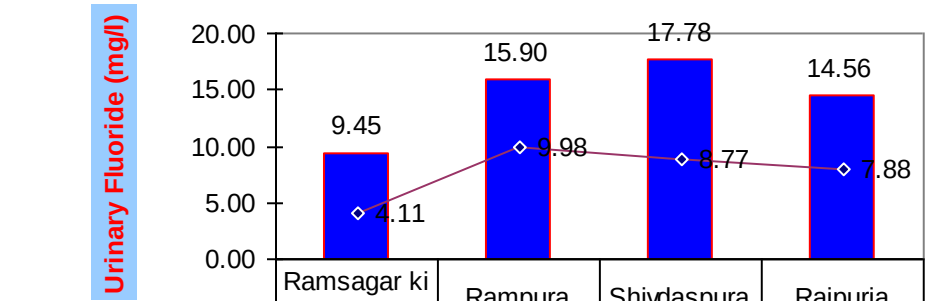
	Ramsagar ki Dhani	Rampura	Shivdaspura	Raipuria
S.Fluoride (Average in mg/l)	0.79	1.10	1.10	1.07
S.Fluoride (SD)	0.21	0.58	0.17	0.17

S.Fluoride (Average in mg/l) — S.Fluoride (SD)

URINARY FLUORIDE

Table 20 presents the values of urinary fluoride in selected four target areas. Figure 20 is a graphical presentation of table 20.

Table 20 & Fig. 20 : URINARY FLUORIDE



	Ramsagar ki Dhani	Rampura	Shivdaspura	Raipuria
Urinary Fluoride (Average in mg/l)	9.45	15.90	17.78	14.56
Urinary Fluoride (SD)	4.11	9.98	8.77	7.88

Urinary fluoride level has been used to estimate the absorbed amount of fluoride and is recognized as one of the best indices of fluoride intake. Fluoride (F) in urine has long been known to reflect intestinal F absorption as well as F liberation from a fluoride-rich skeleton.

The urinary fluoride level, which is being used for the detection of endemic hydrofluorosis and industrial fluorosis, has been shown to be a misleading parameter in certain conditions. The kidney tubules fail to function normally as the disease progresses and a normal limit of fluoride is excreted at late stages of the disease. The above observations have challenged the health reports emerging from industries where urinary fluoride has been used as the main parameter to detect fluoride toxicity.

POST TREATMENT EVALUATION
(Clinical, dental and skeletal)

Table 21

Table 21 indicates the pre treatment (Pre Trt.) and post treatment (Post trt.) grading of clinical, dental and skeletal fluorosis in children *AT RAM SAGAR KI DHANI*.

Table 21

Cases No.	Dental		Clinical		Skeletal		Spina Bifida
	Pre Trt	Post Trt	Pre Trt	Post Trt	Pre Trt	Post Trt	
1	4	1,2	1	0	2	0	Nil
2	3	1,2	1	0	1	0	Nil
3	3	0,1	1	0	0	0	L5
4	2	0	1	0	0	0	L5
5	3	0	1	0	1,2	0	Nil
6	4	2	1	0	0	0	L5
7	3	0.5	1	0	1	0	L5
8	3	1,2	1	0	2	0	Nil
9	0.5	NF	1	NF	0	NF	L5
10	3	0,1	1	0	1	0	L5
11	3,4	0,1	1	0	1	0	L5S1
12	4	3	1	0	3	1,2	Nil
13	1	0	1	0	2	0	Nil
14	2	NF	1	NF	1	NF	Nil
15	3	0	1	0	0	0	Nil
Controls							
1	3	2	1	1	2	0	Nil
2	3	2	1	1	1	0	Nil
3	2	3	1	1	1	0	L5
4	3	1	1	1	0	0	L5
5	0	3	1	1	0	0	Nil
6	3	2	1	1	0	0	Nil
7	0.5	1	1	1	0	1	Nil
8	4	2	1	1	0	0	L5
9	2	3	1	1	0	0	L5
10	0.5	NF	1	NF	0	NF	L5
11	3	1	1	1	0	1	Nil
12	3	2	1	1	1	0	Nil
13	0	NF	1	NF	0	NF	Nil
14	1	1	1	1	0	1	L5
15	2	2	1	1	1	1	Nil

Grading of clinical, dental and skeletal fluorosis before the treatment indicated presentations of varying severity. The presentation of dental fluorosis ranged from grade 0 to 4, clinical fluorosis was of grade 1 and skeletal grade 1. Post treatment examination showed a significant improvement in comparison to controls ($p < 0.05$).

Table 22

Table 22 is indicating the pre treatment and post treatment grading of clinical, dental and skeletal fluorosis in children *AT RAMPURA*

Grading of clinical, dental and skeletal fluorosis before the treatment indicated presentations of varying severity. The presentation of dental fluorosis ranged from grade 0 to 4, clinical fluorosis was of grade 1 and skeletal grade 1. Post treatment examination showed a significant improvement in comparison to controls ($p < 0.05$).

Table 23

Table 23 is indicating the pre treatment and post treatment grading of clinical, dental and skeletal fluorosis in children *AT SHIVDASPURA*:

Grading of clinical, dental and skeletal fluorosis before the treatment indicated presentations of varying severity. The presentation of dental fluorosis ranged from grade 0 to 4, clinical fluorosis was of grade 1 and skeletal grade 1. Post treatment examination showed a significant improvement in comparison to controls ($p < 0.05$).

Table 22: Pre treatment and post treatment grading of clinical, dental and skeletal fluorosis in children AT RAMPURA

Cases No.	Dental		Clinical		Skeletal		Spina Bifida
	Pre Trt	Post Trt	Pre Trt	Post Trt	Pre Trt	Post Trt	
1	1	0	1	0	0	0	Nil
2	3	1	1	0	1	0	L5
3	0	0	1	0	0	0	Nil
4	1	0	1	0	1	0	Nil
5	2	0	1	0	0	0	L5
6	0	NF	1	NF	1	NF	Nil
7	2	1	1	0	0	0	Nil
8	0.5	0	1	0	1	0	L5,S1
9	0	0	1	0	0	0	Nil
10	3	2,3	1	0	1	0	L5
11	2	0,1	1	0	1	0	L5
12	2	NF	1	NF	0	NF	L5
13	1	0	1	0	2	0	Nil
14	2	0	1	0	1	0	L5
Controls							
1	2	2	1	1	0	0	Nil
2	1	2	1	1	0	0	Nil
3	3	3	1	1	0	0	L5
4	0	1	1	1	0	0	L5
5	3	3	1	1	0	0	L5
6	2	2	1	1	0	0	L5
7	0.5	1	1	1	1	1	Nil
8	2	2	1	1	0	0	L5
9	3	3	1	1	0	0	L5
10	0	NF	1	NF	1	NF	Nil
11	1	1	1	1	1	1	Nil
12	2	2	1	1	0	0	L5
13	1	1	1	1	0	0	L5
14	0	1	1	1	1	1	L4,5
15	2	2	1	1	1	1	L5

Table 23*:* Pre treatment and post treatment grading of clinical, dental and skeletal fluorosis in children AT SHIVDASPURA:

Table 23

Cases No.	Dental		Clinical		Skeletal		Spina Bifida
	Pre Trt	Post Trt	Pre Trt	Post Trt	Pre Trt	Post Trt	
1	2	1	1	0	2	0	Nil
2	3	2,3	1	0	1	0	Nil
3	4	2	1	0	0	0	L5
4	3	2,3	1	0	0	0	L5
5	2	1	1	0	1,2	0	Nil
6	3	2,3	1	0	0	0	L5
7	4	2	1	0	1	0	L5
8	3	1,3	1	0	2	0	Nil
9	1,2	0	1	0	0	NF	L5
10	2	1	1	0	1	0	L5
11	2,3	2	1	0	1	0	L5S1
12	2	1	1	0	3	1,2	Nil
13	3	2,3	1	0	2	0	Nil
14	2,3	2,3	1	0	1	NF	Nil
15	2	1,2	1	0	0	0	Nil
Controls							
1	2	3	1	1	0	0	Nil
2	3	3	1	1	0	0	Nil
3	0	3	1	1	0	0	L5
4	3	2	1	1	1	1	Nil
5	0.5	1	1	1	0	0	Nil
6	4	4	1	1	0	0	Nil
7	2	3	1	1	0	0	Nil
8	0.5	1	1	1	0	0	L5
9	3	3	1	1	1	1	L5
10	3	3	1	1	0	0	Nil
11	0	1	1	1	0	0	Nil
12	1	1	1	1	1	1	Nil
13	2	2	1	1	0	0	Nil
14	2	2	1	1	0	0	L5
15	1	1	1	1	0	0	Nil

Table 24

Table 24 is indicating the pre treatment and post treatment grading of clinical, dental and skeletal fluorosis in children *AT RAIPURIA.*

Table 24

Cases No.	Dental		Clinical		Skeletal		Spina Bifida
	Pre Trt	Post Trt	Pre Trt	Post Trt	Pre Trt	Post Trt	
1	3	2	1	0	0	0	Nil
2	4	3	2	1	1	0	Nil
3	3,4	3,4	2	1	1	0	Nil
4	3	3	2	1	2	0,1	Nil
5	4	3	1	0	3	1,2	Nil
6	4	2,3	1	0	0	0	Nil
7	2,3	2	2	1	1,2	0,1	Nil
8	1,2	3	1	0	1	0	Nil
9	3,4	2,3	2	1	0	0	Nil
10	4	4	1	0	2	0	Nil
11	4	2	2	1	3	1,2	Nil
12	3,4	2,3	2	1	1	0	Nil
13	4	3	1	0	0	0	Nil
14	3	2	1	0	0	0	Nil
15	4	3	1	0	0	0	Nil
Controls							
1	3	3	1	1	1	1	Nil
2	2	2,3	2	2	0	0	Nil
3	3	3	1	1	3	3	Nil
4	3	3	2	2	0	0	Nil
5	3,4	3,4	2	2	0	0	L5
6	4	3,4	1	1	0	0	L5
7	4	3,4	1	1	0	0	Nil
8	0,1	3	1	1	2	2	L5
9	2,3	3	1	1	0	0	L5
10	4	4	2	2	0	0	L5
11	2,3	2,3	2	2	0	0	L5
12	4	3,4	2	2	0	0	L5
13	1,2	2,3	1	1	0	0	L5
14	1	1	1	1	1	1	Nil
15	4	4	2	2	0	0	Nil

Grading of clinical, dental and skeletal fluorosis before the treatment indicated presentations of varying severity. The presentation of dental fluorosis ranged from grade 0 to 4, clinical fluorosis from grade 1 to 2 and skeletal fluorosis from grade 1 to 3.

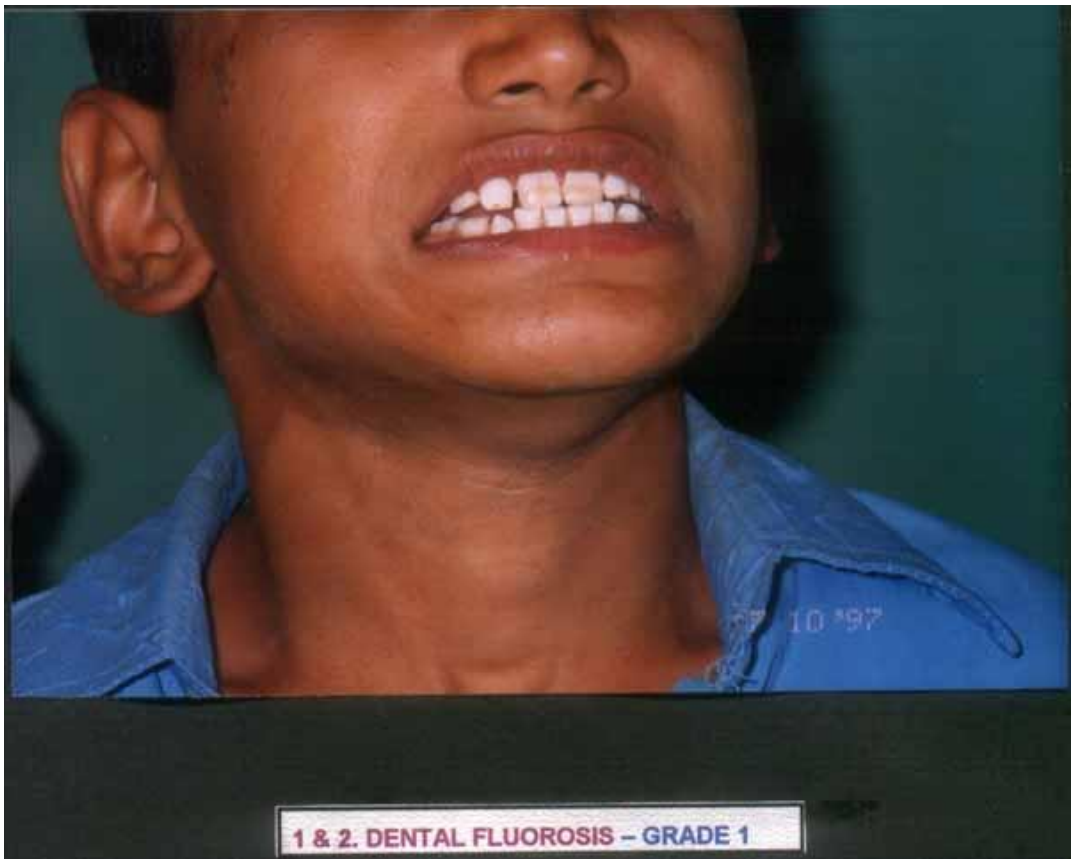
The special and one of the severe presentations of dental fluorosis was observed in this area. About 5 children showed enamel hypoplasia. Apart from the other presentations rarefaction of long and flat bones was one of the important observations of the skeletal fluorosis in this area.

Post treatment examination showed a significant improvement in comparison to controls ($p < 0.05$). The improvement in dental fluorosis was minimal or almost negligible in children presented with enamel hypoplasia. The improvement in skeletal presentation was two folds: a) Improved calcification of the rarefied areas of the long and flat bone. b) Removal of the dense fluoride deposited areas.

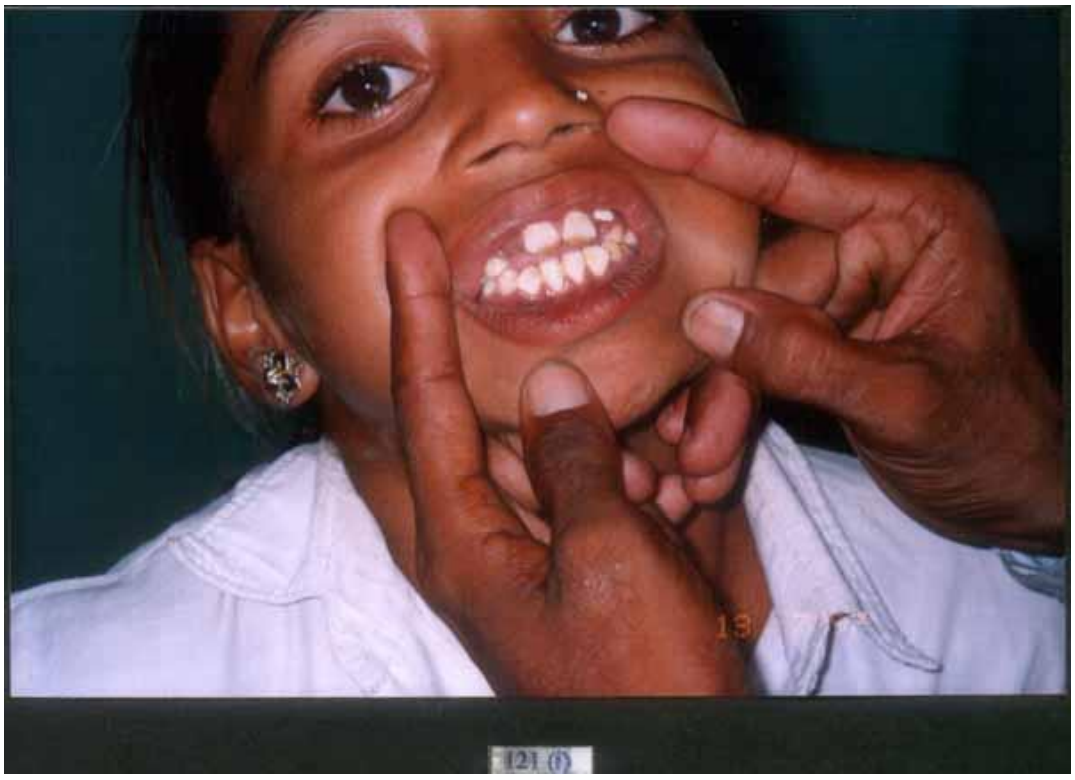
The degree of improvement in dental, clinical and skeletal changes is not much, as compared to other three target areas. This observation indicates that the doses of the drugs (Calcium, Vitamin C & D) needs modification depending on water fluoride concentration and severity of symptoms. This observation has also been reflected in biochemical parameters as already discussed.

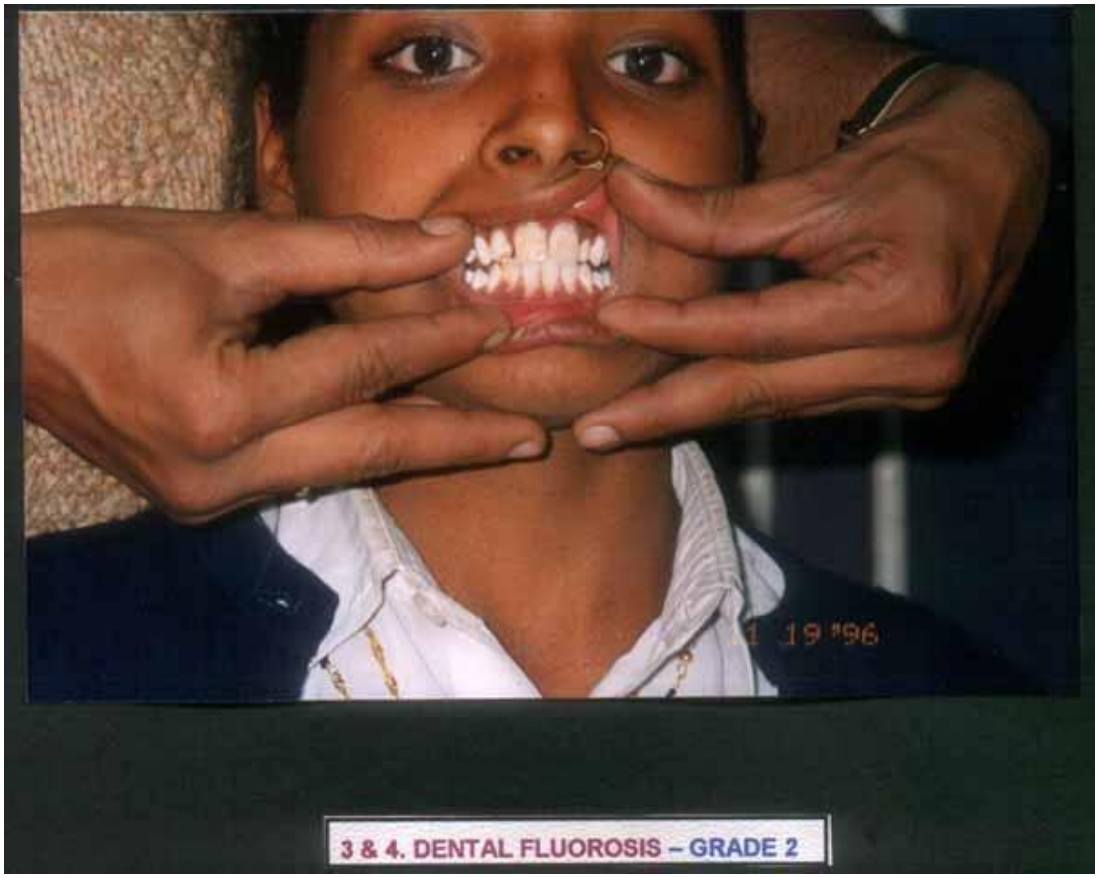
The other presentations were the presence of spina bifida occulta in these children. This observation was in accordance with the observations of previous pilot study.

Post treatment improvements of different grades of dental, clinical and skeletal fluorosis are presented in photographs 19 – 34.

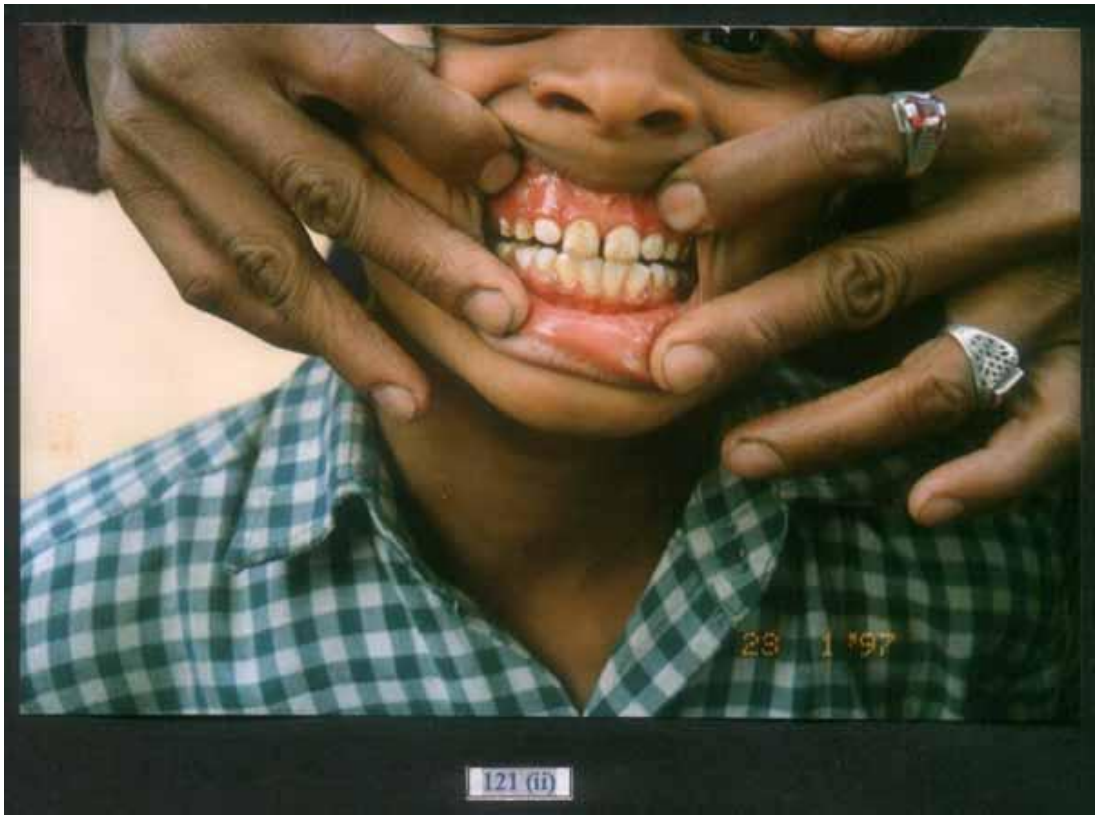


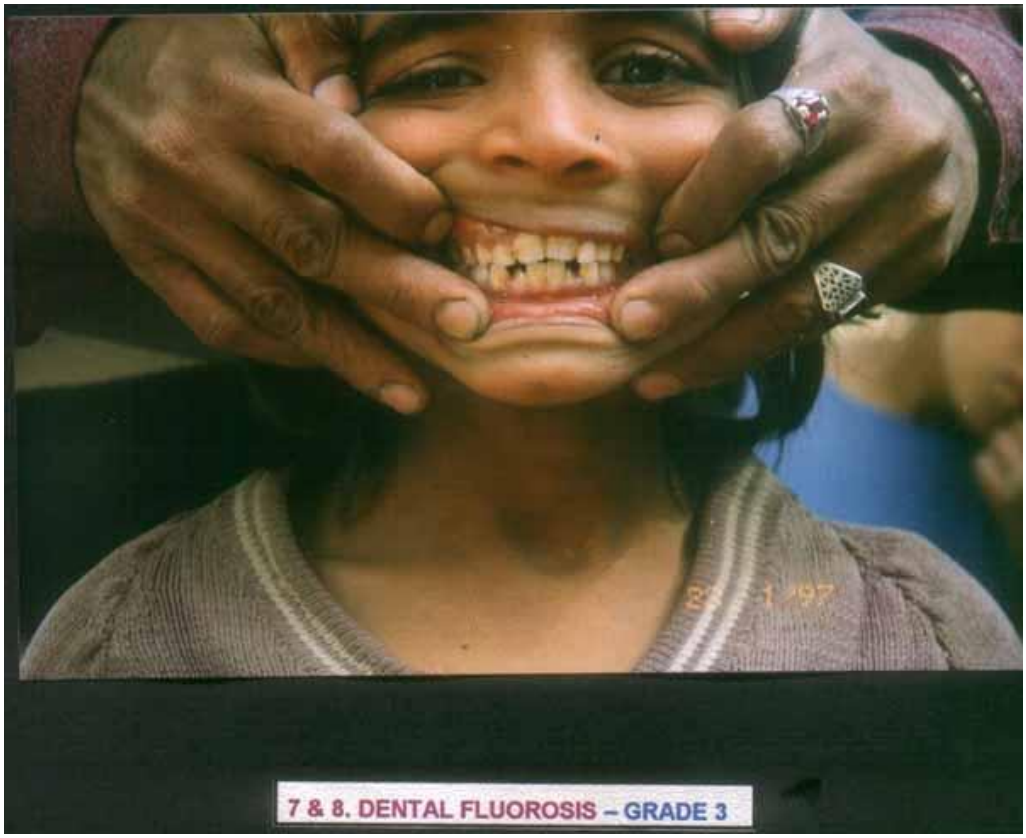
Dental Fluorosis grade I



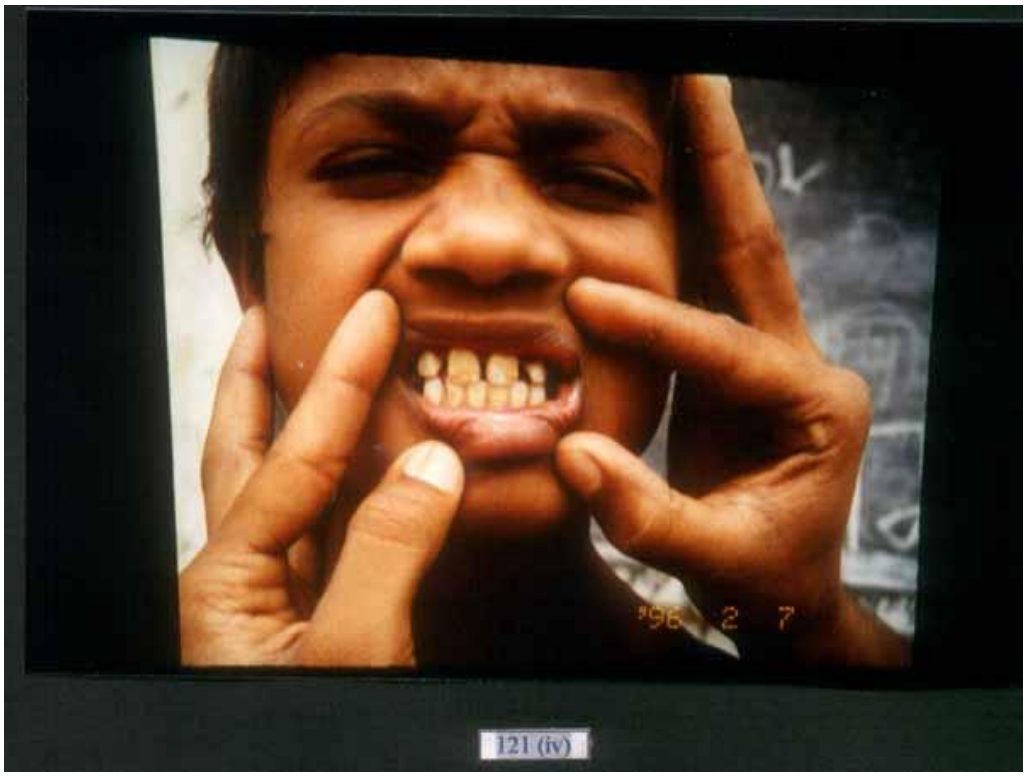


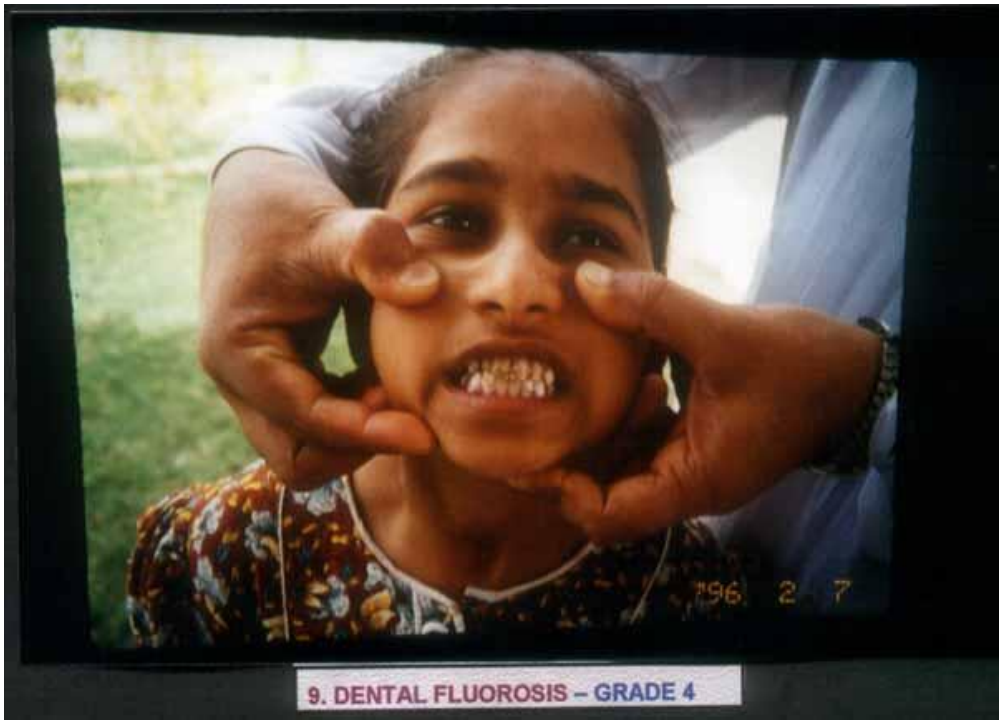
Dental Fluorosis grade II



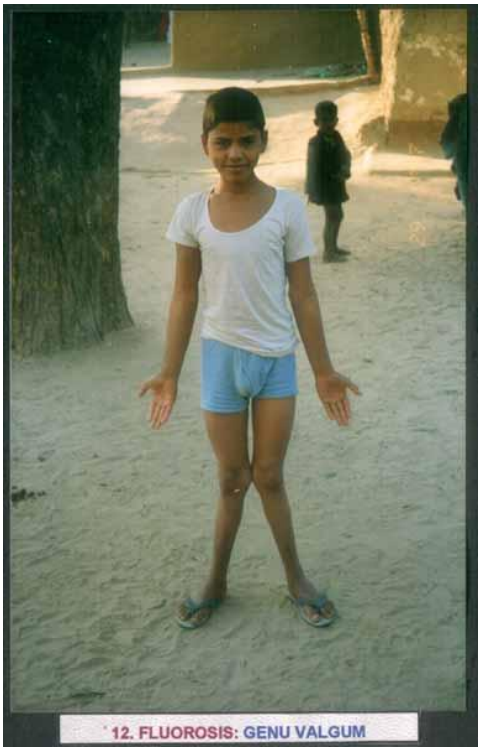


Dental Fluorosis grade III



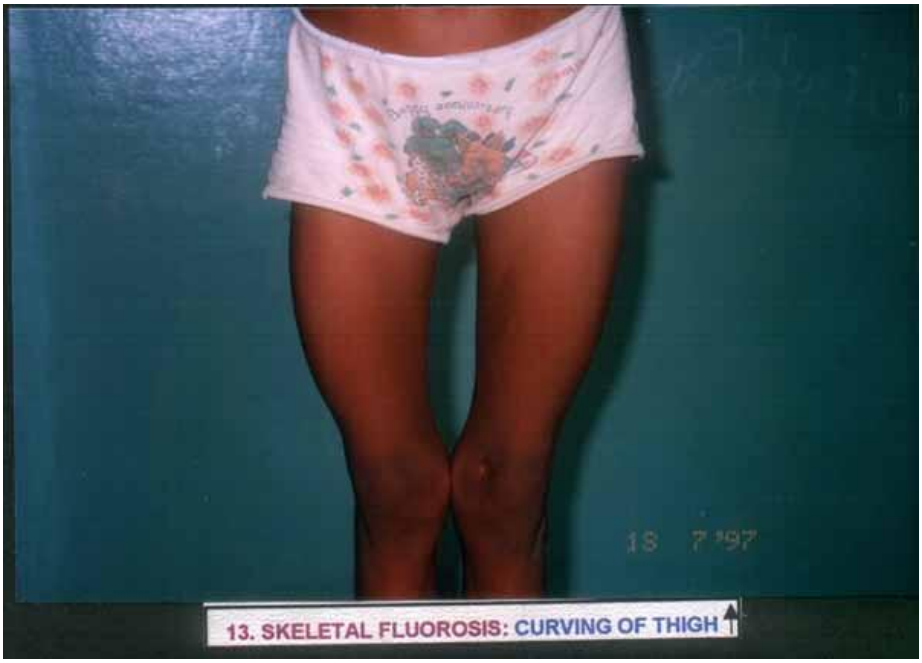


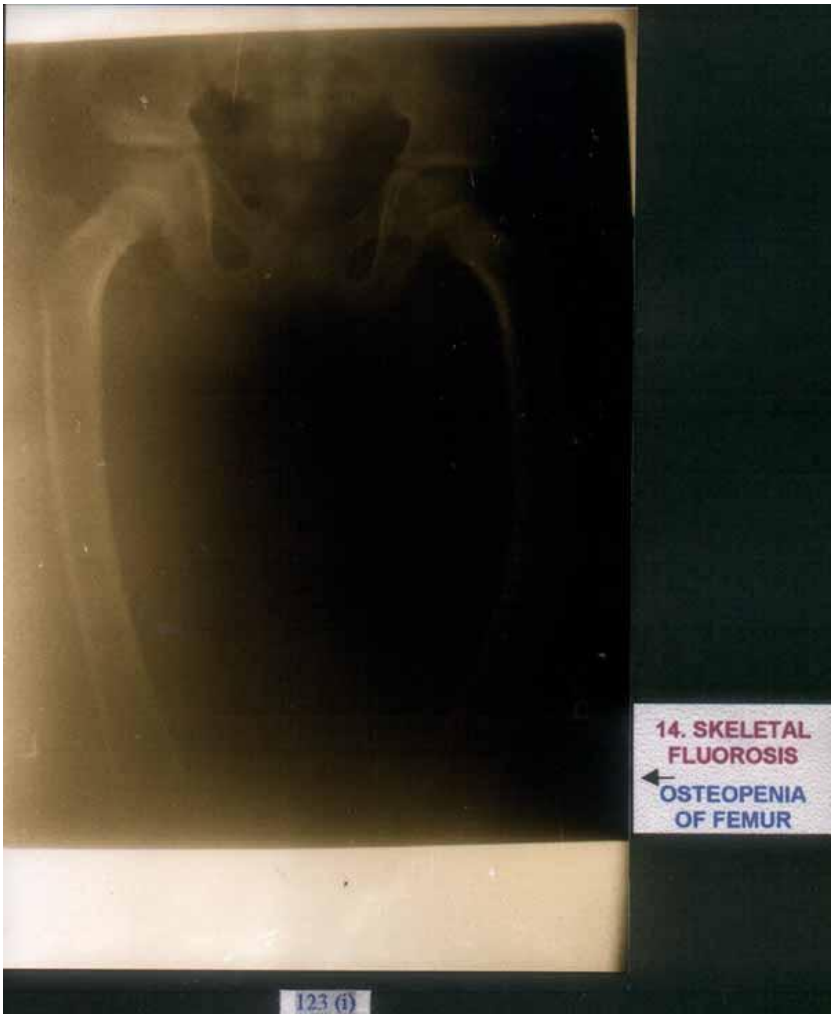
Dental Fluorosis grade IV

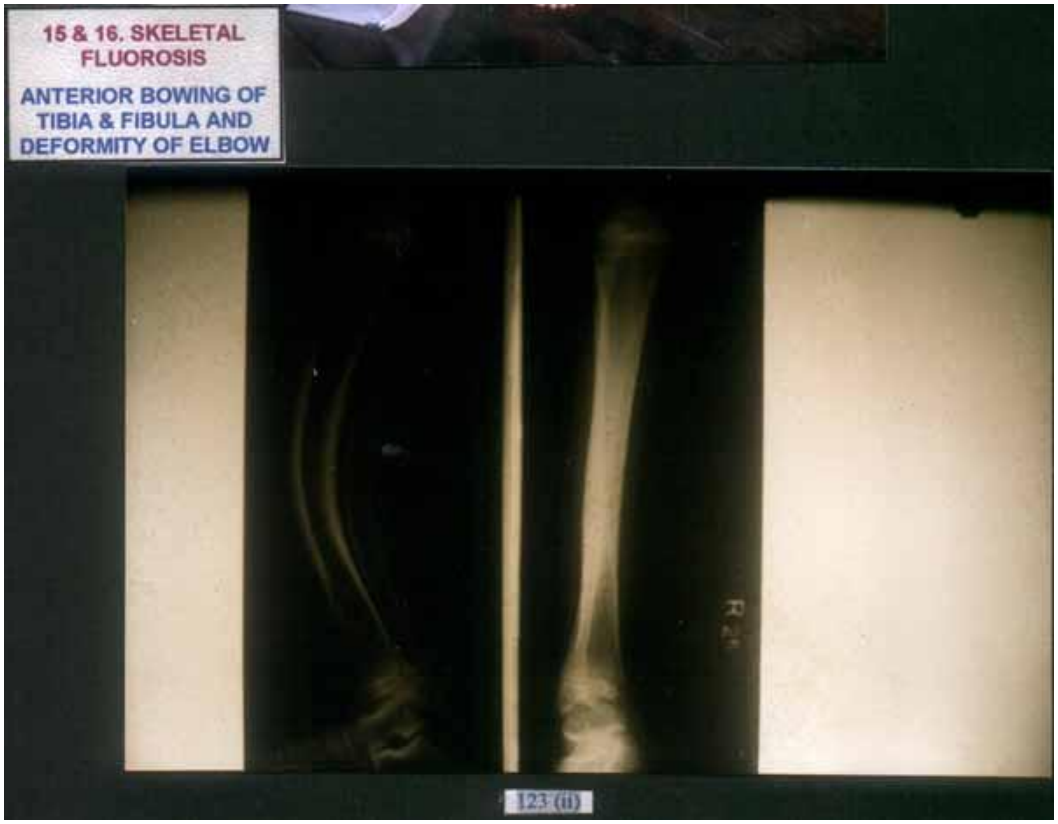


12. FLUOROSIS: GENU VALGUM

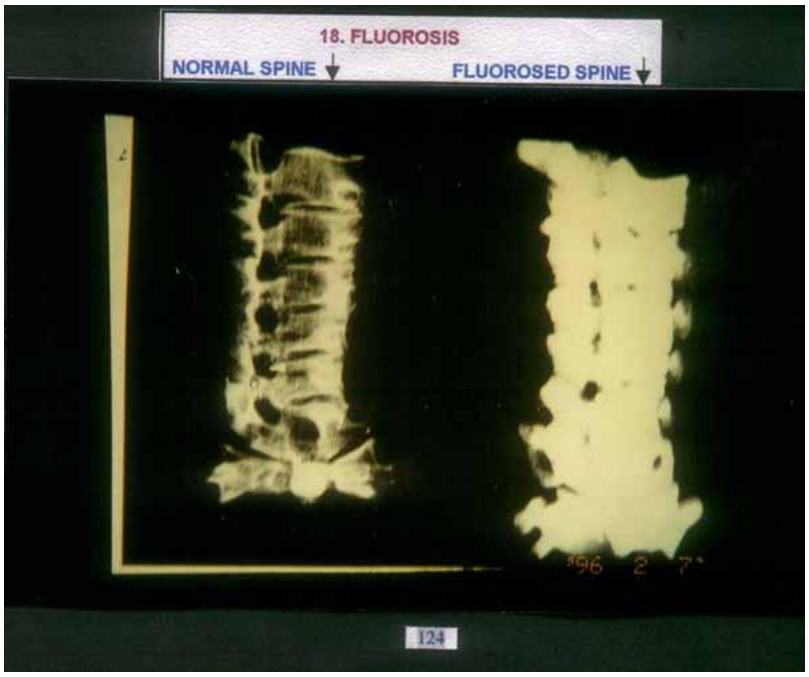
11. FLUOROSIS: MULTIPLE DEFORMITIES


18 7 '97
13. SKELETAL FLUOROSIS: CURVING OF THIGH


14. SKELETAL FLUOROSIS
OSTEOPENIA OF FEMUR
123 (i)


15 & 16. SKELETAL FLUOROSIS
ANTERIOR BOWING OF TIBIA & FIBULA AND DEFORMITY OF ELBOW
123 (ii)


17. SKELETAL
FLUOROSIS
PARAPLEGIA


18. FLUOROSIS
NORMAL SPINE
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Pre Treatment
Post Treatment
Pre Treatment
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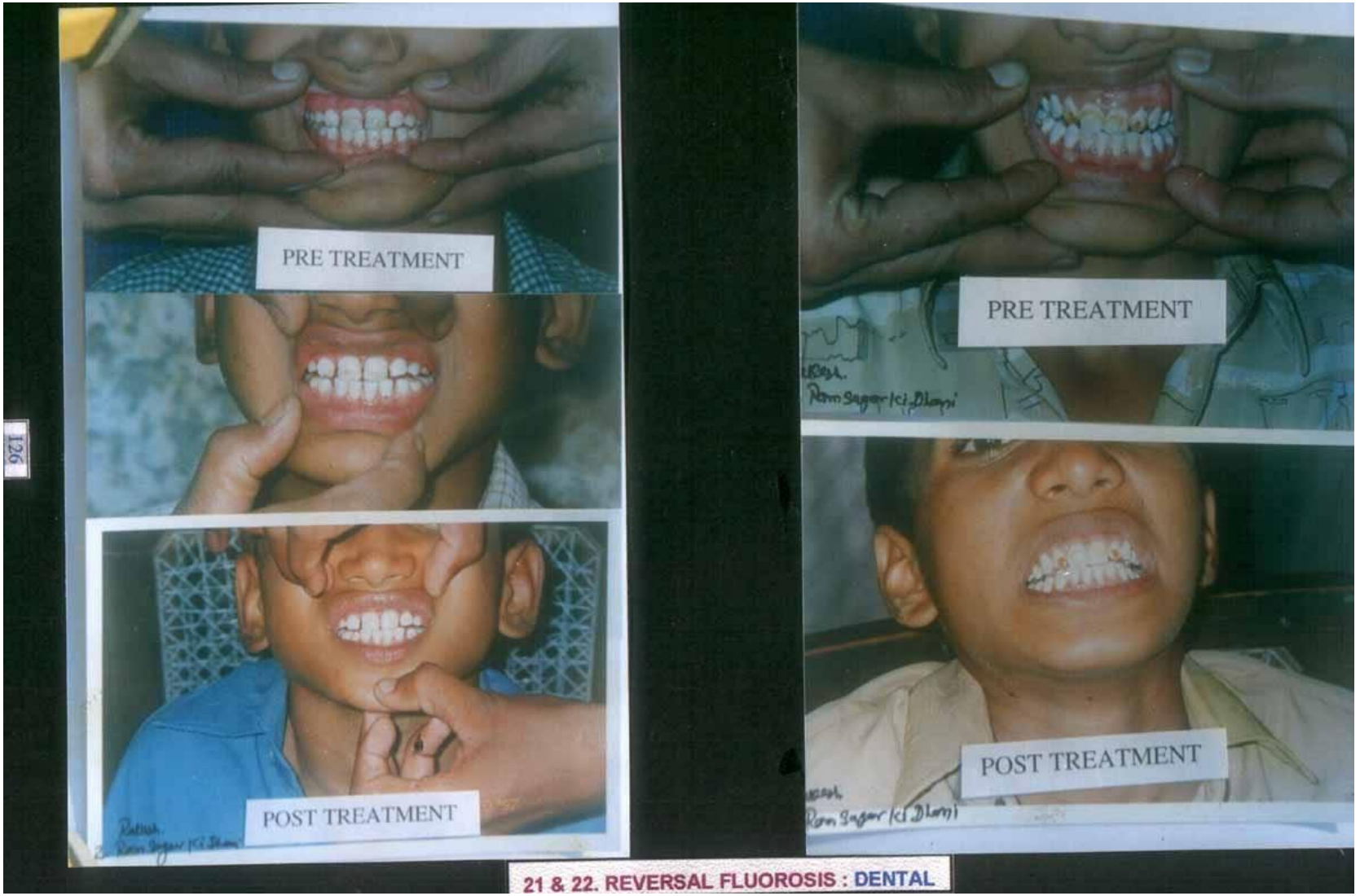
19 & 20. REVERSAL FLUOROSIS : DENTAL



21 & 22. REVERSAL FLUOROSIS : DENTAL

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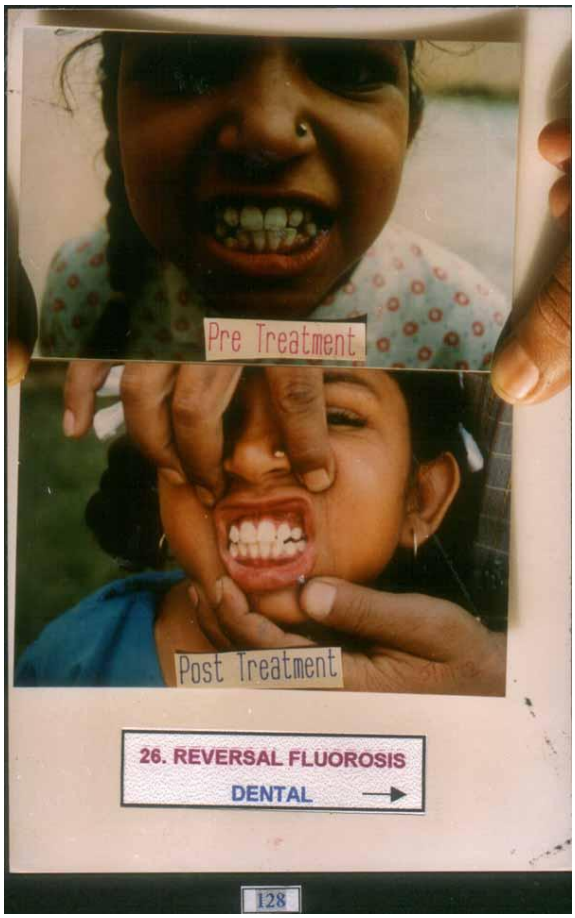
25. REVERSAL FLUOROSIS
CLINICAL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26. REVERSAL FLUOROSIS
DENTAL
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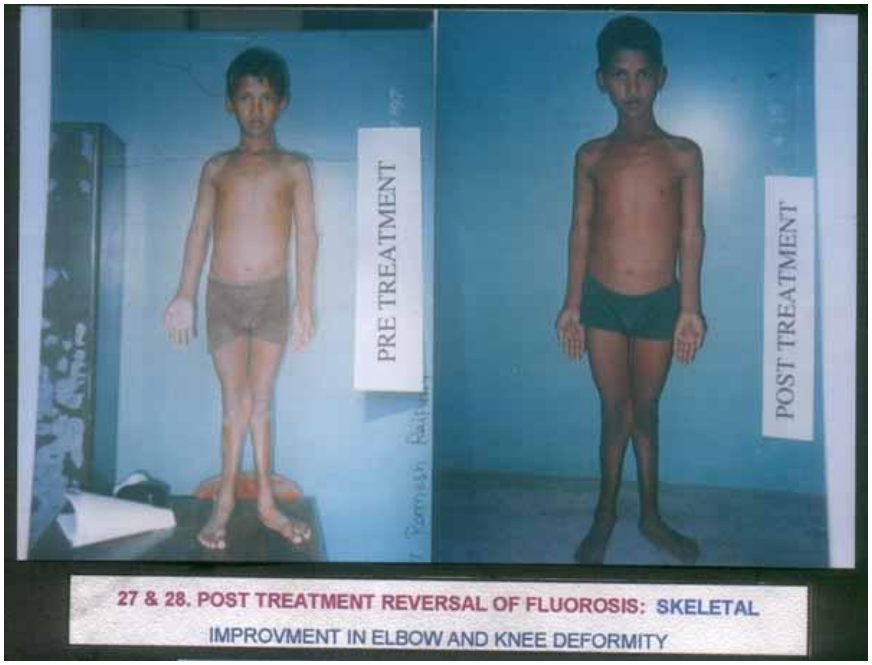
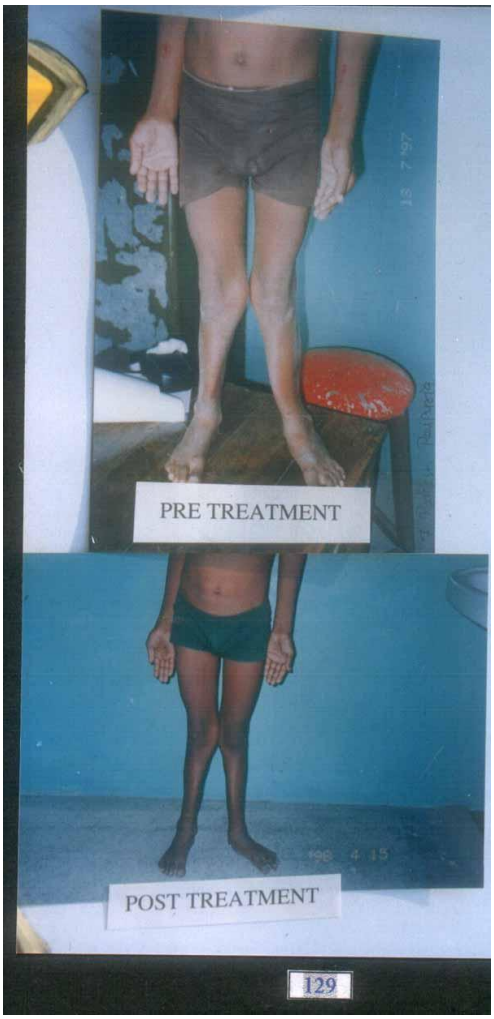
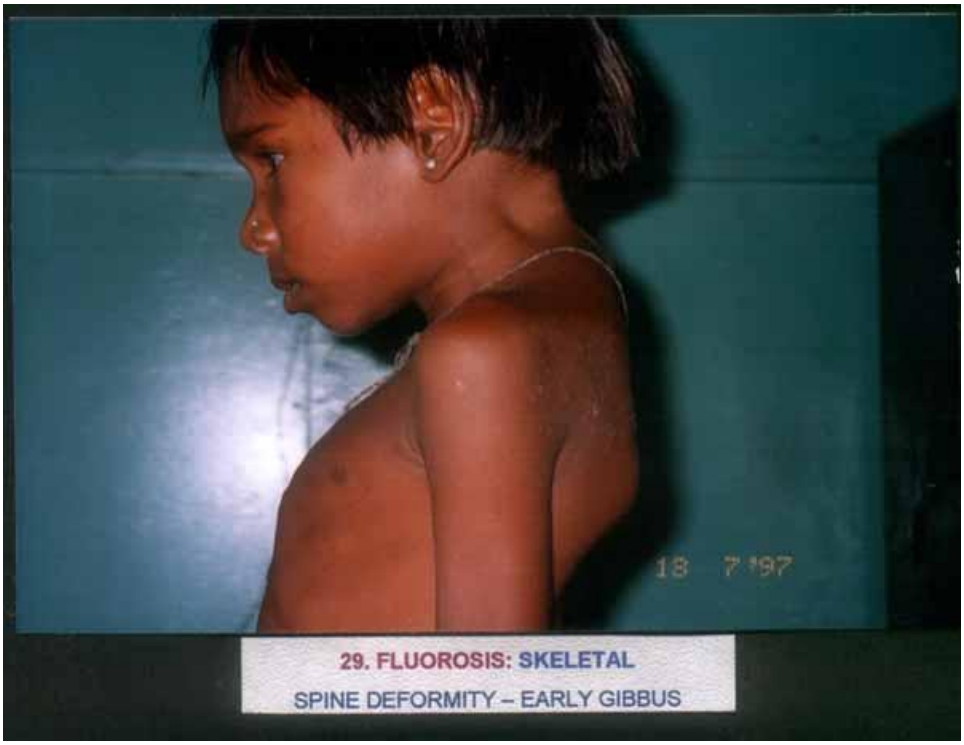


27 & 28. POST TREATMENT REVERSAL OF FLUOROSIS: SKELETAL IMPROVMENT IN ELBOW AND KNEE DEFORMITY

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29. FLUOROSIS: SKELETAL
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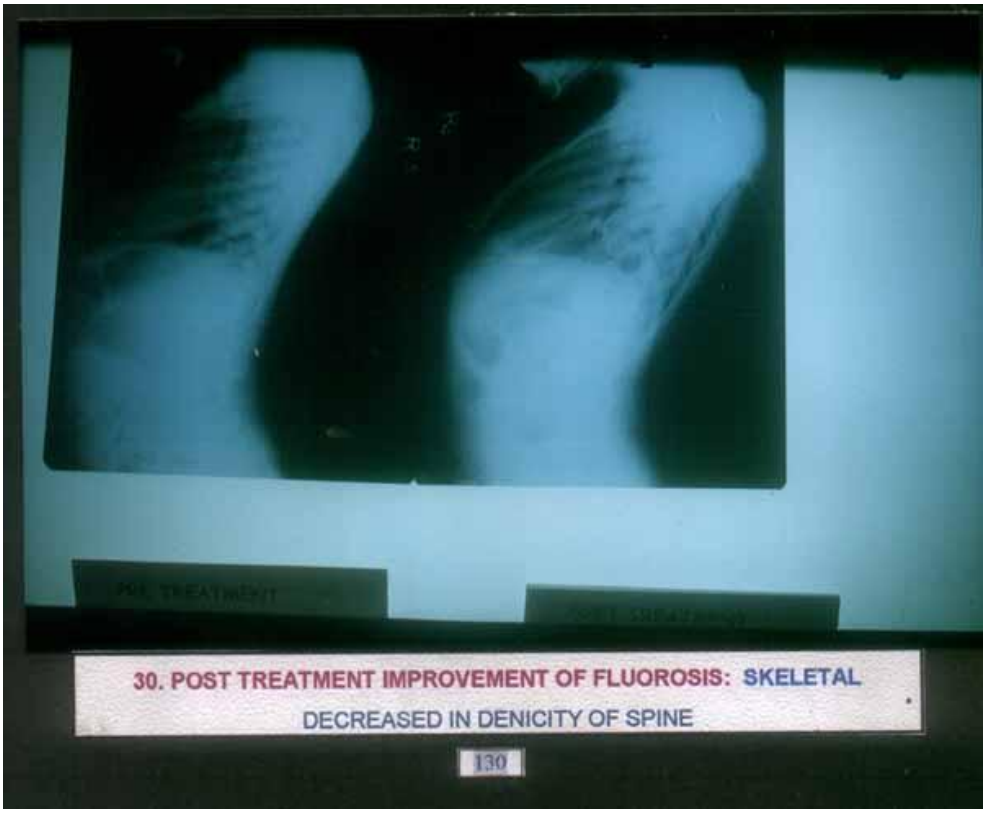
30. POST TREATMENT IMPROVEMENT OF FLUOROSIS: SKELETAL
DECREASED IN DENICITY OF SPINE
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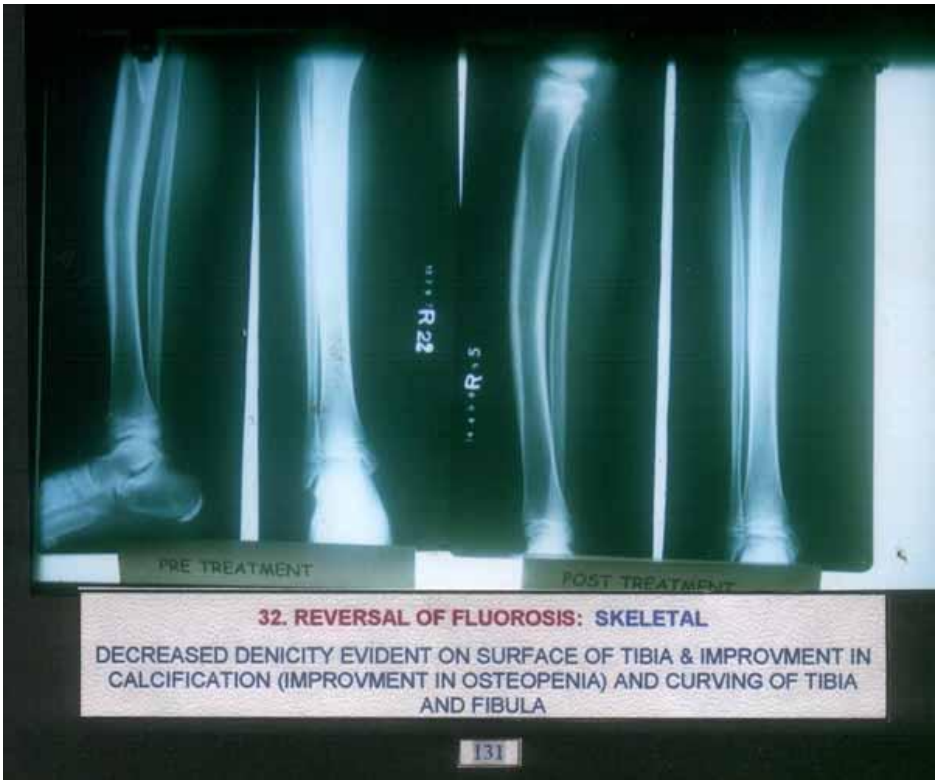
31. REVERSAL OF FLUOROSIS: SKELETAL

DECREASED DENICITY OF SPINE



32. REVERSAL OF FLUOROSIS: SKELETAL

DECREASED DENICITY EVIDENT ON SURFACE OF TIBIA & IMPROVMENT IN CALCIFICATION (IMPROVMENT IN OSTEOPENIA) AND CURVING OF TIBIA AND FIBULA

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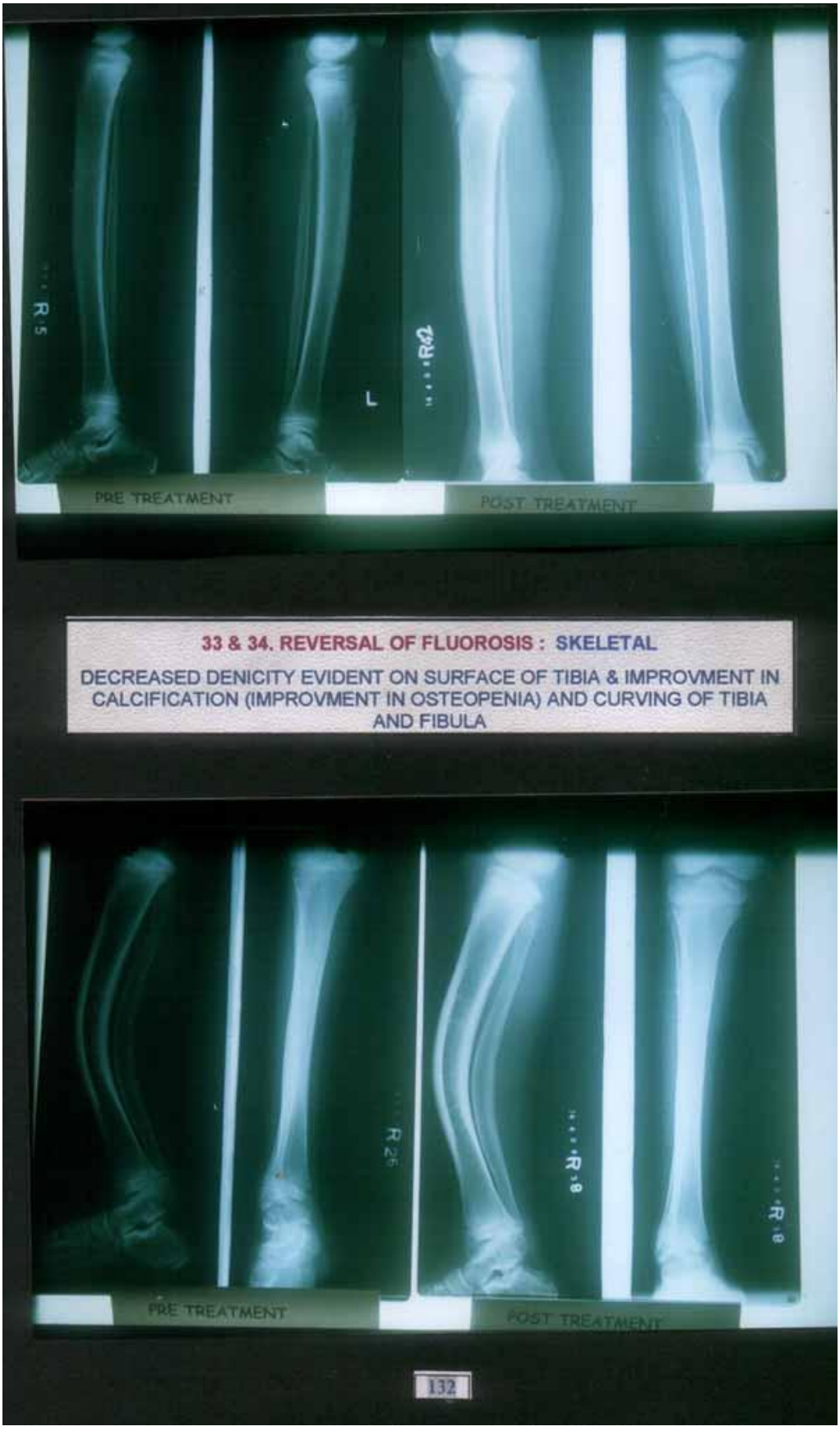
PRE TREATMENT
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DECREASED DENICITY EVIDENT ON SURFACE OF TIBIA & IMPROVMENT IN CALCIFICATION (IMPROVMENT IN OSTEOPENIA) AND CURVING OF TIBIA AND FIBULA
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DISCUSSIONS

Systemic fluorosis is an endemic problem in several developing countries especially in India and Pakistan and has been reported sporadically in other parts of the world. While the WHO (1984) standards permit only 1.5 mg/L as a safe limit for human consumption, people in fifteen states of India are consuming water with fluoride concentrations even up to 44 mg/L (PHED Survey, 1991-93). In many of these area people have no alternative but to drink such water. Worse still, with depletion of limited low fluoride containig ground water sources available in some pockets, more and more people are being forced to consume water rich in fluoride.

Toxic effects of excessive fluoride ingestion take three forms: clinical, skeletal and dental. General manifestations are: dental discoloration, dental as well as skeletal deformities, severe joint pains, general debility, as also psychosocial problems due to bad teeth, body deformities and immobility.

In the past vitamins C or vitamin D, and salts of calcium, magnesium or aluminum were prescribed individually in an attempt to inhibit or reverse these effects. These studies were restricted to adult samples. The results were, however, inconclusive as far as the skeletal fluorosis is concerned. The author has not come across any study indicating reversal of dental fluorosis.

Because of the conflicting observations by various workers (Pandit GC *et al.*, 1940; Pandit CG and Narayana Rao D, 1940; Wadhawani TK., 1954; Venkateswarlu P and Narayana Rao D, 1957; Ekstrand J and Ehrnebo M, 1979; WHO, 1991) there was a need

to further evaluate the possibility of using a combination and substitution of various salts of calcium, Vitamin C and/or Vitamin D for the treatment of fluorosis.

There is an urgent need to overcome the problem of fluorosis. In view of the gravity of the problem this study was designed with the objectives to find out a solution at treatment level and also at preventive level.

Earlier studies conducted for the treatment of fluorosis in children had shown encouraging results (Gupta SK *et al.*, 1994; Gupta SK *et al.*, 1996) . Therefore in this study the selected groups of children were subjected to treatment with Calcium, Ascorbic acid and Vitamin D in non-toxic high doses. The aims of the study were explained to all the patients and their parents. A written free and informed consent and an authority to publish the results of the study and related photographs were obtained from all of them.

AREA SELECTION, EVALUATION OF CHILDREN AND DISCUSSION OF OBSERVATIONS

AREA SELECTION

Four target areas were then selected. The villages representing target areas were Ram Sagar Ki Dhani (drinking water fluoride 2.4 mg/L), Rampura (drinking water fluoride 4.6 mg/L), Shivdaspura (drinking water fluoride 5.6 mg/L) and Raipuria (drinking water fluoride 13.6 mg/L).

CHILDREN SELECTION

50 Fluorosis affected children aged up to 12 years from a cross section of each of the target area (village) comprising different socio-economic groups were chosen. These children were graded for dental, clinical and skeletal fluorosis, and biochemical estimations were done to evaluate the severity of fluorosis. They were also evaluated for nutritional status especially for vitamin C, fluoride and calcium in their diet.

In this study the selected groups of 30 children in each area were subjected to treatment with Calcium, Ascorbic acid and Vitamin D in non-toxic high doses. These children were re-evaluated after completion of nine months of treatment for dental, clinical and skeletal fluorosis.

CHILDREN EVALUATION: (DENTAL, CLINICAL AND SKELETAL FLUOROSIS)

All types of presentations of clinical, dental and skeletal fluorosis were observed in these areas before the treatment. The presentation of dental fluorosis ranged from grade 0 to 4, clinical fluorosis from grade 1 to 2 and Skeletal from grade 1 to 3. The severity of presentation was highest in Raipuria, the area with highest fluoride concentration in drinking water (13.6 mg/L). The severe form of presentations observed in this area were enamel hypoplasia in dental fluorosis, rarefaction of the long and flat bone in skeletal fluorosis and severe form of clinical presentations in clinical fluorosis *(Different grades of dental, skeletal and clinical fluorosis are presented in photographs 1-18).*

. The observation indicated that water fluoride concentration is one of the important parameter for the toxicity and clinical presentations of the disease.

EVALUATION: DIET

The observations have indicated that fluoride toxicity is primarily related to the ingestion of fluoride through water or liquids (e.g. Tea). The study indicated that role of dietary fluoride is not very significant. In general the lack of dietary protein intake, low dietary calcium and ascorbic acid were other factors found to be associated with increased toxicity of fluoride intake.

Fluorosis and Diet

The role of dietary factors in causation of fluorosis depends upon many other factors also e.g. effect of cooking on available fluoride in food, availability of calcium and ascorbic acid in the food etc. So the observations have also indicated that dietary fluoride

is playing a secondary role in causation of fluorosis, whereas the primary role is related with the drinking water fluoride concentration and in liquid diets.

DISCUSSION OF THE OBSERVATIONS:

K *Serum calcium*:

Serum calcium levels in all the four target areas were in low to normal range (7.6 to 10.2 mg/dl). Considering the pathophysiology of fluorosis and the compensatory Parathyroid (PTH) mechanism, the observed findings are well with in the expectations. These observations are also in accordance with the observations of other workers (Jowsey I *et al.*, 1985).

As the calcium is one of the key ions affected by fluoride intake, the role of calcium supplementation is one of the pioneer steps in the treatment of fluorosis. Oral calcium supplementation not only helps in chelating the fluoride ion in the gut forming insoluble compound, but also helps to maintain the calcium homeostasis in the body, and as a net result improves the created conditions of calcium stress due to fluoride ingestion. Therefore the *dose of calcium supplementation should be based on water fluoride levels as well as clinical and pathological changes observed in the patient.*

K *Serum alkaline phosphatase*

The serum alkaline phosphatase (SAP) activity was observed to be high (20-52 kA Units). The analysis of observed values indicates that there is no correlation between SAP activity and fluoride levels in drinking water. Other workers (Rajyalakshmi K *et al.*, 1985; Ming HO YU and Huey Lin Hwang, 1988; Farly JR, 1983) had also observed a similar trend. The SAP activity indicates the osteoblastic activity. It has been observed that osteocalcin is secreted by mature osteoblasts and to certain extent by immature osteoblasts also (Akesson K *et al.*, 1998; Rodan GA Noda M, 1991). It is possible that the serum level of osteocalcin may reflect the number and/or activity of mature and immature osteoblasts. Despite this decrease in bone formation, there is an increase in number of immature osteoblasts (Thompson ER *et al.*, 1975). This increase is associated with an increase in serum osteocalcin (Tanimoto H *et al.*, 1991). Therefore it is obvious that during hypocalcemic phase as well as during recovery phase the osteoblastic activity remains high, which is the responsible factor for increased SAP activity.

K *Serum inorganic phosphorus*

The normal range of Serum inorganic phosphorus (SIP) is 3.7 – 5.6 mg/dl in the age group of 4-12 years. The SIP levels were observed to be well within normal range before starting the treatment. The SIP levels remains well with in normal range, till calcium homeostasis remains well compensated. As decompensation proceeds the SIP levels will also be disturbed due to PTH receptor resistance observed in conditions of chronic hypocalcemia.

K *Ascorbic acid*

The serum ascorbic acid was observed to be in the normal range (0.3-1.8 mg/dl) in all the target areas except group D. The leukocyte ascorbic acid levels (3 - 43 $\mu g/10^8$ WBCs) were well below the normal range in all areas

The leukocyte ascorbic acid levels were well below the normal range in all areas. The lowest values have been observed in the group D, the area of highest fluoride concentration, which correlates well with the observed low values of SAA in the same group.

The low levels of LAA indicate the longstanding depletion in ascorbic acid, in comparison to SAA. Lower values of serum in area D and leucocyte ascorbic acid in all target areas are consistent with observations of Rao *et al.* (1979), Jenkins *et al.* (1970), Pandit *et al.* (1940), who reported the coexistence of severe forms of human fluorosis and vitamin C deficiency. Wadhwani (1954) and Pandit *et al.* (1940) stated that the aggravated condition of fluorosis with vitamin C deficiency as encountered in endemic fluorosis might very well be a complex superimposition of the signs and symptoms of scurvy on those of fluorosis. The coexisting condition of scurvy responds to vitamin C therapy, but it may not affect the basic condition of fluorosis. Animal study reported in ICMR Bulletin (1979) report indicated considerable reduction in cellular ascorbic acid content indicating that fluoride ions interfered with vitamin synthesis pathway of the gland or alternatively with utilization of the vitamin.

K *Sialic acid (N – acetyl neuraminic acid)*

N – Acetyl Neuraminic Acid (Sialic acid), a component of glycoprotein, is an important parameter in detection of fluoride toxicity. The normal value ranges from 59-64 mg/dl in human being. The sialic acid is minimally altered in bone as a result of fluoride ingestion, but its levels are decreased in serum as a result of F-toxicity both in animal and man. The ratio of sialic acid to GAG (SA/GAG) has been found to be a sensitive index to detect fluoride toxicity at very early stages both in human and animal models. The ratio SA/GAG revealed a 30-50% reduction in human sera in fluoride poisoning (Jha M *et al.*, 1983). Lower values of sialic acid (23.5 - 57.2) were observed in this study in all areas.

K *Glucosaminoglycans (GAG)*

The glucosamineglycans (GAG) are components of glycoproteins. The normal values of GAG are 9-11 mg/dl. Elevated values of GAG (15-43 mg/dl) have been observed in all target areas. The maximum elevation have been observed in areas with maximum fluoride concentration i.e. group D. Elevated content of GAG in bone and its reflection in serum can be considered as an index to assess fluoride toxicity and fluorosis at very early stages (Jha M *et al.*, 1983). Until recent time, the only reliable criterion for assessing fluoride toxicity was radiographs. However, radiographs are only helpful to diagnose the disease at late stages when the ligaments are calcified and bones become denser (Jha M *et al.*, 1983).

K *Fluoride*

Blood and serum

High blood (0.5 -1.9 mg/L) and serum fluoride (0.5-1.5 mg/L) levels were observed in all areas. It is obvious to have high fluoride concentration in blood and serum with the ingestion of high fluoride containing water and food.

Urine

Urinary fluoride level has been used to estimate the absorbed amount of fluoride (Ming HO YU and Huey Lin Hwang, 1985; Dinman BD *et al.*, 1976a) and is recognized as one of the best indices of fluoride intake. Increased urinary fluoride levels (4-30 mg/L) in children of all groups were observed.

Fluoride (F) in urine has long been known to reflect intestinal F absorption as well as F liberation from a fluoride-rich skeleton, but blood plasma F has been believed only to show a limited absorption process of gradual F depletion of an F-rich skeleton. Some of the variations of plasma inorganic F may have been obscured by analytical methods due to organically bound plasma fluoride fraction. While the ionizable F (F-) of a young person's fasting plasma is generally as low as 0.01 – 0.02 mg/l, or 0.5 – 1 μM/l, the organically bound F may be 5 to 10 times higher. The nature of organic F bonding in plasma is not yet understood, but it is evident that the ionized or easily ionizable plasma F is the physiologically active fraction (Ericsson Y, 1975).

K **Parathyroid hormone levels (PTH):**

Parathyroid hormone levels (PTH) were high in all groups. The PTH levels were higher (30 – 260 pmol/L) in areas consuming more fluoride in comparison to areas consuming less fluoride in drinking water.

This finding indicates that the calcium, is one of the important regulating ions for PTH secretion, and to maintain the calcium homeostasis, the PTH secretion showed a rising trend with increasing fluoride ingestion in drinking water (Bronner F, 1961; Schwartz P *et al.*, 1994; Adami S *et al.*, 1982; Colin PR *et al.*, 1989; Cunningham J *et al.*, 1989; Felsenfeld AJ, 1992)

The reasons for increased PTH levels due to high fluoride ingestion are multifactorial :

1. Calcium stress

   This calcium stress is due to

   K Ingestion of high fluoride containing water.

   K Simultaneous requirement of calcium for laying down of developing bones in children.

2. PTH receptor resistance (Rizzoli R and Bonjour JP, 1992; Goldsmith RS *et al.*, 1974)

   It has been observed that in chronic hypocalcemic states PTH receptors are showing the resistance.

3. Measuring the M-PTH - Mid molecule of PTH (Goltzman D and Hendy GN)

   The PTH measured in this study was mid molecule. The metabolism of the PTH indicates that the active portion of the PTH have short half life in

comparison with mid molecule which have a long half life. Thus the measurement of mid molecule gives a result of cumulative release of PTH to a certain extent.

4. Compromised Renal function (Goldsmith RS *et al.*, 1974)

   Fluorosis, chronic hypocalcemia, hyperparathyroidism are few conditions associated with compromised renal function, leading to slow clearance of M-PTH.

PROPOSED PATHOPHYSIOLOGY OF FLUOROSIS

CALCIUM METABOLISM:

This regulation of calcium is basically dependent on the calcium sensing receptors (Heath DA, 1998).

Calcium sensing receptor (CaR): (Heath DA, 1998)

These are situated within its extracellular membrane. The CaR consists of an aminoterminal, extracellular domain, a seven membrane-spanning domain and cytoplasmic carboxy terminal domain. As such, it is a member of the large family of G-protein-coupled receptors. The CaR is expressed in a wide variety of tissues with the highest concentrations being in the parathyroid cells, the renal tubular cell (the nephron), C-cells of the thyroid, and certain areas of the brain.

Circulatory calcium is controlled in large part by (Akesson K *et al.*,1998)

a. The balance of calcium absorbed in the intestine and
b. that excreted by the kidney into the urine.

The skeleton is the major reservoir for calcium. Thus, in addition to the intestine and kidney, the skeleton also plays an important role in calcium homeostasis. Hence, any significant changes in the circulatory calcium level would have an impact on bone metabolism. Accordingly, prolonged calcium stress due to insufficient dietary calcium intake, disturbance in in-vivo availability of calcium and/or absorption would have significant deleterious effects on bone mass.

Hypocalcemia will lead to increase in serum PTH (Stauffer M *et al.*, 1973).

EFFECT OF FLUORIDE INGESTION ON CIRCULATORY CALCIUM

Ingestion of fluoride causes decrease in the ionised calcium. This hypocalcemia leads to changes in internal milieu of the body to maintain the calcium levels and leads to secondary hyperparathyroidism. It is well known that ionic calcium is one of the important ions for the initiation and maintenance of the activity of the vital organs and musculoskeletal system. Lowering of the ionised calcium is one of the important stimulants for the release of PTH. Even lowering of blood ionized calcium by an amount as low as 0.02mmol/l within 30 min elicited an immediate large, transient peak release of PTH amounting to 6-16 times the baseline concentration (Schwartz P *et al.*, 1998).

This secondary Hyperparathyroidism gives rise to three main events – bone resorption and abnormal bone deposition on bone surfaces, decreased collagen synthesis and defective ground substance production. These three pathophysiological changes following secondary hyperparathyroidism and hypocalcemia explain most of the clinical presentations of fluorosis.

A brief of the proposed pathophysiology of fluorosis has been described in **figure 1** for a better understanding of the observations made during the study.

EFFECT OF INCREASED PTH ON BONE

Increased PTH will be responsible for:

1. Significant loss of bone mass – It is due to increase in the number and activity of osteoclasts (Stauffer M *et al.*, 1972, wright *et al.* 1994) which then resulted in a stimulation of bone resorption and depletion of bone formation.

PROPOSED PATHOPHYSIOLOGY OF FLUOROSIS

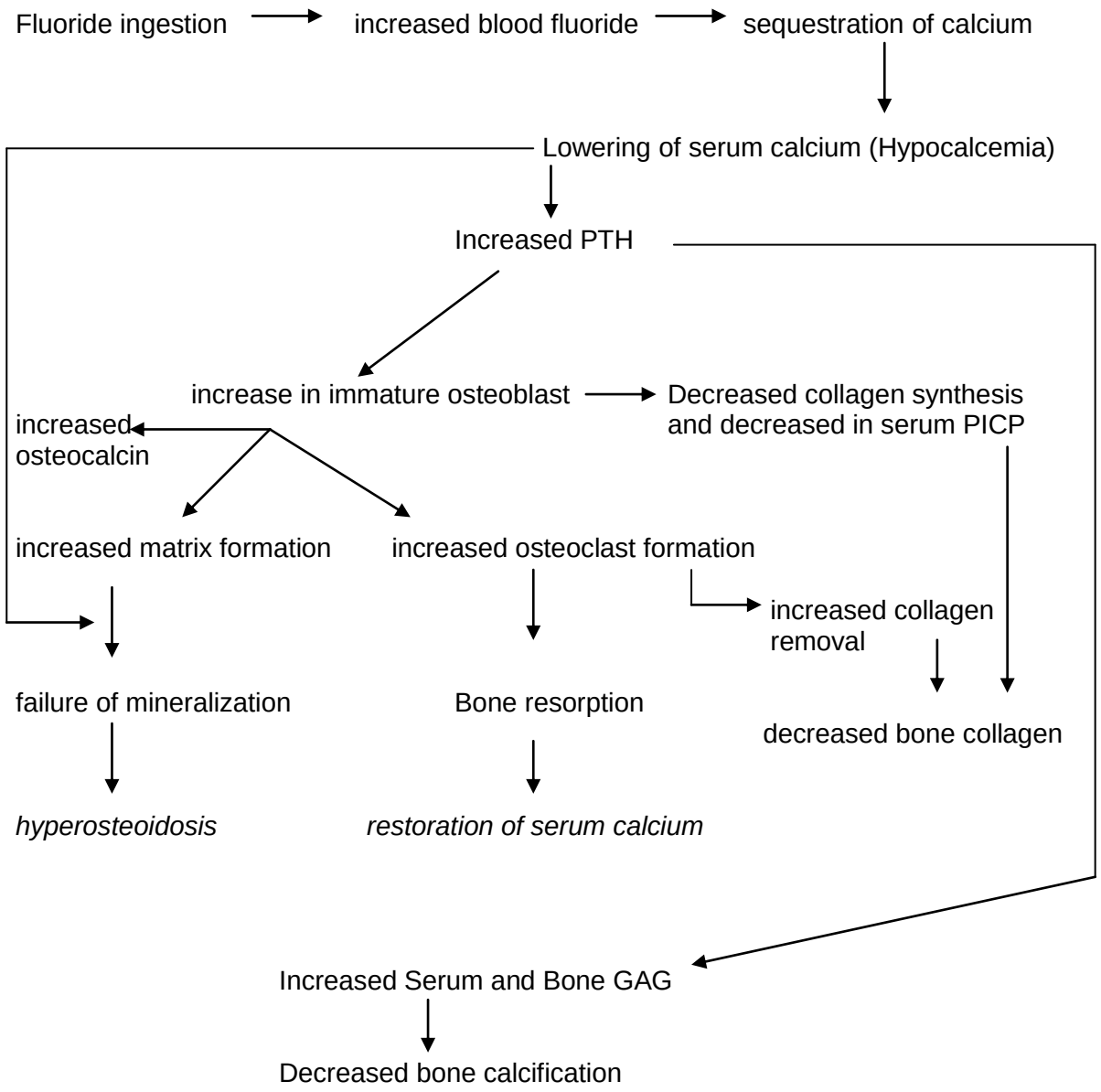

FIGURE 1

Under normal conditions, an increase in bone resorption is coupled with a compensatory increase in bone formation of an equal magnitude to ensure that no net bone mass is lost. However, during depletion when there is a demand to mobilize calcium from bone to counteract the hypocalcemia, the normal bone coupling process becomes compromised.

2. Significant loss of bone mass – It is due to increase in the number and activity of osteoclasts (Stauffer M *et al.*, 1972, wright *et al.* 1994) which then resulted in a stimulation of bone resorption and depletion of bone formation.

   Under normal conditions, an increase in bone resorption is coupled with a compensatory increase in bone formation of an equal magnitude to ensure that no net bone mass is lost. However, during depletion when there is a demand to mobilize calcium from bone to counteract the hypocalcemia, the normal bone coupling process becomes compromised.

3. Depletion of bone formation

   It is reported that despite a significant increase in bone resorption, bone formation not only did not increase, but was significantly inhibited (Stauffer M *et al.*, 1973). Consequently, the combined actions of calcium depletion bone resorption and formation led to a significant loss of bone mass (Stauffer M *et al.*, 1973).

   The changes in bone formation and bone resorption are influenced by increased PTH levels due to decreased serum calcium level and cause two metabolic changes in serum bone formation markers (Akesson K *et al.*,1998)

a. increased the serum level of osteocalcin by a as much as 10%,

As osteocalcin is secreted by mature osteoblasts (Rodan GA *et al.*, 1991), it is possible that the serum level of osteocalcin may reflect the number and/or activity of mature osteoblasts. It is reported that despite this decrease in bone formation, there was an increase in osteoblast number (Thompson ER *et al.*, 1975). This increase in osteoblast number was associated with an increase in serum osteocalcin (Tanimoto H *et al.*, 1991).

b. significantly decreased the serum PICP level by as much as 15%.

PICP is a product of bone collagen synthesis (Risteli L *et al.*, 1993). It is presumed that the PICP concentration may reflect the total amount of bone matrix synthesized. This finding indicates a marked reduction in collagen synthesis.

Thus there was dissociation between collagen synthesis and osteoblast number as well as osteocalcin synthesis during calcium depletion.

EFFECT OF INCREASED PTH ON COLLAGEN FORMATION AND GROUND SUBSTANCE

Calcium and matrix (collagen + glucosaminoglycans as proteoglycans + glycoproteins) are essential structural components of bone and teeth. About 80-85% of bone matrix is constituted by Collagen and glucosaminoglycans (proteoglycans) & glycoproteins constitute about 15-20 %. All three components of bone and teeth that is collagen, proteoglycans and calcium are adversely effected by ingestion of high quantity of fluoride for prolonged duration (ICMR, 1979; Jha M *et al.*, 1983). Degradation of collagen and ground substance in the body leads to symptoms of fluorosis like, delayed eruption of teeth, dental fluorosis, clinical fluorosis, premature aging etc.

Based on the survey of the literature and Author's earlier observations it was postulated that the ingestion of fluoride leads to a decrease in serum calcium levels (hypocalcemia) and increases in the serum PTH (Dinman BD *et al.*, 1976a). The system attempts to correct the hypocalcemia by leaching out calcium from the body reserve mainly in the bones. This calcium is bound to the ground substance (collagen matrix). An increase in the PTH action degrades the ground substance by increasing the sulphation of chondroitin sulphate, not only in the bones, but all over the body (Bronner F, 1961; Cramer CF *et al.*, 1961; Bordier PHJ and Chot ST, 1972) . This in turn greatly enhances the leaching of calcium from bones (bone resorption) and also increases the levels of hyaluronic acid in bone and other tissues body (Bronner F, 1961; Cramer CF *et al.*, 1961). Increased degradation and excretion of hydroxyproline, glucosaminoglycans, phosphates and pyrophosphates have also been observed with increased PTH (Bronner F, 1961; Cramer CF *et al.*, 1961; Bordier PHJ and Chot ST, 1972). This impairs the laying down of collagen fiber. It has been observed that in fluoride toxicity there is decrease in hydroxyproline (effects the solubility of collagen), decrease in lysine (decreases the collagen cross linkage and increases the solubility of collagen protein) and an increase in proline residue (due to impairment in hydroxylation process).

In teeth the major changes associated with fluorosis are appearance of dermatan sulphate with decrease in molecular size of the hydroxyappetite crystal, which may be due to some catabolic events leading to degradation of large parent molecule and inhibition of the biosynthetic assembly of such molecule (Bronner F, 1961).

To summaries the fluoride ingestion will cause hypocalcemia, secondary hyperparathyroidism causing increased bone resorption, defective bone formation and

defective collagen (ground substance) formation explaining all clinical, dental and skeletal presentations of fluorosis.

It is therefore hypothesized that all these effects are mediated through hyperpapathyroidism secondary to increased fluoride ingestion.

TREATMENT OF THE DISEASE

The treatment is based on the studies conducted by the Gupta *et al.* (1994 & 1996) with supplementation of Calcium, Vitamin C and Vitamin D in higher doses but well below the toxic doses.

A part of the study group was supplemented with the treatment in this study as well. 30 children (15 cases with 15 control) from each group were provided with the treatment of 9 months. Improvement in clinical and dental fluorosis was observed in all areas, but the degree of improvement in skeletal fluorosis was varied in different areas (*Post treatment improvements of different grades of dental, clinical and skeletal fluorosis are presented in photographs 19 – 34).*

It was observed that the duration of treatment (9 months therapy) was not sufficient in areas with very high fluoride concentration in drinking water. So the duration of therapy needs revision in these areas.

The observations also indicated that the dose of the drugs (Calcium, Vitamin C & D) needed modification depending on water fluoride concentration and severity of symptomatology.

The improvement in skeletal presentation of fluorosis was two folds:

a) Improved in calcification, of the rarefied areas of the long and flat bone.

b) Removal of the dense fluoride deposited areas.

RATIONALE OF THE TREATMENT

Based on the pathophysiology, appropriately the therapy should be aimed at improving the serum calcium levels and reducing the PTH action. Thus calcium supplementation is indicated for reducing the bone resorption and improving the bone formation. It should also be ensured that the body effectively absorbs the oral calcium

intake. Further, conditions favorable to bone formation from the available calcium in the system must be present.

Role of calcium supplementation:

Apart from improving serum calcium levels the presence of extra calcium in gut directly inhibits the absorption of fluoride ions, by forming insoluble complex of calcium fluoride. It also inhibits the excessive release of parathyroid hormone thereby preventing excessive activation of osteoblasts thus preventing hyperosteoidosis and osteopenia (Tortora JG & Anagnostakas NP, 1990). Availability of adequate calcium would also help in laying down of bones and teeth mineralisation.

Role of Vitamin D supplementation

Vitamin D3 in low doses enhances calcium absorption and retention without causing hypercalcemia. It also inhibits the excessive release of parathyroid hormone by improving hypocalcemia thereby preventing excessive activation of osteoblasts thus preventing hyperosteoidosis and osteopenia.

Role of vitamin C supplementation

Vitamin C provides the Conditions favorable for laying down of bones and collagen. The reduced activity of the PTH due to calcium supplementation will also reduce the accumulation of hyaluronic acid and citric acid, responsible for degradation of bone and decreased formation of abnormal bone matrix by reducing the sulphation of sulfated mucopolysaccharides. This will also provide the ground substance either of neutral or slightly alkaline media, required for laying down of bone.

Another important condition required for laying down of bone and other tissues is collagen synthesis. The ascorbic acid (Robertson WVB, 1961) improves the hydroxylation of proline, one of the important prerequisites for the collagen formation. Presence of

ascorbic acid in adequate amount will induce massive and rapid collagen synthesis by making hyaluronic acid molecule depolymerised and more diffusable.

Sulphation of mucopolysaccharides (MPS) and accumulation of hyaluronic acid are associated with deficiency of ascorbic acid and is responsible for creating acidic environment in the ground substance. These biochemical changes can partly be prevented by supplementation with ascorbic acid. It also improves the laying down of collagen by promoting the hydroxylation of proline an amino acid. Collagen maintains the teeth structure and is also essential for bone formation.

Hence a regime of calcium, vitamin D3 and ascorbic acid supplementation well below the toxic dosage without changing the quality or quantity of food or water consumption was adopted.

The observations in Raipuria village (Group 4) indicated that the duration of treatment considered in this study (9 months therapy) was not sufficient in this area, having very high fluoride concentration in drinking water. So the duration of therapy needs revision in these areas.

To summarize, before going for treatment special emphasis should be given in deciding the dose of drug, which depends upon the drinking water fluoride concentration and also on other factors viz. fluoride concentration in drinking water, severity of clinical presentation and biochemical parameters.

PREVENTION BY DIETARY SUPPLEMENTATION

It is well recognized in India that consuming fluoride contaminated water and / or food for a period of 6 months to 1 year is adequate to have the ill effects on health. Water borne fluoride is known to cause “hydrofluorosis”; whereas food borne fluoride is known to

cause “Food borne fluorosis”. The fact remains that it is not an easy task to differentiate these two forms; however, it is known that fluoride entry through food and water causes fluorosis. (RGDWM, 1993, Rugg-Gunn-AJ *et al.*, 1997).

Many infant foods were found rich in fluoride. In one study fluoride concentration of 238 commercially available infant foods, fluoride concentrations ranged from 0.01 to 8.38 micrograms of fluoride per gram, with the highest fluoride concentrations found in infant foods containing chicken. (Heilman JR *et al.*, 1997). Apart from the food items other sources e.g. use of soy-based formulas, toothpaste ingestion, and dietary fluoride supplements etc. reported to have different concentrations of fluoride ranging from 0.0 to 4.4 were found. which was high enough to cause dental fluorosis.(Levy SM & Zarei MZ, 1991;Rock WP & Sabieha AM, 1997). Cao J *et al.* (1997) observed that milk tea made from brick tea water, contains high concentrations of fluoride (2.58-3.69 mg/litre).

Therefore for prevention and treatment of fluorosis apart from defluoridation of fluoride rich water, diet can have two folds of action:

1. Reduce the intake of fluoride by avoiding the intake of fluoride rich food.
2. As it is not possible to avoid the intake of fluoride more then the recommendations, the ingestion of Protein, Vitamin C (ascorbic acid) and Calcium diet will play a vital role in prevention of fluorosis.

Diet of the selected children (children selected were of comparable group in weight and age, to the extent possible, in all four areas) were recorded for three consecutive days. Detailed analysis of the diet was conducted for daily protein, calcium and vitamin C intake was calculated using reference “Nutritive value of Indian foods” published by National institute of Nutrition (Gopalan *et al.*, 1993). Details of daily fluoride intake through water and food were also calculated using details given in Annexure 2.

It was observed that

- Daily protein intake in these children was less then the required amount. The average protein intake was 0.5 – 0.76 gm per Kg day.
- Daily calcium (average 230 – 430 mg/ day) and vitamin C (less then 100 mg/day) intake was less then the desired amount, in all four areas.
- There was no significant difference in total daily intake of protein, Calcium and vitamin C intake among all four areas.
- Fluoride intake through food was more or less same in all four villages in comparison to fluoride intake through water. The fluoride through food was about 30% of the total daily fluoride intake in low fluoride areas (Ram
- Sagar Ki Dhani – water fluoride content 2.4 ppm), whereas in high fluoride areas (Raipuria- 13,6 ppm) the food is contributing only 7% of daily Fluoride intake.
- Clinical presentations vary with the total fluoride intake.
- The severity of dental fluorosis is almost same in three areas (Ram sagar ki dhani – 2.71, Rampura – 1.73 and Shivdaspura – 2.44) except in Ramsagar ki dhani, with drinking water fluoride concentration 2.4 to 5.6 ppm. Daily total intake ranged from 7 – 14 ppm in these three areas. More severity of dental fluorosis at Ramsagar ki dhani among all three areas, (even with lowest fluoride concentration in drinking water and lowest total daily intake among all four selected areas) can well be explained by poor dental hygiene indicated by high prevalence of dental caries in this area.
- There is abrupt increase in severity of dental fluorosis in area (Raipuria) with drinking water fluoride concentration 13.6 ppm, and total average daily fluoride intake is about 30.00 mg. These observations have indicated that the presentation of dental fluorosis varies with the total fluoride intake.

- The severity of clinical and skeletal starts rising as the total fluoride intake starts rising more then 12 mg per day (Fluoride from food – 14%), but as the total daily fluoride increases more then 25 mg (Fluoride from food – 7%), the severity of clinical and skeletal fluorosis increases abruptly.

Considering the above observations the following conclusions can be drawn:

1. These observations indicated that food fluoride plays a vital role in causation of dental fluorosis even in areas with low fluoride in drinking water. Whereas it has substantial role in causing clinical and skeletal fluorosis, where drinking water fluoride is playing its major role.
2. These observations indicated that simple restriction of ingestion of dietary fluoride in areas with less fluoride concentration in drinking water will prevent the people from developing the dental fluorosis.
3. In areas with high fluoride concentration in drinking water restriction of dietary fluoride have to supplemented with defluoridation of drinking water and vitamin C and Calcium rich diet.

So to summarize the fluorosis is best prevented by reducing the intake of fluoride with food and water and by choosing a proper diet that mitigates the ill effects of fluoride.

1. *REDUCTION IN INTAKE OF FLUORIDE*

Fluoride intake can be reduced by:

- **Avoiding water containing more than 1.5 mg/l of fluoride** by using domestic defluoridation
- **Avoiding consumption of food rich in fluoride**: There are certain food items, which are very rich in fluoride e.g. Tea, Jwar, Onion, etc. If the population is well informed they will avoid the intake of such items.

2. *INTAKE OF APPROPERIATE DIET*

A diet rich in Calcium and Ascorbic acid (Vitamin C) is highly conducive to the mitigation of ill effects of fluorides. Changing the dietary habits without disturbing the available resources of food, and the customs, so as to get protein, calcium and vitamin C rich diet and changing cooking processes (e.g. cooking practices which destroys Vitamin C during cooking) will help to avoid the reemergence of the disease also once treated. Tables narrating the details of commonly available Indian foods rich in Protein (annexure 3), Calcium (annexure 4), and Ascorbic acid (annexure 5), along with their quantity available in that food are presented.

Both of the above strategies call for an action-oriented plan of **Health education.**

PREVENTION BY USING DEFLUORIDATION TECHNIQUES SUITABLE FOR DOMESTIC USE ESPECIALLY IN VILLAGES

Considerable work has been done all over the world on treatment of Fluorosis. Unfortunately the results indicated that the effects of fluorosis are irreversible. According to the Gupta *et al.*, 1994 this condition can be cured, at least in children, by a treatment which are inexpensive and easily available (Gupta *et al.*, 1994; Gupta *et al.*, 1996). Numerous people have conducted surveys on the problem of fluoridation and treatment options available for defluoridation processes (Killedar DJ and Bhargava DS, 1988; Killedar DJ and Bhargava DS, 1988a; Solsona F, 1985). But, however ,a safe, efficient and cost effective defluoridation technique / process needs to be developed in order to prevent the occurrence of fluorosis.

Methods of domestic deflouridation recommended so far are aimed at bringing the fluoride levels to the WHO standards (1984) and are cumbersome and difficult to use by our villagers. Deflouridation on community scale is capital intensive and requires continuous skilled supervision. Some experiments conducted to reduce the fluoride levels by simple means in the field have provided encouraging results. They call for only minor changes in the water storage system of the people within their social customs and living standard. Although there are several methods available they have to be tailored to suit specific site conditions. Considering the conditions obtaining in our country the following should be the minimum desirable characteristics of the ideal defluoridation process. The process should:

- Not add undesirable toxic substances (eg. Aluminum) to treated water

- Be cost-effective
- Be independent of input Fluoride concentration, alkalinity, pH, temperature
- Be easy to handle/operate by rural population - the major sufferer
- Not affect taste of water

Various commonly available processes for defluoridation with salient advantages and disadvantages are given below.

1. *Nalgonda process* (Nawlakhe WG,1975) :

   This process involves direct addition of lime in water to maintain the pH of water and addition of a known quantity of alum in water directly depending on water fluoride content. The quantity of lime and alum depends upon alkalinity, and fluoride content of water, therefore it is difficult to specify the alum dose universally as it is different for each source of water. It is a cumbersome technique. And hence is not suitable for use by unskilled persons, who needs it most. Further, the process can be used only for water having a fluoride content of less than 10 mg/l. There is a high free residual aluminum content in output water, when treated with alum in this process and over this, when this treated water is boiled in aluminum utensils (a common practice in Indian kitchens) It further aggravates the free available aluminum concentration in treated water. It is reported that the residual aluminum ranges from 2.01 mg/l to 6.86 mg/l in this process (Selvapathy P, and Arjunan NK , 1995).

   It is relevant to note that aluminum is a neurotoxin and concentration as low as 0.08 mg/l of aluminum in drinking water is reported to have caused Alzheimer's disease

(Davidson AM *et al.*, 1982; Martyn CN *et al.*, 1989). In this process maximum permissible limit of 0.2 mg/l for aluminum as prescribed in IS 10500 (1983) is normally exceeded. Also the taste of the treated water is generally not acceptable.

2. *Activated alumina process* (Bulusu KR and Nawlekhe WG, 1988; Bulusu KR and Nawlekhe WG, 1990; Venkobachar C and Iyengar L, 1996)

   It is an expensive process. Reactivation of filter material is cumbersome (done by treatment of bed by acid and alkali) and it requires the help of trained persons, who are generally not available in most of our villages. This process also results in moderately high residual aluminium in output water ranging from 0.16ppm to 0.45ppm.

3. *Other processes* ; (Killedar DJ and Bhargava DS, 1988a; Solsona F, 1985; Nawlakhe WG *et al.*, 1975) [7,8,9]:

   Processes like Electro-dialysis, Reverse Osmosis etc. require special equipment, high power input, specially trained persons to operate, and a lot of maintenance besides being very expensive.

   Considering the one or the other drawbacks of these available defluoridation processes, a new defluoridation process (Gupta SK, 1997; Gupta SK at al, 1999) , named as (KRASS defluoridation process) was developed, which is safe, efficient, easy to use by our rural population and cost effective The defluoridation study were conducted in columns (Agarwal KC, 1997) using a support material (under patent) in a down flow mode. In this study fluorides from water could be removed for influent pH range of 4.3-9.0 and influent fluoride concentration upto 24 mg/l.

Aluminium was found in traces in the treated water through this process. Cost of treated water was found equivalent to that of Nalgonda process.

4. *KRASS Process*

In this process the fluoride contaminated water passed through the media as in the case of a slow sand filter to get the defluoridated water. This process differs from the known processes in its simplicity, cost effectiveness and **very low traces of residual aluminum in outlet water**. There is no limit on fluoride concentration in input water. Temperature, pH, alkalinity and Total Dissolved Solids of input water do not effect this process. The ambient conditions like atmospheric temperature and humidity do not have any effect on this process.

This is a defluoridation process, which is easy to use by illiterate villagers, requires minimal involvement of technical personnel, is harmless and is cost effective. In the process, once the filter is installed, no maintenance is required till the media is exhausted. The fluoride free water flows out by gravity. The exhausted media bed can be easily recharged again without replacing the material at least up to 40 cycles. As a byproduct, the process achieves a better removal of suspended matter, betters clarity and maintains taste of water. The treatment cost is about 0.6 – 0.8 paisa per liter at 10 ppm of influent fluoride.

To sum up the following are the salient features of the commonly available defluoridation processes:

NALGONDA TECHNIQUE

- ADDS aluminum & TDS to treated water
- Cost-effective
- NOT independent of input Fluoride concentration, alkalinity, pH
- NOT easy to handle/operate by rural population - the major sufferer
- AFFECTS taste of water

ACTIVATED ALUMINA

- ADDS moderate amount of aluminum to treated water
- NOT Cost-effective
- INDEPENDENT of input Fluoride concentration, alkalinity, pH
- NOT easy to handle/operate by rural population - the major sufferer
- DOES NOT affect taste of water

KRASS TECHNIQUE

- Does not add any toxic substance (e.g. Aluminum) to treated water
- Low-cost Technique
- Easy to handle/operate by rural population
- Independent of input Fluoride concentration, alkalinity, pH, temperature
- Does not affect taste of water
- Other characteristics are:

K Filter bed recharging using alum solution. It can be recharged for 30-40 cycles and then discarded. Initial F retaining capacity of filter material not high. It is highly portable and can be sited at anywhere to suit a user.

CRITICAL ANALYSIS RELATING TO COST EFFECTIVENESS OF DIFFERENT FIELD DEFLUORIDATION PROCESSES

A critical analysis of the cost effectiveness of all the commonly advisable defluoridation indicated that KRASS process is most cost effective. Apart from the capital cost the cost of treated water is as follows:

KRASS	: 0.8 paise/l
Nalgonda	: 2.3 paise/l
Activated Alumina	: 4.3 paise/l

In almost all defluoridation processes basically aluminum salts have been used. Aluminum, which was not given much importance for health point of view till few years back, has now been identified as one of the most toxic neurotoxin. Before discussing the residual aluminum in various defluoridation processes, it is mandatory to discuss the toxic effects of aluminum in brief.

ALUMINUM AND HUMAN HEALTH (WHO, 1997):

Environmental hazard of various heavy metals particularly lead, Mercury and zinc were not unknown to our ancestors. Toxic effects of heavy metals like Iron, Lead, Mercury, Arsenic and Cadmium is well known for years. However, the global significance

and human health impact of *Al* metal pollution have become an issue of public concern only in last few years.

Aluminium is the third most common element in the earth's crust, after oxygen and silicon. It accounts for approximately 8.1% of the crust by weight. Al is a lightweight and cheap metal and so extensively used in various manufacturing industries for example electrical, aeronautical, building and utensils industry. Because of its widest application in the industrial sector it is now being regarded as the metal of Twenty first (21^{st}) century. Also due to its abundance in the nature, it can not be regarded as a contaminant in the usual sense of words. However intensive mining operations and processing of Al metal to meet the ever increasing needs of our industrial society have generated the problems related to environmental pollution in specific locations all over the world.

World Health Organisation (WHO) have recommended the secondary maximum contaminant level of the Al as 0.2 mg/l. (WHO, 1984) in the drinking water. At present, the level of Al is ever exceeding the permissible limits in water, air, soil and food.

Various sources of *Al* consumption in environment are water, air, soil and food (Abercrobie, DE, Fowler RC, 1997; Athar MD and Vohra SB, 1995). High concentration of Al residuals is observed in municipal drinking water treated with excessive dosage of alum. De-fluoridation of water with Nalgonda technique in fluoride affected areas also contribute high residual Al in finished water. Other probable sources of Al intake into human body are tea, berries, spinach, Al pan and utensils for making food.

Although Al is ubiquitous in the environment, it serves no known purpose in the human body. At present, Al is widely regarded as a neuro-toxin (Davison AM and Walker GS, 1982; Driscoll CT and Letterman RD, 1988; Singer SM *et al.*, 1997) and far from

innocuous. Elevated levels of Al in potable water have human health impacts. Medical research studies have indicated the high Al into body as a causative agent in neurological disease (Albrey AC, 1976; Crapper DR, 1973, Flaten TP *et al.*, 1996) such Alzheimer's disease and pre-senile dementia (Letterman RD and Driscoll TC, 1988; Martyn CN and Barker PDJ, 1989; Mclachlan DR, 1996; Savory J *et al.*, 1996; Rane RD, 1994).

Al severely restricts the growth and presence of plant species. Fishes and amphibians have been directly connected to Al toxicity. Indirect effects on birds and animals also have been identified.

Toxic effects of aluminum

- CNS: Neuro-toxin-Alzheimer's Disease, Encephephalopathy, Impair Cognitive & motor function, Peripheral Neuropathy , Myopathy,
- BONES: Osteomalacia, Rickets, Pathological # (Zhu P *et al.*, 1993)
- X-ray of BONE: Non healing #, Osteopenia, Reduction in calcified tissue
- ALLERGY : Itchy dermatitis axilla
- RESPIRATORY TRACT: Pulm. Fibrosis, Asthma, Ch. Bron.
- BLOOD: Microcytic Anemia

The permissible limit of aluminum in drinking water is :

- Desirable Limit : 0.03 mg/l
- Absolute Maximum Permissible Limit : 0.2 mg/l

Considering the toxic effects of aluminum a critical analysis was conducted to evaluated the residual aluminum in treated water in commonly advisable defluoridation processes:

KRASS	: Traces
Nalgonda	: 2.01 - 6.86 mg/L
Activated Alumina	: 0.16 - 0.45 mg/L

Considering all the above facts the KRASS defluoridation process seems to be most suitable for our rural population.

STRATEGIES TO OVERCOME THE PROBLEM OF FLUOROSIS IN HUMAN BEING

Based upon the above observations the following strategy can be adopted to achieve a global control of fluorosis at community level:

1. **TREATMENT OF THE CHILDREN**:

   By the use of Vitamin C, Calcium and Vitamin D in non-toxic doses.

2. **USE OF SAFE DOMESTIC DEFLUORIDATION TECHNIQUE AT HOUSEHOLD LEVEL**.

   The defluoridation process, which is easy to use by illiterate villagers, requires minimal involvement of technical personnel, is harmless and is cost

effective. KRASS process is most suited in the present scenario. In the process, once the filter is installed, no maintenance is required till the media is exhausted. The fluoride free water flows out by gravity. The exhausted media bed can be easily recharged again without replacing the material at least up to 40 cycles. As a byproduct, the process achieves a better removal of suspended matter, betters clarity and maintains taste of water. The treatment cost is about 0.6 – 0.8 paisa per liter at 10 ppm of influent fluoride.

3. HEALTH EDUCATION

Prevention of fluorosis can be done by simple health education to effect minor changes in the diet. It can be achieved by:

(a) Avoiding use of fluoride rich water,

(b) Taking diet rich in Protein, Calcium and Vitamin C.,

(c) Changing dietary habits (e.g. cooking practices that destroy Vitamin C during cooking) of the population within their social system and available resources.

This is only being possible if this program can be made community oriented rather than Government driven. That is the program must be **“For The People And By The People”**

CONCLUSIONS

THIS STUDY EXPLAINS THE

- **Pathophysiology of fluorosis.**
- **Treatment and prevention of fluorosis**

Pathophysiology

The proposed mechanism adequately explains the pathophysiology of fluorosis.

Treatment

The double blind control study has effectively established that fluorosis is reversible, at least in children, by a therapeutic regime of Calcium, Vitamin C and Vitamin D. The choice of the therapy adopted was based on the following logic. The presence of calcium in gut directly effects the absorption of fluoride ions and will also improve serum calcium levels. Vitamin D3 in low doses enhances calcium absorption and retention without causing hypercalcemia and thus directly reduces the absorption of fluoride ions. It also inhibits the excessive release of parathyroid hormone thereby preventing excessive activation of osteoblasts thus preventing hyperosteoidosis and osteopenia. Ascorbic acid controls collagen formation, maintains the teeth structure and is also essential for bone formation. These structures are adversely effected by higher fluoride intake. The proposed therapy can be used with confidence in fluoride rich areas to alleviate the sufferings of the children that per force consume fluoride rich water.

Prevention

It is possible to reduce the intake of fluoride by avoiding intake of water containing more than 1.5 mg/l of fluoride and avoiding food rich in fluoride. Where fluoride free sources of water are not available domestic defluoridation must be used. Food items rich in fluoride should be avoided. The population may be educated to avoid such food items.

Changing the dietary habits without disturbing the available resources of food and the customs, so as to get protein, calcium and vitamin C rich diet and changing cooking habits will help to avoid the reemergence of the disease once treated.

Out of the three processes of defluoridation suitable for domestic use especially in Indian villages the KRASS defluoridation technique has negligible residual Aluminum toxicity, is the simplest to operate and maintain and is the cheapest.

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ANNEXURES

Annexure 1

PROFORMA

1. Name of the Child Age Sex
2. Educational Status
3. No. of Brothers/Sisters
4. Father's Name
5. Occupation Income (Rs. per Month)
6. Socio-economic status
7. Residential Address
8. Source of Water Supply Tube well/Well/Tap/Hand Pump/ PHED Supply/Pond/River/Any other
9. Fluoride Content of that water supply (ppm)

1. COMPLAINTS

- Bone-joint pain
- **Stiffness/Rigidity Restriction of movement at spine/joint**
- Deformities of spine/limbs/Knock-knee/Crippling bed ridden state/Bowing of leg.

2. GENERAL EXAMINATION

- Built Nutrition
- Hair
- Fontanelle Anterior Open/closed

- K Posterior Open/closed
- K Evidence of Anemia
- K Evidence of Vitamin A Deficiency
- K Evidence of Vitamin D Deficiency
- K Evidence of Vitamin C Deficiency
- K Evidence of Malnutrition / Kwashiokor
- K Evidence of Spinal deformities congenital Acquired
- K Evidence of Any other bony deformity (Specify)
- K Evidence of Dental Caries,

3. **ANTHROPOMETRY**

- K Wt. (kg.) Ht.(cm.)
- K HC (cm.) MAC. (cm.)

4. **DETAILED DIETARY HISTORY**

Day 1

Day 2

Day 3

5. CVS EXAM Resp. rate
B.P.
Pulse

6. RESP. EXAM.

7. GIT EXAM

8. ENT/OPHTH EXAM.

9. DENTAL FLUOROSIS

Gr. (O) (0.5) (1) (2) (3) (4)

10. **INVESTIGATION** I visit II visit III visit

- K Hb.
- K TLc.
- K DLc.
- K (P/L/E/M/B)
- K ESR
- K PBF
- K Urine
- K Serum fluoride conc.
- K Blood fluoride
- K Urinary fluoride
- K Serum Calcium
- K Serum Inorganic Phosphorus
- K Serum alkaline phosphatase
- K Ascorbic acid, (Serum)
- K (Leucocytes)
- K Serum Sialic acid
- K Serum Glucosamine glycan
- K Serum Parathyroid hormone

11. **RADIOLOGICAL EXAM :**

K Spine

K Skull

K Others

12. **FOLLOW UP**

Visits → I II III

→

Date

K Dental Grade →

K Clinical Grade →

K Radiological Grade →

Annexure 2

FLUORIDE CONTENT IN AGRICULTURAL PRODUCTS AND OTHER EDIBLE ITEMS

(Fluoride in ppm)

Food Item	A Sengupta and Pal (1971)	B Lakdawala and Puneker (1973)	C Chari et al (1975)	D Rajyalaxmi (1982)
Place of study	Calcutta	Bombay	Podli (AP)	Andhra Pradesh
Fluoride content in drinking Water (ppm)	0.4	0.15-0.48	2.5-5.0	1.0-12.0
Cereals and Tubers				
Cereal	4.6	2.59-3.3	--	--
Wheat	5.9	3.27-14.03	2.9-3	--
Rice	--	1.72-2.23	2.82	74.0
Maize	5.6	--	--	--
Pulses & Legumes				
Bengal Gram	6.2	3.84-4.84	--	14.8
Green Gram Dal	2.5	2.34-4.84	--	21.2
Red Gram Dal	3.7	2.34-4.84	--	52.8
Soyabean	4.0	--	--	--
Leafy Vegetables				
Spinach	2.0	0.77-4.14	--	--
Cabbage	3.3	1.28-2.29	--	--
Amaranth leaves	5.8	4.91-7.14	--	--
Lettuce	5.7	--	--	--
Mint	4.8	--	--	--
Bathua Leaves	6.3	--	--	--
Chowli leaves	--	1.79-7.33	--	--
Other Vegetables				
Cucumber	4.1	2.57-3.58	--	--
French beans	--	1.07-1.96	--	--
Tomato	3.4	1.00-2.08	0.33	--
Brinjal	1.2	1.62-2.48	1.24	--
Ladies finger	4.0	2.2-3.62	1.74	--
Snake Gourd	2.3	2.16-3.44	0.75	--
Roots & Tubers				
Beet root	4.2	--	--	--

Carrot	4.1	1.9-4.9	--	--	
Potato	2.8	1.27-2.92	--	--	
Onion	3.7	1.00-3.00	--	--	
Sweet Potato	3.2	--	--	--	
Fruits					
Banana	2.9	0.84-1.58	--	--	
Dates	4.5	--	--	--	
Grapes	--	0.84-1.74	--	--	
Figs	4.2	--	--	--	
Mango	3.7	0.8-1.80	--	--	
Apple	5.7	1.05-2.2	--	--	
Guava	5.1	0.24-0.52	--	--	
Nuts & Oil seeds					
Almond	4.0	--	--	--	
Cashewnut	4.1	--	--	--	
Coconut	4.4	--	--	--	
Mustard Seeds	5.7	--	--	--	
Groundnut	5.1	--	--	--	
Beverages					
Tea (Dry leaves)	--	39.8-68.59	--	--	
Tea infusion (1 gm boiled for 5 min. in 125 ml. water)	--	18.13-56.19	--	--	
Tea infusion (1 gm in 125 ml) of hot water)	--	11.13-37.34	--	--	
Aerated drinks	--	0.77-1.44	--	--	
Coconut Water	--	0.43-0.60	--	--	
Spices & Condiments					
Corriander 2.3	--	--	--	--	
Cumin Seeds	1.8	--	--	--	
Garlic	5.0	--	--	--	
Ginger	2.0	--	--	--	
Tamarind pulp	3.8	--	--	--	
Termeric	***3.3***	**--**	**--**	**--**	
Food from Animal sources					
Mutton	--	3.0-3.5	--	--	
Beef	--	4.0-5.0	--	--	
Pork	--	3.0-4.5	--	--	
Fish	--	1.0-6.5	--	--	

FLUORIDE CONTENT OF SPICES IN PPM (RGDWM, 1993)

Cardamom (Big ilachi)	14.4
Red Pepper (Kashipuri)	10.7
Small Cardamom (Small ilachi)	8.3
Red Pepper (Patna)	8.07
Coriander Powder	8.3
Coriander Leaves	0.3-0.9
Ajwain(Omum)	2.9
Dal Chini	2.4
Turmeric Powder	4.6
Cumin Seeds	4.6
Cumin Seeds (Black)	3.8
Pepper (Black)	3.6
Cloves	3.2
Fenugreek Seed	3.6

FLUORIDE CONTENT IN AGRICULTURAL CROPS, EDIBLES AND OTHER ITEMS
(ppm)

Arecanut (Supari)	3.8-12.0
Betal Leaf (Pan)	7.8-12.0
Tobacco	3.1-38.0
Bajra	74.
Bengal gram	14.80
Green gram dal	21.2
Cabbage	1.28-2.29
Bathua leaves	6.3
Tomato	0.33-2.08
Brinjal	1.2-2.48
Ladies finger	1.74-4.00
Carrot	1.90-4.90
Potato	1.27-2.92

Onion	1.00-3.00
Banana	0.84-1.58
Grapes	0.84-1.74
Mango	0.80-1.80
Apple	1.05-2.2
Tea (dry leaves)	39.8-68.59
Coconut water	0.43-0.60
Garlic	5.00
Ginger	2.00
Mutton	3.00-3.50
Beef	4.00-5.00
Pork	3.00-4.50
Fishes	1.00-6.50
Dalchni	2.40

Fluoride contents in 6 brands of Tea (RGDWM, 1993)

S.No.	*Brand Name*	*Fluoride content (mg/kg)*
1.	Lamsa	Highest
2.	All Dusi	Second Highest
3.	Three Roses	Third Highest
4.	Red Lable	Forth Highest
5.	Tajmahal	Fifth Highest
6.	Super	Sixth Highest

Annexure 3

PROTEIN RICH DIET

S. No.	Name of Food / Item	Protein (per 100 gm of food)
1.	Bajra	11.6
2.	Barley	11.5
3.	Jowar	10.4
4.	Rice, Parboiled	8.5
5.	Wheat, whole	11.8
6.	Wheat, Flour (whole)	12.1
7.	Wheat, bread (brown)	8.8
8.	Green Gram, Dhal	24.5
9.	Peas green	7.2
10.	Bathua leaves	3.7
11.	Cabbage	1.8
12.	Carrot Leaves	5.1
13.	Cauliflower greens	5.9
14.	Mustard Leaves	4.0
15.	Radish Leaves	3.8
16.	Carrot	0.9
17.	Onion Big	1.2
18.	Potato	1.6
19.	Radish Pink	0.6
20.	Turnip	0.5
21.	Bitter Gourd	1.6
22.	Brinjal	1.4
23.	Cauliflower	2.6
24.	Cucumber	0.4
25.	Ladies Fingers	1.9
26.	Mango, Green	0.7
27.	Papaya, Green	0.7

28.	Pumpkin Fruit	1.4
29.	Tinda, Tender	1.4
30.	Tomato, green	1.9
31.	Coconut fresh	4.5
32.	Groundnut	18.3
33.	Watermelon Seeds (Kernal)	34.1
34.	Chillies dry	15.9
35.	Chillies green	2.9
36.	Garlic dry	6.3
37.	Pepper dry	11.5
38.	Apple	0.2
39.	Bael Fruit	1.8
40.	Banana ripe	1.2
41.	Guava, country	0.9
42.	Lemon	1.0
43.	Mango, Ripe	0.6
44.	Melon, Musk	0.3
45.	Melon, Water	0.2
46.	Orange	0.7
47.	Pine Apple	0.4
48.	Milk Buffalo’s	4.3
49.	Milk Cow’s	3.2
50.	Khoa (whole buffalo)	14.6

Annexure 4

CALCIUM IN FOOD PRODUCTS (mg./100 gm)

Ajwayan	1525	Gajar Sag	340
Til	1450	Samp Machli	330
Skimmed Milk Powder	1370	Kala Til	300
Mar (Agathi)	1130	Lobia	290
Jira	1080	Kulthi (Horse Gram)	287
Khoa	990	Chironji	279
(Skimmed Buffllo Milk)		Mooli Ka Sag	265
Whole Milk Powder	950	Rajmah	260
Kantewali Chaulai	800	Cholai-Ki-Dandi	260
Long Dry	740	Bhatmas (Soyabean)	240
Shalgam Ka Sag	710	Pan Ka Patta	230
Hing	690	Badam	230
Dhania	630	Bhains Ka Doodh	210
Phool Gobee Sag	626	Chana	202
Bathuva	520	Moth	202
Rai	490	Gendhri	200
Panner	480	Pudina	200
Kali Mirch	460	Hara Dhania	184
Coconut Dry	400	Imli	170
Chaulai Sag	397	Bakri Ka Doodh	170
Chukandar Ka Sag	380	Karonda	160
Methi Sag	395	Mirch Dry	160
Kaddu Ka Sag	392	Methi	160
Arbi	380	Sarson-Ka-Sag	155
Chumli Sag (Arai Keerai)	364	Urad Dal	154
Bargad Ka Phal	364	Bakri Ka Gosht	150
Madua (Ragi)	344	Haldi	150
Chana Sag	340	Bathua Sag	150

Dahi	149	Nimbu Bada	70
Pista	140	Shahtoot	70
Ilaychi	130	Bathak Ka Anda	70
Munakka	130	Masur Dal	69
Guar Ki Phalli	130	Gai Ka Ghosht	68
Falsa	129	Rice Bran	67
Mung	124	Bhindi	66
Gay Ka Doodh	120	Sem	60
Khajur	120	Arwi Ki Dandi	60
Gay Ka Doodh	120	Bara Sem	60
Kalni Sag	110	Murgi Ka Anda	60
Imli Patte	101	Bhuna Chana	58
Sarson Ki Dandi	100	Arhar, Tender	57
Akhrot	100	Chane-Ki-Dal	56
Katchua Ka Anda	93	Sayan Ki Phalli	51
Chilgoza	91	Muli	50
Moong Phali	90	Zimikand	50
Neembu	90	Sem	50
Kishmish	87	Fras Bean	50
Bel (Bael Fruit)	85	Kathal Seeds	50
Matar Roasted	81	Lasson	50
Gadhe Ka Doodh	80	Supari	50
Gud (Cane)	80	Kaju	50
Lauki Ka Sag	80	Amla	50
Gajar	80	Atta	48
Anjeer	80	Tamator	48
Matar Dry	79	Pyaz (Onion) Big	46
Mung Dal	75	Shakarkand,Sweet	
Lobia	77	Potato	46
Arhar Dal	73	Chotee Gobee	43
Palak	73	Bajra	42

Gehun	41
Wheat Gram	40
Arwi	40
Pyaz Small	40
Mausambi	40
Raspberry	40
Band Gobee	39
Wheat Bulgar (Parboiled)	37
Amra	36
Phul Gobi	33
Kharbooja	32
Kangni (Italian Millet)	31
Shalgam	30
Mirch Green	30
Lehson Dry	30
Malta	30
Suar Ka Gosht	30
Lassi	30
Makhan	00

Annexure 5

VITAMIN C IN FOOD PRODUCTS(mg./100gm)

Amla	600	Tamator	27
Shalgam Ka Sag	180	Pudina	27
Mar (Agathi)	169	Sem	27
Hara Dhania	135	Kharbooja	26
Band Gobee	124	Arhar, Tender	25
Mirch Green	111	Shakarkand,Sweet Potato	24
Chaulai Sag	99	Fras Bean	24
Mooli Ka Sag	81	Falsa	22
Gajar Sag	79	Amra	21
Chotee Gobee	72	Muli	17
Chukandar Ka Sag	70	Kathal Seeds	14
Neembu	63	Bhindi	13
Phul Gobi	56	Lehson Dry	13
Malta	54	Bathuva	13
Methi Sag	52	Bara Sem	12
Mirch Dry	50	Shahtoot	12
Mausambi	50	Ari	11
Guar Ki Phalli	49	Lasson	11
Shalgam	43	Pyaz (Onion) Big	11
Nimbu Bada	39	Cholai-Ki-Dandi	10
Kalni Sag	37	Gadhe Ka Doodh	10
Bathua Sag	35	Bel (Bael Fruit)	8
Kantewali Chaulai	33	Coconut Dry	7
Sarson-Ka-Sag	33	Lobia	4
Rusbhary	30	Pan Ka Patta	5
Palak	28		

Anjeer	5
Skimmed Milk Powder	5
Whole Milk Powder	4
Chana	3
Imli Patte	3
Gajar	3
Arwi Ki Dandi	3
Jira	3
Imli	3
Khajur	3
Samp Machli	3
Moth	2
Pyaz Small	2
Suar Ka Gosht	2
Gay Ka Doodh	2
Chane-Ki-Dal	1
Kulthi (Horse Gram)	1
Munakka	1
Kishmish	1
Bhais Ka Doodh	1
Bakri Ka Doodh	1
Dahi	1