

Reversal of fluoride induced cell injury through elimination of fluoride and consumption of diet rich in essential nutrients and antioxidants

A.K. Susheela and Madhu Bhatnagar

Fluorosis Research and Rural Development Foundation, Delhi, India

Abstract

The objective of the present communication is to address the issues concerning reversal of fluoride induced cell injury and disease (i.e. fluorosis) through the elimination of fluoride and consumption of a diet containing essential nutrients and antioxidants. Humans afflicted with fluorosis, as a result of consuming fluoride contaminated water or food, have been investigated. Hospital based diagnostic procedure for early detection of fluorosis, through retrieval of history, clinical complaints, testing of blood, urine and drinking water for fluoride using ion selective electrode technology, along with X-ray of the forearm have been carried out. Confirmed cases of fluorosis were introduced to an intervention protocol consisting of (1) provision of safe drinking water with fluoride levels less than 1 mg/L and (2) counselling on nutritional supplementation with focus on adequate intake of calcium, vitamins C, E and antioxidants. The patients were monitored at frequent intervals up to one year and the results are reported. With a standardized early diagnosis, elimination of fluoride intake and supplementation of a diet rich in essential nutrients and antioxidants, we have shown that the fluorosis can be reversed. (*Mol Cell Biochem* 234/235: 335–340, 2002)

Key words: reversal of fluorosis, nutritional supplementation, antioxidant therapy

Introduction

Incidence of fluorosis in India, a disease caused by ingestion of fluoride mainly through drinking water and other sources such as agricultural crops, food, drugs, and dental products, is a major cause for concern. It is one of the major public health problems affecting the rural Indian population, who are dependent on ground water drawn through hand pumps in 19 out of the 35 states and Union Territories in India. The fluoride content in drinking water, ranges from 1.1 mg to 48.0 mg/L and the number of people afflicted with dental, skeletal and non-skeletal fluorosis run into several million [1].

Fluoride ingestion through naturally occurring sources of water, and the incidence of fluorosis affecting young and old, men and women is not confined to India, but occurs in 23 other nations around the globe.

According to WHO's guidelines for drinking water, a fluoride level of 1.5 mg/L is the desirable upper limit. According to US EPA, the maximum allowable content of fluoride in

drinking water is 4.0 mg/L. However, these norms for fluoride content are being increasingly questioned and new norms are being formulated by different nations. Senegal reduced the upper permissible limit of fluoride in drinking water from 1.5 to 0.6 ppm based on the prevalence of dental fluorosis at a level 1.0 mg/L of fluoride in drinking water [2]. India reduced the upper limit of fluoride in drinking water from 1.5 to 1.0 mg/L with a rider that less is better [3]. This is due to extremes in climatic conditions and the diet being deficient in essential nutrients (calcium, vitamins C, E and antioxidants) in the rural communities of India.

Although dental and skeletal fluorosis have been recognized in India as early as 1937 [4], the non-skeletal entity of fluorosis, affecting the soft tissues and organs of the body, is relatively a new condition, confirmed through different studies carried out in India during the last two decades. It is now an established fact that fluoride ingestion over a period of time can affect the structure and function of cells, tissues, organs and systems resulting in a variety of clinical manifes-

tations [5–7]. The following manifestations may be due to fluoride toxicity: (1) aches and pain in the joints, i.e. neck, back, hip, shoulder and knee without visible signs of fluid accumulation [8], (2) non-ulcer dyspepsia such as nausea, vomiting, pain in the stomach, bloated feeling or gas formation in the stomach, constipation followed by diarrhea [9–14], (3) polyurea (tendency to urinate more frequently) and polydipsia (excessive thirst), (4) muscle weakness, fatigue, anemia with low hemoglobin level, (5) complaints of repeated abortions/still birth, (6) complaints of male infertility with abnormality in sperm morphology, oligospermia (deficiency of spermatozoa in the semen), azoospermia (absence of spermatozoa in the semen) and low testosterone levels [15–17].

The basis for these manifestations possibly is the derangement that might take place at the molecular level. It is now well established that toxic levels of fluoride inhibit protein biosynthesis (actin and myosin filaments are not formed in skeletal muscle resulting in atrophy of the myofibrils). Collagen biosynthesis is also deranged [16, 17]. The glycosaminoglycans (GAG), which constitute an important entity in matrix molecules of osseous tissues responds to fluoride adversely and is tissue specific. In calcified systems, one of the sulphated isomers of GAG, namely dermatan sulphate, which normally does not exist in the mature and fully developed tissue, begins to reappear, resulting in demineralized or cartilaginous lesions. This is evident in both cancellous bone and tooth, but not in cortical bone. It is also true that in non-osseous tissues such as ligament, tendon, skin and the aorta, where the dermatan sulphate isomer of GAG is normally found in high concentrations, it gradually declines in concentrations leading to ectopic calcification [18–26].

It is well known that excess amounts of fluoride in drinking water and food affect bone remodeling and increase the density of the inorganic constituents of bone; fluoride also affects the GAG of bone matrix [27]. Three types of bone responses generally occur when exposed to high levels of fluoride: (1) an increase in the number of osteoblasts, (2) a decrease in the rate of bone formation, and (3) an increase in serum alkaline phosphatase activity [28]. The structural and molecular derangements taking place in the bone and the teeth as a result of fluoride toxicity are not reversible; however, soft tissue manifestations and the resulting clinical complaints are reversible.

A close association between chronic fluoride toxicity and increased oxidative stress has been reported in humans [29–31]. Oxidative stress is commonly measured in erythrocytes since they are susceptible to oxidative reactions because of the presence of polyunsaturated lipid-rich plasma membrane [32]. Fluoride has been demonstrated, both *in vivo* and *in vitro*, to increase lipid peroxidation in human erythrocytes [29]. In erythrocytes of children afflicted with skeletal fluorosis, increases in malondialdehyde (MDA) levels, decreases in glutathione (GSH) levels, increases glutathione peroxidase

(GSH-px) activity and decreases in superoxide dismutase (SOD) activity were reported [33]. Fluoride inhibits the activities of SOD and GSH-px causing a heavy accumulation of free radicals and hydrogen peroxide resulting in damage to various cells [34]. Wang *et al.* reported that fluorosis is associated with an increase in lipid peroxides and a decrease in antioxidants in patients with skeletal fluorosis [35] and suggested that elimination of fluoride source and treatment with SOD and vitamin E can play an important role in the prevention of fluorosis [35]. Data obtained from dietary supplementation studies suggested that inadequate levels of ascorbic acid and calcium are related to the manifestation and severity of fluorosis [36]. Fluorosis has no treatment but can be prevented through appropriate intervention if the disease is diagnosed at an early stage. Toxic effects of fluoride can be reversed by withdrawing the fluoride source and by providing a diet adequate in protein, calcium, vitamins C, E, D and other antioxidants [36]. Antioxidants play a protective role in fluorosis [34, 36–38]. Beta carotene and superoxide dismutase (SOD) were effective in mitigating impaired growth due to fluoride toxicity in rats [35].

As fluoride is a powerful oxidizing agent, intervention focusing on antioxidant intake has been attempted. The results of such an approach are reported here.

Materials and methods

Ten patients (6 males, 4 females, ages ranging from 8–60 years) having clinical manifestations suggestive of fluoride poisoning were referred to the Foundation by clinicians from hospitals in New Delhi and the neighboring states. Blood, urine and drinking water samples were collected for measuring fluoride levels.

Modules developed for early detection of fluorosis: Diagnostic procedures hospital and laboratory based

The following complaints from patients ought to alert the physician to consider fluoride toxicity as one of the possible reasons [8]. These complaints include: aches and pain in the joints, viz. neck, back, hip, shoulder and knee without visible signs of synovial fluid accumulation; non-ulcer dyspepsia and gastrointestinal complaint such as nausea, vomiting, pain in the stomach, bloated feeling/gas formation in the stomach, constipation followed by diarrhoea; polyurea and polydipsia; muscle weakness, fatigue, and anemia; complaints of repeated abortions or still birth, male infertility. Any loss of shine, or discoloration of the enamel surface in the front row of teeth of the patient or discoloration away from the gums and appearing as horizontal streaks or spots on the enamel surface, may invariably be due to dental fluorosis.

This is an important clue for follow up of the members of the family as they may be drinking fluoride contaminated water.

In view of the information provided, the tests that need to be carried out to arrive at a definitive diagnosis of skeletal and non-skeletal fluorosis are: (1) fluoride levels in the blood (serum), urine and drinking water. Although, 24 h urine is ideal, it is impractical to collect such samples from the rural population, therefore a spot sample of urine was collected for testing. Samples were collected in plastic containers and not glass bottles as there is a possibility of fluoride in the sample reacting with the silica in the glass resulting in unreliable data; (2) radiographs of the region or joint where the patient had complaints such as pain, and stiffness and (3) forearm X-ray to look for interosseous membrane calcification. The forearm X-ray is essential for diagnosis of fluorosis at early stages as well as for differential diagnosis of fluorosis from other orthopedic conditions. This is an important message as forearm X-ray is only requested for diagnosing fluorosis. In patients with fluorosis and osteomalacia, increases in bone mass and bone density may not appear, but ligaments would reveal calcification.

Field and home based investigations

In rural areas, a field-based approach was introduced where diagnostic facilities do not exist. One can either get the fluoride content of drinking water tested, or use existing fluoride data. Every district water-testing laboratory has been provided with an Ion meter with fluoride ion selective electrodes, a preferred method for testing fluoride in water [40].

If the drinking water had high fluoride levels, then the following procedure was adopted: (1) any discoloration of the teeth, due to dental fluorosis in the children of the family was recorded, (2) three physical tests to assess whether there are aches and pain in the joints were performed (Fig. 1), (3) history from the members of the family to assess whether they have non-ulcer dyspeptic complaints, polyurea and or polydipsia was recorded, (4) to confirm whether the health complaints in the family were due to fluoride, the family was diverted to a safer source of water, for cooking and drinking, and the non-ulcer dyspeptic complaints were followed-up. If the complaints were due to fluoride, they would disappear within 10–15 days following the switch to safe water. It is known that GI mucosa can regenerate and non-ulcer dyspeptic complaints disappear. The earliest relief the patients experienced was from non-ulcer dyspepsia.

This approach was followed in a rural village setting where facilities did not exist for diagnosing fluorosis by testing body fluids and taking radiographs. If the disease was confirmed as fluorosis, the patient was monitored following the intervention.

Intervention protocol

Upon diagnosis of the disease the patients were introduced to the following intervention protocol: (1) safe defluoridated water was provided for consumption on a sustainable basis through existing safe sources in the village and or through domestic filters with activated alumina which adsorbs the fluoride. These filters were indigenously fabricated, (2) nutritional counseling was provided for consuming a diet adequate in calcium, vitamins C, E and other antioxidants and to avoid intake of food or other substances containing high fluoride [41–45]. If the intervention protocol consisted of only safe water, it was observed that the process of recovery was slow. However, if the protocol consisted of both safe water and nutritional supplementation, the recovery process was faster.

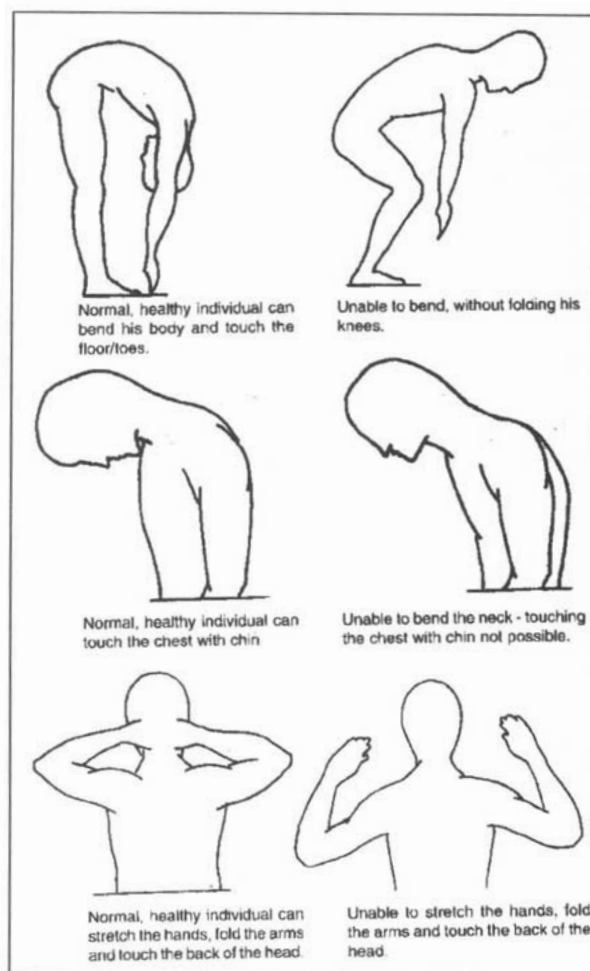


Fig. 1. The three physical tests for assessing aches and pain in the various joints of the body.

Impact assessment

After introducing the intervention protocol patients were monitored up to one year by measuring fluoride levels in blood, urine and the intensity of health complaints at frequent intervals.

Results

The data obtained from the patients with fluorosis before and after treatment are reported in Table 1. It was observed from the present study that some patients with complains associated with fluorosis were consuming safe water but the health complaints were due to fluoride ingested through food and or other fluoride containing items.

A reduction in fluoride content in urine, blood and health complaints were observed in the patients following the intervention. Non-ulcer dyspeptic complaints or gastrointestinal complaints were observed in all of the patients before treatment. During the first impact assessment reduction in health complaints, especially in gastrointestinal discomfort, was most striking. Most of the patients ~ 70% showed relief in gastrointestinal complaints during first impact assessment. During the second impact assessment all of the patients showed relief from gastrointestinal complaints (Table 2).

A significant reduction in fluoride levels in urine and serum and relief of joint pain was observed after the second impact assessment. After the third impact assessment complete relief from polyurea, muscle weakness and joint pain were observed. A significant reduction in the urine and serum fluoride levels before and after the first, second and third impact assessments are shown in Fig. 2.

Discussion

Generation of free radicals, lipid peroxidation and altered antioxidant defense system are considered to play an important role in the toxic effect of fluoride [46–48]. The present investigation reveals that withdrawal of the fluoride source and nutritional supplementation showed improvement in health and a significant reduction in fluoride levels in urine and serum of the patients. It is evident from the earlier studies that fluorosis can be prevented through appropriate intervention if the disease is diagnosed early. Consumption of a diet adequate in protein, calcium, vitamins C, E and other antioxidants can minimize the adverse effect of fluoride toxicity [36]. Ascorbic acid is one of the most important antioxidants in the plasma [49, 50] and is also an anti-stress factor [38]. Ascorbic acid plays a significant role in the amelioration of fluoride-induced toxicity [39]. Earlier studies in pa-

Table 1. Fluoride level in patients with Fluorosis before and during intervention

Patient no.	Fluoride in drinking water (mg/l)		Fluoride in serum (mg/l)			Fluoride in urine (mg/l)				
	Before intervention	During intervention	Before intervention	During intervention			Before intervention	During intervention		
				1 st IA	2 nd IA	3 rd IA		1 st IA	2 nd IA	3 rd IA
1.	3.00	0.27	0.08	0.03	0.03	0.02	8.00	4.50	1.60	0.60
2.	5.80	0.90	0.12	0.10	0.08	0.02	9.00	1.80	1.00	0.21
3.	26.07	0.55	0.22	0.13	0.09	0.05	24.10	15.00	6.00	0.58
4.	1.74	0.55	0.08	0.04	0.03	0.03	2.21	1.16	0.80	0.31
5.	29.00	0.80	0.63	0.40	0.10	0.08	5.00	4.11	1.00	0.50
6.*	1.06	1.06	0.20	0.16	0.11	0.03	2.50	1.46	1.00	0.70
7.*	0.38	0.38	0.09	0.04	–	–	1.00	0.90	–	–
8.	2.00	0.38	0.04	0.04	–	–	2.00	0.80	–	–
9.*	0.14	0.14	0.09	0.04	–	–	0.70	0.51	–	–
10.	0.90	0.52	0.09	0.04	–	–	1.27	1.00	–	–

Permissible limit of fluoride in drinking water: 1.0 mg / L or less. Normal upper limit of fluoride in serum: 0.02mg/L [53]. Normal upper limit of fluoride in urine: 0.1 mg/L [53]. IA – Impact assessment. *Food contaminated with fluoride.

Table 2. Health improvements: expressed by the patients (n = 10)

Manifestations	Percent affliction before intervention	Percentage recovery during intervention		
		1 st impact assessment	2 nd impact assessment	3 rd impact assessment
Gastro-intestinal complaints	100	70	100	–
Muscular Weakness	60	40	50	Complete recovery
Polyurea	30	20	30	Complete recovery
Polydypsea	50	20	40	Complete recovery
Pain and rigidity in the joints	90	30	60	Complete recovery

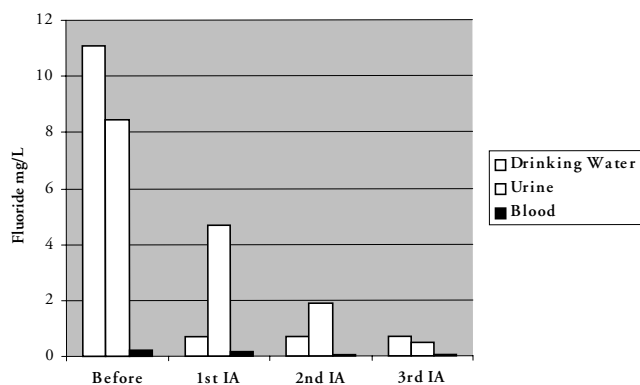


Fig. 2. Average concentration of fluoride in urine, serum and drinking water among the patients before and during impact assessment (IA).

tients with fluorosis indicated that drinking water containing high fluoride concentrations reduced the levels of GSH and GSH-px. However, drinking water with low fluoride concentration would return these to normal [51].

Oxidative stress produced by free radicals and hydrogen peroxide is greater if fluoride impairs the production of free radical scavengers [46]. Studies have shown a decrease in the activity of serum SOD and GSH-Px [31] and increased level of GSH in people living in areas for endemic fluorosis [30]. Increased lipid peroxidation and altered levels of antioxidants in the blood of fluorotic children were reported [33]. The present study indicates that consumption of a diet adequate in essential nutrients and antioxidants has a significant beneficial role in the reversal of fluoride-induced toxicity. This may be due to the fact that increasing the levels of free radical scavengers results in reducing the vulnerability to toxicity induced by free radicals.

Conclusions

The developments in health sciences, in particular the issues focusing on fluoride and fluorosis mitigation achieved in India in a short span of 13 years, have been considerable. Fluorosis has become an easily preventable disease. Efforts have been made for conducting a continuing medical education program on fluorosis mitigation and management of patients. Dissemination of information to school teachers, clinicians, hospitals and medical rural health facilities in areas of endemic fluorosis is important. Water-quality monitoring and importance of surveillance are emphasized. Similarly, health, engineering and education professionals are also being updated on recent developments on water quality testing, quality control and fluoride removal procedures, so that the community has access to safe drinking water in endemic areas for fluorosis.

The need to view and assess the issues related to fluoride and fluorosis in developing countries has become extremely important as the populations afflicted with fluoride poisoning are ever increasing. In the last 4–5 years, the Foundation has received a large number of requests from UK and USA seeking assistance for definitive diagnosis of fluorosis as well as management. Some of the requests are from parents of children who have severe dental fluorosis diagnosed by dentists. This may have resulted from giving fluoride tablets to the children to prevent dental caries. Abstention from fluoride is recommended in such cases. Pediatricians need to be educated about fluorosis. Perhaps water fluoridation and indiscriminate promotion of fluoridated dental products in the name of prevention of dental caries need to be reviewed. In this report we present two standardized procedures to diagnose fluorosis at an early stage, ideally suited for hospital and field-based environments. We also describe an intervention protocol to prevent fluorosis and the necessary monitoring practices.

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