

# Epidemiological, Clinical, and Biochemical Study of Endemic Dental and Skeletal Fluorosis in Punjab

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**Summary:** The incidence of dental fluorosis in 46,000 children in the Punjab was assessed and compared with the fluoride content of their water supplies. Ten villages were selected for more detailed studies of skeletal as well as dental fluorosis.

Factors other than the fluoride content of the drinking water which were found to influence the incidence and the severity of fluorosis were the chemical composition of the water and in particular its calcium content; the duration of exposure; sex and occupation; nutritional status; and climatic and geological variations. The role of fluoride-containing foodstuffs has yet to be assessed.

## Introduction

The Department of Medicine, Medical College, Patiala, has been actively engaged in the epidemiological, clinical, and biochemical studies of endemic fluorosis for the past nine years. These studies have been carried out in Punjab, which is one of the most highly endemic areas in the world. Extensive data on dental, skeletal, and neurological aspects of fluorosis have been collected and fully reported (Singh and Jolly, 1961a; Singh *et al.*, 1961, 1962a, 1963). The object of the present communication is to evaluate the role of various factors causing fluoride toxicity. Even with identical levels of fluoride in the water, the incidence of fluoride intoxication varies, thus clearly pointing to the existence of factors other than the concentration of fluoride.

## Material and Methods

An epidemiological survey to determine the incidence of dental fluorosis was carried out in 358 villages in Punjab. Children aged 5 to 17 years were examined for dental mottling and characteristic dental pigmentation.

Apart from this dental survey, 10 villages from the endemic fluorotic area of Punjab with different fluoride concentrations in drinking-water were selected to assess the effect of various factors on fluoride intoxication. Children and adults (both males and females) of these villages were subjected to thorough clinical and radiological examination. Interosseous membrane calcification was taken as a definite radiological index of skeletal fluorosis.

The waters of these 10 villages were analysed for various important chemical constituents such as total hardness, calcium and magnesium hardness, and alkalinity. In Indian villages there is no regular central water supply. Almost each house has its own hand pump, and the fluoride concentration of the water shows a wide variation in the same village because of the different depths of the wells. Moreover, the farmers and labourers (the subjects of this study) work in the fields away

from the villages and ingest water from different places. It is therefore not possible to evaluate precisely the incidence of fluorosis according to a particular concentration of fluoride in the sample of water. However, the mean fluoride concentration of all water supplies was taken for the purpose of determining the incidence of fluorosis.

## Observations

About 46,000 children were examined in 358 villages in Punjab. The incidence of dental involvement as correlated to fluoride concentration is shown in Table I. The incidence of dental fluorosis was found to rise with increasing fluoride concentration, but there was no rigid linear relationship between fluoride concentration and the incidence of dental involvement. The incidence of dental mottling in children and adults in the 10 villages chosen for special study is given in Table II.

TABLE I

No. of Villages	Maximum Concentration of Water Fluoride (p.p.m.)	Incidence of Dental Mottling (%)
210	1.4	0-10
96	2.3	10-30
52	> 2.3	> 30

TABLE II.—Incidence of Mottling in the 10 Villages

Village	Fluoride Concentration (Mean) (p.p.m.)	Incidence of Dental Mottling (%)	
		Children	Adults
Gharachaon .. ..	1.4	22.6 (124)	13.8 (87)
Laluwala .. ..	2.4	30.6 (49)	60.2 (74)
Dhaipai .. ..	3.0	24.5 (57)	47.6 (107)
Bhodipura .. ..	3.0	55.9 (34)	31.2 (64)
Rajthal .. ..	3.3	47.0 (133)	10.0 (160)
Bhikki .. ..	3.3	53.4 (146)	52.5 (160)
Sanghera .. ..	3.6	27.4 (317)	49.4 (154)
Ramuana .. ..	5.0	52.7 (93)	56.6 (90)
Gangigulabsingh ..	8.5	81.4 (43)	55.6 (58)
Khara .. ..	9.7	66.0 (50)	70.7 (232)

Figures in parentheses denote the number of cases.

## Skeletal Fluorosis

The skeletal changes of fluorosis are best described under the headings of gross changes in the skeleton, radiological changes, the chemical composition of bones, the histopathology, and deformities. These changes have been described in detail elsewhere (Singh *et al.*, 1962b). However, the skeletal fluorosis may be completely asymptomatic in the early stages, and the only method of detecting it earlier is by radiological study. Later on it may lead to generalized bone pains, particularly in the spine and joints, and also result in exostosis and deformities such as kyphosis, flexion deformity of the knees, etc. An attempt was made to correlate the incidence of skeletal fluorosis with water fluoride concentration. For this purpose an extensive survey was carried out in the 10 villages. The results are shown in Table III.

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TABLE III.—Incidence of Skeletal Fluorosis

Village	Concentration of Fluoride in Water (p.p.m.)		Incidence of Skeletal Fluorosis (%)	Crippling Fluorosis
	Mean	Range		
Gharachaon ..	1.4	0.9-2.5	2.4 (82)	Negative
Laluwala ..	2.4	1.0-5.5	23.0 (74)	Negative
Dhaipai ..	3.0	1.1-5.5	19.6 (107)	Negative
Bhodipura ..	3.0	1.3-5.2	42.2 (64)	Positive
Rajthal ..	3.3	0.5-6.5	10.0 (160)	Negative
Bhikki ..	3.3	1.0-5.9	45.6 (160)	Positive
Sanghera ..	3.6	1.1-5.8	33.1 (154)	Positive
Ramuana ..	5.0	1.5-11.5	60.0 (90)	Positive
Gangigulabsingh ..	8.5	3.7-14.0	58.9 (56)	Positive
Khara ..	9.7	6.0-16.2	70.7 (232)	Positive

Figures in parentheses indicate the total number examined.

The incidence of skeletal fluorosis was practically nil at a mean concentration of 1.4 p.p.m. but rises with the increase of fluoride concentration in water. The concentration of fluoride alone is not responsible for the incidence of skeletal fluorosis. Thus crippling fluorosis was evident in a village with a mean fluoride concentration of 3 p.p.m., but in another village with the same concentration it did not occur.

Neurological Manifestations of Fluorosis

In our earlier studies it was reported that in some cases of skeletal fluorosis the neurological complications of radiculomyelopathy occur owing to compression of the spinal cord and roots as a result of irregular bone deposition in and around the spinal canal. This has been convincingly shown in a macerated skeleton (Singh et al., 1962b). So far we have studied 62 proved cases of skeletal fluorosis with neurological manifestations. This is the largest published series. The details of neurological manifestations are being compiled separately, but a summary is shown in Table IV. It is worth emphasizing that only five cases of neurological fluorosis were seen in women against 57 in men.

Factors Influencing Toxicity

Concentration of Fluoride

It is generally agreed that fluoride ingestion produces toxic effects, but the concentration which may have deleterious effects is a subject of controversy. The minimal threshold has not

TABLE IV.—Summary of 62 Cases with Neurological Manifestations

Type of Neurological Lesion	Males	Females
Cervical radiculomyelopathy .. .. .	31	—
Cervical radiculomyelopathy with deafness ..	1	—
*Cervical radiculomyelopathy with cerebellar involvement .. .. .	1	—
Purely cervical myelopathy .. .. .	4	1
Cervicodorsal myelopathy .. .. .	2	1
Dorsal myelopathy .. .. .	12	3
Cervical radiculopathy .. .. .	1	—
Peripheral neuritic type .. .. .	3	—
*Fluorosis associated with cerebrovascular accident .. .. .	2	—
Total .. .. .	57	5

\* These cases were probably due to compression of the vertebral artery as it passed through the cervical spine.

yet been definitely established. In some studies from India lower concentrations of fluoride have been shown to be associated with well-marked fluorosis. The present survey shows that concentrations of fluoride from 0.9 to 2.5 p.p.m. are associated with an incidence of only 2.4% skeletal fluorosis, but that crippling fluorosis was seen in some villages in which the fluoride range was 1.3 to 5.2 p.p.m. The belief that the incidence of skeletal fluorosis is dependent only on the fluoride concentration is shown to be fallacious when two villages, Rajthal and Bhikki, are compared. These villages have practically the same fluoride concentration but show a marked difference in the incidence of skeletal fluorosis. In Bhikki a large number of cases of crippling fluorosis were observed, but not a single similar case was detected from Rajthal—clearly showing the existence of factors other than fluoride concentration.

Duration of Fluoride Exposure

The period of fluoride intoxication has a definite influence on the development of endemic fluorosis, because with a similar fluoride concentration the incidence was found to increase with age. In villages having a low fluoride concentration skeletal fluorosis was detected in an older age group than in villages with a high fluoride concentration.

Sex and Occupation

Sex and occupation also have some influence on the development of endemic fluorosis, particularly in relation to severe

TABLE V.—Chemical Constituents of Water

Village	Fluoride (p.p.m.)	Total Hardness	Calcium Hardness	Magnesium Hardness	Alkalinity	Chloride	Incidence of Skeletal Fluorosis
Normal	(Mean .. .. .)	0.28	287	96	13	271	—
	(Range .. .. .)	0.1-0.7	120-558	38-208	0.5-59	88-404	—
	(S.D. .. .. .)	0.066	103	32	11	73	—
Gharachaon	(Mean .. .. .)	1.4	235	71	191	588	2.4
	(Range .. .. .)	0.9-2.5	180-470	44-116	110-294	480-760	80-182
	(S.D. .. .. .)	0.03	99	19	57	80	32
Laluwala	(Mean .. .. .)	2.4	116	48	—	798	23.0
	(Range .. .. .)	1.0-5.5	38-300	20-98	—	360-1164	56-356
	(S.D. .. .. .)	1.2	57	24	—	197	—
Dhaipai	(Mean .. .. .)	3.0	375	108	268	753	19.6
	(Range .. .. .)	1.1-5.5	200-696	46-200	106-558	536-952	204-320
	(S.D. .. .. .)	1.4	109	41	116	131	33
Bhodipura	(Mean .. .. .)	3.0	287	73	205	448	42.2
	(Range .. .. .)	1.3-5.2	125-450	36-184	89-308	310-680	10-46
	(S.D. .. .. .)	1.2	88	25	82	141	12
Rajthal	(Mean .. .. .)	3.3	601	358	344	519	10.0
	(Range .. .. .)	0.5-6.5	308-1136	120-800	108-506	368-740	40-1040
	(S.D. .. .. .)	0.5	61	57	40	32	96
Bhikki	(Mean .. .. .)	3.3	136	56	80	300	45.6
	(Range .. .. .)	1.0-5.9	32-312	6-190	26-198	138-569	12-200
	(S.D. .. .. .)	0.3	20	98	10	31	31
Sanghera	(Mean .. .. .)	3.6	237	81	185	460	33.1
	(Range .. .. .)	1.1-5.8	120-389	36-160	56-316	260-696	20-224
	(S.D. .. .. .)	1.5	88	44	73	120	71
Ramuana	(Mean .. .. .)	5	101	27	62	869	60.0
	(Range .. .. .)	1.5-11.5	30-160	8-72	25-120	680-1100	32-98
	(S.D. .. .. .)	2	43	13	28	123	19
Gangigulabsingh	(Mean .. .. .)	8.5	30	14	17	851	58.9
	(Range .. .. .)	3.7-14	15-55	4-24	0-46	500-1010	20-110
	(S.D. .. .. .)	2.3	13	5	12	102	20
Khara	(Mean .. .. .)	9.7	94	34	61	391	70.7
	(Range .. .. .)	6-16.2	28-304	10-140	12-164	46-1160	44-190
	(S.D. .. .. .)	1.0	21	11	13	113	16

varieties, such as neurological and crippling fluorosis, because there were only five female as compared with 57 male cases of neurological fluorosis admitted to hospital. The disease was far more common in labourers and farmers who do hard manual work and carry heavy loads on the head; this perhaps is one of the factors accounting for a severe type of disease in men and the high incidence of neurological and crippling fluorosis.

### Chemical Composition of Water

Besides the fluoride content, a number of other constituents in water are important, such as calcium, magnesium, and alkalinity. We found that fluoride concentration bears an inverse relation to total hardness and calcium hardness. In areas having low fluoride content the water was harder. The incidence of endemic fluorosis depended to a great extent on the hardness of water. This point was nicely illustrated by comparing the analysis of water constituents from the villages of Bhikki and Rajthal (Table V). In these two villages the water has the same fluoride content but the incidence of endemic fluorosis varies widely. The other factors—nutritional status, climatic effect, duration of fluoride exposure, sex, profession, etc.—were identical; the only difference was in calcium hardness and magnesium hardness.

In America most of the water containing fluoride is hard water and the fluoride/hardness ratio is more than 1 in 500, but in our study the fluoride/hardness ratio was much less (Table VI). It is therefore clear that both the calcium and the magnesium content have a protective influence on the absorption of fluoride and its subsequent deposition in the skeleton (Marier *et al.*, 1963). In India the lower toxic limits of fluoride may be related to water that is less hard, as shown in the analysis of water constituents in some of the endemic areas.

TABLE VI

Village	F : Total Hardness	F : Ca Hardness	F : Alkalinity	Ca : Alkalinity
Normal .. ..	1 : 1025	1 : 340	1 : 97	1 : 2.8
Gharachaon ..	1 : 167.8	1 : 51	1 : 420	1 : 8.2
Laluwala ..	1 : 48	1 : 20	1 : 332	1 : 16.6
Dhalpai ..	1 : 125	1 : 36	1 : 251	1 : 6.9
Bhodipura ..	1 : 96	1 : 24	1 : 162	1 : 6.6
Rajthal ..	1 : 182	1 : 108	1 : 159	1 : 1.4
Bhikki ..	1 : 41	1 : 17	1 : 91	1 : 6.4
Sanghera ..	1 : 66	1 : 22	1 : 128	1 : 5.6
Ramuana ..	1 : 20	1 : 5	1 : 194	1 : 32.1
Gangulabsingh ..	1 : 4	1 : 2	1 : 101	1 : 60
Khara ..	1 : 10	1 : 3	1 : 40	1 : 11.5

### Nutritional Status

It has been alleged that the higher incidence of endemic fluorosis in India is partly related to malnutrition, because no case of fluorosis has been detected in other parts of the world with a similar fluoride concentration—for example, Texas (Leone *et al.*, 1954). Our studies in Punjab have not borne this out, because that State is the best-nourished area in India and yet has the highest incidence of fluorosis. However, this needs further elucidation. It has recently been shown by calcium balance studies (Srikantia and Siddiqui, 1965) that cases of fluorosis retain larger amounts of fluoride, and this increased retention is due to lower excretion both in the urine and in the faeces.

### Climatic and Geological Factors

Cases of endemic fluorosis were observed only from those areas where the soil was sandy and the climate was hot and dry. The average temperature during summer was above 100° F. (37.8° C.) and the rainfall was rather scanty. Moreover, cases of fluorosis were observed only in those villages where the sub-soil water was rather superficial. In deeper wells the fluoride concentration of water was not so high; this has been suggested to be a preventive factor.

### Fluoride from Food and Beverages

Besides the fluoride ingested in drinking-water, considerable quantities of fluoride may be ingested from foods grown in soil rich in fluoride, from tea, and from wines (Soriano and Manchón, 1966). We have still not precisely evaluated these factors in our endemic areas, but it is quite possible that they may aggravate fluoride intoxication, because the villager of Punjab consumes a great deal of tea and alcoholic drinks.

### Method for Fluoride Estimation in Water

#### Reagents

**Standard Sodium Fluoride Solution.**—Dissolve 0.221 g. of sodium fluoride in distilled water and dilute to 1,000 ml. Dilute 100 ml. of this stock solution to 1,000 ml. with distilled water; 1 ml. is equivalent to 0.01 mg. of fluoride.

**Zirconium Alizarin Solution.**—Dissolve 0.3 g. of zirconium oxychloride octahydrate in 50 ml. of distilled water in a one-litre glass-stoppered flask. Dissolve 0.07 g. of sodium alizarin monosulphonate (alizarin red S) in 50 ml. of distilled water and pour slowly into the zirconium solution while stirring. The resulting solution clears on standing for a few minutes.

**Mixed Acid Solution:** Dilute 101 ml. concentrated HCl to 400 ml. with distilled water. Add carefully 33.3 ml. concentrated H<sub>2</sub>SO<sub>4</sub> to 400 ml. of distilled water. After cooling, mix the two acids.

**Acid Zirconium Alizarin Reagent.**—To the clear zirconium alizarin solution in the one-litre flask add the mixed acid solution, add distilled water up to mark of one litre, and mix. The reagent changes in colour from red to yellow within an hour and is then ready for use. If stored away from direct sunlight the reagent is stable for at least six months.

**Sodium Arsenite Solution.**—Dissolve 5 g. of sodium arsenite and dilute to 1 litre with distilled water.

#### Procedure

**Sample Pretreatment.**—If the sample contains residual chlorine, remove it by adding 1 drop (0.05 ml.) of arsenite for each 0.1 mg. of chlorine and mix.

**Preparations of Standards.**—Prepare a series of standards by diluting various volumes of standard fluoride solution (1 ml. = 0.01 mg. of fluoride) to 100 ml. in Nessler tubes. The standards should be chosen so that there is at least one with lower and one with higher fluoride concentration than the unknown sample. An interval of 0.05 mg./l. is usually sufficient.

**Colour Development.**—Adjust the temperature of samples and standards so that the deviation between them is no more than 2° C. A temperature near that of the room is satisfactory. A constant temperature should be maintained throughout the determination. To 100 ml. of the clear sample or an aliquot diluted to 100 ml. and to the standards in Nessler tubes add 5 ml. of the acid zirconium alizarin reagent from a volumetric pipette. Mix thoroughly and compare the sample and standards after one hour.

$$\text{Calculation: mg./litre fluoride} = \frac{\text{mg. F} \times 1,000}{\text{ml. sample}}$$

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