



RESEARCH ARTICLE

FLUOROSIS INDUCED ANEMIA IN FLUORIDE ENDEMIC ZONE OF BANKURA DISTRICT, WEST BENGAL, INDIA

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ABSTRACT

Fluorosis is a major public health problem in India. The present cross-sectional observational study has been conducted in the Hirbadh block, one of the fluoride endemic zones of Bankura district, West Bengal, India, on 77 subjects by using simple random sampling in which 41 were cases for evaluating the role of fluorosis in development of anemia. The blood and urine samples were collected from the cases and serum and urinary fluoride levels were estimated by Ion-Meter, blood hemoglobin levels were estimated by Sahli's Hemoglobinometer and then were compared with age and sex matched controls. The serum and urinary fluoride were found to have statistically significant relationship with hemoglobin in the cases. The serum and urinary levels of fluoride were higher in the cases compared to the comparison group, while the hemoglobin levels were much less in cases than in controls. We found that 21.43% were suffering from mild anemia and 71.43% had moderate anemia of all female cases. While 29.63% had mild anemia and 62.96% had moderate anemia of total male cases.

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INTRODUCTION

Fluorosis is an important public health problem many parts of the world including India. Of the 85 million tons of fluoride deposits on the earth's crust, 12 million are found in India (Teotia, 1984). Hence it is natural that fluoride contamination is widespread, intensive and alarming in India. It has been estimated that the total population consuming drinking water containing elevated levels of fluoride is over 66 million (FRRDF, 1999). Endemic fluorosis resulting from high fluoride concentration in groundwater is a public health problem in India (Kotecha et al., 2012). The recommended level of fluoride in drinking water in India is 0.5 to 0.8 mg/L (Park K 2011). The available data suggest that 15 States in India are endemic for fluorosis (fluoride level in drinking water >1.5mg/L), and about 62 million people in India suffer from dental, skeletal and non-skeletal fluorosis. India was one of the worst fluorosis affected countries, with large number of people suffering as a large number of Indians rely

On groundwater for drinking purposes and water at many places is rich in fluoride (https://www.novapublishers.com/catalog/product_info.php?product_id=15895). World Health Organization (WHO) has set the upper limit of fluoride concentration in drinking water at 1.5 mg/L (Andezath et al., 2000), and the Bureau of Indian Standards, has laid down as 1.0 mg/L as the maximum permissible limit of fluoride (https://www.novapublishers.com/catalog/product_info.php?products_id=15895). The normal upper limit of fluoride in urine is 0.1mg/L and normal upper limit for fluoride in serum is 0.02 mg/L (Susheela, 2005). In Bankura District out of 22 blocks 17 blocks are affected with dental and non-skeletal fluorosis symptomatic cases (National programme for prevention and control of fluorosis bankura, 2015). It is known when fluoride is ingested; it will also accumulate on the erythrocyte membrane, which in turn loses calcium content. This change causes formation of echinocytes. The life span of this echinocytes is less than the normal life span of RBC, and hence early destruction of RBCs in form of echinocytes causes anaemia. The membrane which is deficient in calcium content, is pliable and is thrown into folds. The RBCs attain the shape of an amoeba with pseudopodia like folds projecting in different directions. Such RBCs are termed as Echinocytes.

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The Echinocytes will be found in circulation in large numbers, depending upon the extent of fluoride poisoning and duration of exposure to fluoride. RBCs, in human, although have a life span of 120-130 days, the echinocytes undergo phagocytosis (eaten-up by macrophages) and are eliminated from circulation. This would mean that RBCs in individuals exposed to fluoride poisoning, shall not live the entire life span, but are likely to be eliminated as echinocytes. This would lead to low hemoglobin levels in patients chronically ill due to fluoride toxicity (RGNDWM, 1993). So aim of the study was to determine the hemoglobin levels in patients with endemic fluorosis to check the effect of fluorosis on it and the occurrence of anemia among the fluorosis affected people.

MATERIALS AND METHODS

Study area: This cross-sectional observational study was conducted in Hirabandh block of Bankura district. The tests were carried out at department of Biochemistry, B.S. Medical College, Bankura (District fluorosis detection laboratory).

Study subject: 77 subjects were selected by simple random sampling among them 52 were male and 25 were female. In that particular block, the endemic fluorosis was diagnosed according to the clinical diagnosis criteria, as described by Wang *et al.* (1994). The criteria for study are (1) people living in the endemic fluorosis region since birth, (2) having mottled tooth enamel, indicating dental fluorosis, (3) consuming water with fluoride levels above 1.2 mg/L (normal 1 mg/L), and (4) a urine fluoride level greater than 1.5 mg/L. Control subjects living in the same area without showing above mentioned signs were designated as control.

We have chosen the subjects from age 5 to age 65 years.

The exclusion criteria

- History of hematological or other malignancy,
- History of any active infection,
- History of any chronic illness such as chronic kidney disease or chronic liver disease,
- Pregnant women.
- Severely malnourished individuals.

The case and the control subjects were screened and physically examined and information regarding demography, nature of illness were collected from them using a predesigned and pretested questionnaire. Informed consent was taken from each of the participant. The study design was approved by institutional ethical committee.

Study Period: This study was conducted from January 2016 to May 2016.

Laboratory investigations: The blood and urine samples were collected from the 77 subjects and stored at -20°C before analyzing. Urinary and serum fluoride levels were estimated by Ion Meter (ORION-9609BNWF). Estimation of blood hemoglobin levels was done by Sahli's Hemoglobinometer.

Statistical analysis

The data were compiled in MS excel and analyzed by different statistical methods. Data display was done by charts and tables. Data were described by proportion, mean, SD, range etc. Statistical tests like independent 't' test, Pearson's correlation coefficient (r) etc. were used to explore the relationship between variables. P value of <0.05 was considered significant to discard the null hypothesis at 5% precision and 95% confidence interval.

RESULTS

The average age of the participants was 37.64 ± 3.93 (mean \pm SD) years with a range of 60 years. Out of 77 participants 25 were female and rest were male. The sex wise distribution of the cases and controls revealed clearly that men were dominant, 27 out of 41 in case group i.e. 65.85% and in control group it was 69.44% (25 out of 36) (Figure 1).

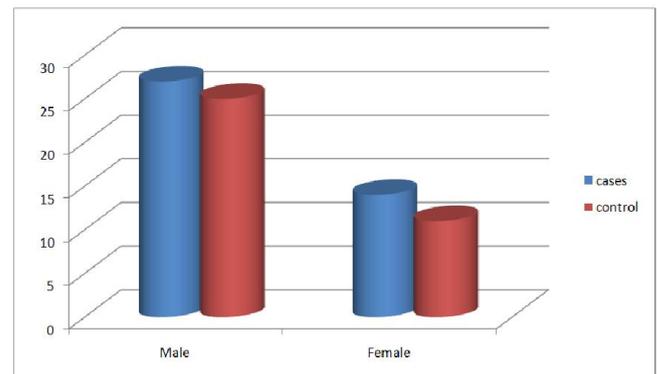


Figure 1. Distribution of study subjects according to gender

The case group was found to have higher average serum and urinary fluoride levels as well as blood hemoglobin level compared to that of the comparison group i.e. control group and the difference was statistically robust (Table 1).

Table 1. Levels of Haemoglobin and Fluoride (Urinary and Serum) in Cases and control

S.No	Parameters	Control (n=36) (Mean \pm SD)	Fluorosis subject (n=41) (Mean \pm SD)	P Value	Significance
1	Haemoglobin (g/dL)	13.78 \pm 0.67	10.75 \pm 0.55	0.006	Highly Significant
2	Serum Fluoride (mg/L)	0.0331 \pm 0.0029	0.2475 \pm 0.0018	0.017	Significant
3	Urinary Fluoride (mg/L)	0.8019 \pm 0.1677	1.8778 \pm 0.4015	0.000	Highly Significant

Table 2. Relationship between the serum and urinary fluoride level wit hemoglobin level among cases

Parameter	Group	Correlation coefficient (r)	Significance (p value)
Serum fluoride Vs Hemoglobin	Case	0.532	0.000
Urinary fluoride Vs Hemoglobin	Case	0.702	0.000

** . Correlation is significant at the 0.01 level (2-tailed).

The serum and urinary fluoride were found to have a statistically significant positive linear relationship with hemoglobin among the subjects belonged to the case group (Table 2). The age wise distribution of serum fluoride showed that serum fluoride level was highest in age group 26-35 years which group has also minimum blood hemoglobin level (Table 3).

Table 3. Mean serum fluoride and hemoglobin levels in different age groups level among cases

S.No.	Age in years	Serum fluoride level in cases in mg/L (Mean± SD)	Hemoglobin level in cases in g/dl (Mean± SD)
1	5-15	0.2245 ± 0.0014	11.80 ± 0.48
2	16-25	0.2400 ± 0.0009	10.43 ± 0.35
3	26-35	0.3048 ± 0.0021	09.98 ± 0.95
4	36-45	0.2575 ± 0.0022	11.37 ± 0.23
5	46-55	0.2321 ± 0.0017	10.89 ± 0.76
6	56-65	0.2261 ± 0.0032	10.03 ± 0.53

According to WHO guide line for defining anemia, only 5 -15 years of age group was able to escape from including within the anemic group. Rest of the age groups irrespective of sex was included as anemic (Table 4).

Table 4. Age wise and sex wise distribution of hemoglobin level in cases

Age in years	Sex		Hemoglobin in g/dL									
	M	F	> 13		12 – 12.9		11 – 11.9		10 – 10.9		< 10	
			M	F	M	F	M	F	M	F	M	F
5 - 15	2	1	0	0	1	0	1	0	0	0	0	0
16 - 25	5	3	0	0	0	0	0	0	5	3	0	0
26 - 35	9	5	0	0	0	0	0	0	4	3	5	2
36 - 45	7	2	0	0	0	0	7	2	0	0	0	0
46 - 55	2	2	0	0	0	0	1	1	1	1	0	0
56 - 65	2	1	0	0	0	0	0	0	2	0	0	1

So, the occurrence of anemia in fluorosis endemic zone is very high i.e. 38 out of 41(92.68%).

DISCUSSION

India is one of the worst fluorosis affected country. Fluorosis is a major health problem in many parts of the world now a day. Many studies were conducted on effect of fluoride on hematological parameters in experimental animals; a few studies have been conducted to investigate the effects of chronic fluorosis on hematological parameters in human subjects with endemic fluorosis Hb is a hemoprotein with a primary function of transporting oxygen from the lungs to the body tissue. In human anemia has multiple cutoff values for different age groups- in children of 5-11 years is is < 11.5 g/dL, in children 12-14 years it is < 12 g/dL, in non-pregnant women it is < 12 g/dL, in pregnant women it is < 11 g/dL and in adult male it is < 13 g/dL (WHO). Some research has been published that excessive fluoride developed anemia and eosinophilia and some studies showed no influence of fluorosis on various indices of hematopoiesis. A study conducted by Karadeniz *et al*, showed that a significant fluoride associated decrease in RBC and WBC counts, along with decrease the values of haematocrit, and the levels of hemoglobin in rats. The fluorosis-induced anemia observed in that study might be

resulted from inhibition of globulin synthesis (Obrig *et al.*, 1971), depression of erythropoiesis or a decrease in the level of blood folic acid (Cetin *et al.*, 2004 and Hoogstraten *et al.*, 1965). In a study done by Machalinski *et al* observed that sodium fluoride had marked negative effects on hematopoiesis. Choubisa *et al.*, (Choubisa *et al.*, 1996) also found that, decreased RBC and hemoglobin were present in an endemic fluorosis zone. Hillman *et al*. reported that cattle afflicted with fluorosis developed anemia and eosinophilia. But Uslu (Uslu, 1981) did not find anemia in rats after 45 days of exposure to 30 and 100 ppm fluoride in their drinking water. In our study we observed that serum fluoride and urinary fluoride levels were more in cases than controls where as the hemoglobin level in cases was lower than that of controls. The serum and urinary fluoride levels had a strong positive correlation with the hemoglobin level in cases. So, in our study we have found that fluorosis can affect hematological system resulting in anemia. Age group 26-35 years has also minimum blood hemoglobin level along with highest level of fluoride level among the all age groups. We found that 21.43% of all female cases were suffering from mild anemia (11 – 11.9 g/dL) and 71.43% of total female had moderate anemia (8 – 10.9 g/dL).

While 29.63% of total male cases had mild anemia (11 – 12.9 g/dL) and 62.96% of total male cases had moderate anemia (8 – 10.9 g/dL).

Conclusion

Our study suggested that the increased serum and urinary fluoride concentration can damage hematological system resulting anemia. Hence general awareness should be generated to the people for using fluoride free drinking water and further studies are required for determining the proper effect of fluorosis on hematological system in human being.

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