# Fluorosis is linked to anaemia

# Andezhath K. Susheela<sup>1,\*</sup>, Nisith Kumar Mondal<sup>1</sup>, Rashmi Gupta<sup>1</sup>, Mohini Sethi<sup>2</sup> and Ravindra Mohan Pandey<sup>3</sup>

<sup>1</sup>Fluorosis Foundation of India, B-1, Saransh Apartment, 34-I. P. Extension, Patparganj, Delhi 110 092, India
 <sup>2</sup>S-150 (First Floor), Greater Kailash-II, New Delhi 110 048, India
 <sup>3</sup>Department of Biostatistics, All India Institute of Medical Sciences, New Delhi 110 029, India

We report here a simple, easy-to-practice treatment procedure for anaemia, by focusing on withdrawal of fluoride consumption and promotion of nutrients through diet. The approach to improve nutrient intake as supplementation of iron and folic acid or iron tonic does not yield beneficial results. The reason being highly destructive F<sup>-</sup> enters the body through food, water, habit forming substances and dental products destroys the lining of the intestine and prevents absorption of nutrients. Testing of Hb, F<sup>-</sup> in urine, drinking water and body mass index are necessary to assess the problem. The study was made on 2420 adolescent students from 6 schools in Delhi. F<sup>-</sup> removal through diet editing and improved nutrients through counselling without prescription of drugs led to correction of anaemia. This treatment procedure is beneficial to pregnant and lactating mothers and patients in hospital OPDs due to ill health issues aggravated due to low Hb/anaemia.

**Keywords:** Anaemia, fluoride toxicity, school children, treatment procedure.

ANAEMIA is a serious health problem among school children. The National Family Health Survey (NFHS-4) results showed that over 58% of children below 5 years of age were anaemic<sup>1</sup>. Hence they suffer from insufficient haemoglobin (Hb) in blood, get exhausted and become vulnerable to infections; brain development is possibly affected. The NFHS-4 survey also revealed that 38% of children in the same age group were stunted (low height to age), 21% wasted (low weight to height) and 38% underweight (low weight to age). There is a linkage between underweight children and anaemia in mothers during pregnancy. The survey showed over half of all pregnant women were anaemic. Keeping in view the seriousness of the problem, a scheme implemented by the Government of India (GoI) in 2012 began addressing the issue by weekly iron and folic acid supplementation (WIFS) to cover 12 crore adolescents across the country. The efforts in raising Hb by iron and folic acid (IFA) supplementation, midday meal schemes and providing iron-fortified food through State and National Programmes are yet to yield desirable results.

Our major concern is that fluoride (F<sup>-</sup>) ingestion through water and food may cause serious health problems as India is a severely endemic nation for fluorosis<sup>2</sup>. Excess ingestion of F<sup>-</sup> is one of the major reasons for causing anaemia during pregnancy, resulting in low-birthweight babies<sup>3,4</sup>. Non-absorption of nutrients, including IFA supplementation is due to F<sup>-</sup> causing structural derangements of the gastrointestinal mucosa<sup>5-7</sup>. Fluoride causes biochemical derangements as the element fluorine is a powerful enzyme inhibitor, hormone disruptor and neurotoxin<sup>8,9</sup>. Hillman et al.<sup>10</sup>, as early as 1979, showed the presence of anaemia in cattle as a result of F<sup>-</sup> toxicity and fluorosis. They also showed reduced serum folic acid and vitamin  $B_{12}$ , which are essential constituents for Hb biosynthesis due to F<sup>-</sup> poisoning. Inadequate thyroid hormone production  $(T_3, T_4)$  resulted in low red blood cells (RBCs) production. Therefore, on these counts excess consumption of F<sup>-</sup> is a cause for concern. The aim of this article is to highlight a simple treatment procedure for anaemia through reduced consumption of F- from drinking water and food. Simultaneously promotion of nutrients through diet was followed.

#### Materials and methods

The study was conducted in six schools in the National Capital Territory of Delhi (NCTD), involving 2420 students both boys and girls, in the age group 10-19 years and studying in classes VI-X. The schools that catered to children from the lower socio-economic strata of society were chosen from east and south Delhi. Children of low income group can be addressed effectively to treat anaemia, then it can be a lot more easier to extend the protocol to children of high income group schools. The schools were selected based on the following procedure: Letters were written to the principals of the schools, to ascertain their willingness to participate in the project. Those who responded and sought more details were contacted. At a time convenient to the students, teachers and management, a talk on 'Anaemia: control and prevention without iron tablet supplementation' was delivered. With the overwhelming response received, the project was launched in those schools where the principal provided a

<sup>\*</sup>For correspondence. (e-mail: frnrdf.aks@gmail.com)

'No objection certificate'. A coordinator was nominated by the principal in each school to facilitate the activities. Two pre-requisites to be fulfilled were placed before the principals. First, the students were dewormed and the school provided us with a class-wise list of students dewormed during the preceding one year was provided. Second, informed consent of the parents was obtained through a note prepared in Hindi provided to the class teachers. This was explained to the students, and they were directed to inform their parents to sign or affix thumb impression on the consent form. The students returned the forms to the investigating team.

## Inclusion criteria

Students who were anaemic, dewormed and whose consent along with those of their parents have been obtained.

## Exclusion criteria

Students who were unwell or on medication, and those who did not obtain consent of the parents to join the study group.

# Haemoglobin estimation

This was done by the investigating team at the Fluorosis Foundation of India, New Delhi using a portable digital hemoglobinometer (HemoCue 201<sup>+</sup>, Angelholm Sweden)<sup>11,12</sup>.

# Fluoride estimation of urine

Fluoride in fresh urine samples of students collected in plastic bottles was tested at the Foundation using the potentiometric method with  $F^-$  ion specific electrode technology<sup>13</sup>. Results were reported in milligrams per litre. Girls who had their menstrual period were directed not to provide urine samples.

# Fluoride estimation in drinking water

A cup of water (approx 50 ml) collected in a plastic bottle directly from domestic water source and not from collected or stored water, was tested for fluoride content. Besides, the school water sample was also tested for fluoride using an ion meter with a  $F^-$  ion specific electrode. Results were expressed in milligrams per litre.

# Nutrition education to mothers

Nutrition education was imparted to mothers of students class-wise in the presence of their children and class

teachers during the parent-teacher meetings (PTMs). An additional time slot was granted for addressing the issues.

The nutrition education session was conducted as described below.

- Information about the health status of children in terms of Hb level, complaints of ill-health such as cold, cough, fever, leave of absence from the school record was provided to the parents (mothers).
- Information on the prevailing infrastructure of the kitchen at home, dietary habits, methods of cooking and information on food items served for breakfast, lunch and dinner in the family was retrieved from the mothers. The quantity of food consumed and frequency of food served at home were also ascertained.
- Changes in the diet introduced following discussions with the mothers also explained how the modified diet would improve Hb levels of children and subsequently their general well-being, performance in school and extra-curricular activities.
- It was noted every family possessed a pressure cooker, a grinding stone (silbatta) for making masala, chutney, etc. and a churner (also known in local language as muddler/mathini/ghotni/phirni) used in earlier times for making lassi when electrical appliances were not in vogue. The concept was introduced that with these three gadgets, the tastiest food could be prepared at home.

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Discussions were held about different recipes for breakfast that are simple, nutritive and can be easily prepared at home. The mothers were shown pictures of ready-to-serve food items, like aloo paratha with dahi, uppma with dhania chutney, etc. which they learnt to prepare easily. Pictures of various inexpensive but nutrient-rich raw vegetables and fruits were also shown, which could be served as salad with lunch. It was suggested that vegetables like beetroot, raw papaya, carrot and peas could be steamed and used with salad dressing to enhance the taste. The salad dressing was prepared by mixing lime juice with water in the ratio 1:3, with addition of crushed garlic, salt and sugar to taste. Similarly, it was suggested that for dinner, mixed vegetable soup be served. Soups could be prepared with tender leaves of beetroot, radish and carrot, that are normally discarded, along with other inexpensive and nutrient-rich vegetables, viz. bottle guard (lokhi), pumpkin, etc. These are pressure-cooked, blended using a churner, and a cup of milk added to the soup along with salt and pepper to taste. The mixed-vegetable soup must be served an hour prior to dinner. The proffered recipes for breakfast, lunch and dinner ensure maximum daily intake of vegetables and fruits. They are rich in nutrients, affordable and can be consumed on a daily basis. Students were advised to avoid consuming food from street vendors as they might be unhygienically

prepared and are likely to have toxic contaminants. Home-cooked food is the best option for improving health. For snacks, the students were advised to eat chutney sandwiches, cooked vegetables rolled in soft chapatis, steamed corn, home-made 'momos' using wheat flour (instead of maida). The terms 'soup', 'salad' and 'sandwich' were demystified at the very outset. Thus the stage was set with ample confidence among the parents for a better dietary approach for improving the health of their children.

#### Intervention for improving haemoglobin

#### Diet editing

Mothers, students and teachers were advised to avoid consumption of F<sup>-</sup> from all sources. It was pointed out that water samples from the schools and homes were tested for F<sup>-</sup> and found safe for consumption with F<sup>-</sup> < 1.0 mg/l. It was also suggested that street food and beverages containing black rock salt, such as soft drinks (jaljeera), water with lemon squeezed, salty snacks, churans and ready-made masala packets containing black rock salt (CaF<sub>2</sub>) should be avoided as they are highly toxic  $(F^{-}=157 \text{ ppm})^{14}$ . Fluoridated toothpaste was to be replaced with herbal toothpastes which are with less Farising as an impurity from the raw materials used for manufacture. Harmful effects of F- ingestion result in various health problems in students, like high blood pressure, renal failure and diabetes. Anaemia during pregnancy was explained in detail<sup>4</sup> as well as the consequences of low-birth-weight babies born, so that adolescent girls understood the importance of maintaining Hb > 12.0 g/dl.

#### Diet counselling

This was carried out to ensure that students received a reasonably good diet with adequate proteins, carbohydrates through cereals, essential nutrients, micronutrients, antioxidants and vitamins through dairy products, vegetables and fruits. While cooking vegetables and lentils, it was recommended to use garlic, ginger, onion and green chilies as spices instead of the readymade powdered spices with black rock salt. To ensure adequate vitamin B<sub>12</sub> production in the gut, consumption of probiotic-rich curd, lassi and/or chaach was suggested.

Mothers were appraised on various cooking procedures which preserved nutrients, compared to other rigorous conventional methods. Steaming, sautéing, grilling over coal, roasting and pressure cooking were recommended. The necessity to avoid oil-rich fried food was emphasized. It was stressed that breakfast should have adequate proteins, carbohydrates and fats. Pictorial depictions were shown to make the mothers understand the final dishes/ meals that they served their children and family. A pictorial booklet in Hindi, carrying information on inexpensive fruits and vegetables, high in nutritive value and recipes for a healthy diet, was provided on the day of counselling. The children read and explained to their parents the importance of nutritive diet. Students, particularly girls, were advised to increase their daily intake of food from 2 to 3 or 4 times. There was enthusiastic participation from students, parents and teachers in the discussions leading to changes in their lifestyle.

The mothers were convinced of the impact of simple procedures adopted to address ill-health among children and other members of their family, as they spoke about it during the counselling sessions. Nutritional education was tailor-made to the needs of the poorer sections of society and for those who were illiterate. The interventions practised and the impact were reassessed at intervals of 1, 3 and 6 months. Each time, when rise in Hb followed by reduction in urine fluoride level (UFL) was recorded, it was explained to the students and teachers.

#### Body mass index

The results obtained regarding body mass index (BMI), for boys and girls, age-wise were assessed and recorded.

#### Monitoring and impact assessment

After introducing the interventions to the sample and control groups, the students were monitored three times at an interval of 1, 3 and 6 months by measuring Hb and UFL. However, BMI was assessed at baseline and 6 months post-intervention and results reported for girls and boys separately<sup>15</sup>.

#### Other factors

The yardsticks used for socio-economic status were as follows: (i) Literacy status of the parents (both father and mother): illiterate/matriculation/graduate/postgraduate. (ii) Occupation: unskilled/skilled/factory worker/not working. (iii) Total members in the family: parents/ children/grandparents/any other members. (iv) Living in a developing colony/slum/village. (v) Monthly income (Rs) of the earning members of the family was ascertained to understand the capacity of the family to spend on food.

#### Statistical analysis

The data were analysed using Stata 11.2 software and presented as mean  $\pm$  SD and frequency (%). Data analysis was done using two methods (i) 'Intention-to-treat' (ITT) analysis (a method of analysis for randomized trials in which all subjects/individuals randomly assigned to one of the treatments/interventions are analysed together,

regardless of whether or not they completed that treatment/intervention). (ii) 'Per protocol' (PP) analysis (a method which restricts the comparison of treatments/ interventions to the ideal subjects/individuals, i.e. those who adhered perfectly to the clinical trial instructions as stipulated in the protocol). Change within the continuous variable was assessed by repeated measure ANOVA. Categorical variables were compared by chi-square/ Fisher's exact test. *P*-value <0.05 was considered as statistically significant.

As ITT analysis is recommended as the least biased method to estimate intervention effects in randomized trials, results obtained through ITT analysis are reported here, although similar results were also obtained in PP analysis.

#### **Results and discussion**

The results of 2420 boys and girls in the study group investigated for Hb followed by UFL, drinking water  $F^-$  level (DWFL) and BMI are reported. Table 1 shows the study design used here.

The basic characteristics of the six schools chosen which were categorized as sample (n = 4) and control (n = 2) schools have certain commonalities on socioeconomic status of the families.

Table 2 shows the results obtained for Hb during baseline survey and prevalence of anaemia in boys and girls. Table 3 shows the Hb status subsequent to practice of the two interventions at the three intervals. Hb in sample group (n = 445) ranges from 5.8 to 12.0 g/dl. The maximum rise in Hb after practice of interventions at the three intervals, i.e. 1, 3 and 6 months is 13.5, 14.2 and 14.7 g/dl respectively. The *P* value is highly significant at <0.001.

The students in the two groups were further categorized as follows: (i) non-anaemic, i.e. Hb > 12.0 g/dl, (ii) moderate and mildly anaemic, with Hb ranging from 7.0 to 12.0 g/dl, and (iii) severely anaemic, i.e. Hb < 7.0 g/dl(Table 4). During commencement of the study, in sample group under non-anaemic category, there were no students (0%). In the moderate and mildly anaemic category, there was 98% and severely anaemic there was 2% students. The results obtained 1 month post-intervention revealed that the number of students in the non-anaemic category increased from nil (0%) at baseline to 29.5%; 3 month post-intervention the increase in the number of students with raised Hb levels was 35.8% and 6 month post-intervention, it was 45.3%. In the moderate and mild category, 1 month post-intervention the number of students reduced from 98% to 70.2%; after 3 months the number further reduced to 64% and after 6 months to 53.2%. Among the students with severe anaemia, reduction in their number one month post-intervention was from 2% to 0.3%; 3 month post-intervention to 0.2%; and 6 month post-intervention 1.5% respectively. The rise in Hb levels category (i) is as a result of reduction in categories (ii) and (iii) respectively. The *P*-value is significant at < 0.0001.

Figure 1 shows the non-anaemic students with Hb > 12.0 g/dl at baseline being '0', the rise in Hb (%) postintervention at 1, 3 and 6 months are shown in the students in 4 sample and 2 control group schools. The rise in Hb levels in the range 12-14.7 g/dl was observed in a high percentage of the students (41-57), which was achieved merely by improving the diet. In the control group students, Hb did not increase beyond 21.4%-27%. The hypothesis that diet editing to withdraw toxic fluoride consumption and strengthening the diet with nutrients appears to be a promising avenue to address anaemia. The success of the approach depends upon effective diet editing and diet counseling considering the requirements of the target group and total involvement of the family as they appreciated the benefits accrued to the children and other members.

The UFL status in 445 sample group students at baseline was  $1.39 \pm 0.46$  mg/l (mean  $\pm$  SD). Subsequent to practice of the two interventions at three intervals (1, 3 and 6 months) the UFL mean value reduced to 1.13, 0.99 and 1.02 mg/l respectively (Table 5). The UFL in sample group (n = 445) ranged from 1.01 to 3.75 mg/l. The *P*value was significant at <0.001.

The students in the sample and control groups were further categorized as follows: (i) those having UFL > 1.0 mg/l and (ii) those having UFL < 1.0 mg/l. In sample group 100% students were in category (i) and 0% in category (ii) (Table 6). Among the 340 sample group students who participated in the interventions, 1 month postintervention 42.4% students had UFL >1.0 mg/l, whereas in 57.6% students UFL reduced to <1.0 mg/l. The results recorded for UFL 3 months post-intervention, among 408 students who participated, 44.1% had UFL >1.0 mg/l, whereas 55.9% students had reduced UFL <1.0 mg/l. It was also reassessed 6 months post-intervention among students who participated; 35.5% had UFL 397 >1.0 mg/l, whereas in 64.5% students UFL had reduced <1.0 mg/l. These observations recorded are in contrast to control group students.

Figure 2 shows the percentage reduction of UFL school-wise in students of all six schools following postintervention and assessed at three intervals. The lowest percentage reduction 34.5% was found that 3 months post-intervention among students of school 2. The highest percentage reduction 79% was found 6 months postintervention among students of school 3. High compliance in practising the intervention was thus evident.

Figures 3 and 4 show results obtained for BMI. At age 10, girls (n = 7) and boys (n = 6) in the control group had mean BMI 16.2 and 13.9 respectively. Girls had higher BMI compared to boys. In the case of girls and boys in sample group the difference in BMI was maintained but certain peaks in the graph appear at a particular age. Girls

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·	Table 1.         The study design	
Study group comprising of 2420 students from six schools in Delhi, India	Sample schools $(n = 4)$ (Students from sample schools were considered as sample group)	Control schools $(n = 2)$ (Students from control schools were considered as control group)
Total no. of students screened for haemoglobin (Hb)	1815	605
Total no. of students with low Hb (<12.0 g/dl) and prevalence of anaemia (%)	907/1815 (50.0%)	345/605 (57.0%)
Total no. of anaemic students with high urine fluoride level (UFL), i.e. F <sup>-</sup> > 1.0 mg/l (%)	479/860* (55.7)	225/326* (69.0)
All drinking water samples from home and school tested for fluoride content (%)	410/479** (85.6)	214/225** (95.0)
Range of fluoride content in drinking water samples (mg/l)	0.114-0.873	0.014-0.985
Interventions introduced after baseline study		
<ul><li>(i) Diet editing (withdrawal of fluoride intake from all sources)</li></ul>	Diet editing: introduced	Diet editing not introduced, i.e. no withdrawal of fluoride
<ul> <li>(ii) Diet counselling (enhanced intake of essential nutrient through daily diet)</li> </ul>	s Diet counselling: introduced	Diet counselling: same as sample group
Reassessment of students carried out post-intervention by rechecking Hb, UFL and body mass index (BMI) based	Hb and UFL after 1, 3 and 6 months	Hb and UFL after 1, 3 and 6 months
on sex and age	BMI after 6 months only	BMI after 6 months only

\*Urine sample of girl students during menstrual period not collected.

\*\*Many families were sharing public water sources (viz. hand pump, tube well and municipal supply).

Schools had either one or two sources of water for consumption.

Table 2. Prevalence of anaemia in boys and girls among the students of six schools

	Total students screened for anaemia $n = 2420$	Total number of boys $n = 876$	Total number of girls $n = 1544$
Prevalence of anaemia (%)	50.3	38.6	56.9
Prevalence of anaemia (%): range	37–63	30–56	48–69

All students participated in baseline screening for Hb, but not for post-intervention assessment due to vacation, festivals and personal reasons.

Table 3. Haemoglobin of the sample and control student groups at baseline and post-interventions at intervals of 1, 3 and 6 months

			Hb post	-intervention at inte	ervals of	
Group	Hb level (g/dl)	Hb at baseline	1 month	3 months	6 months	<i>P</i> -value within the group
Sample* (n = 445) Control (n = 225)	Mean ± SD (range) Mean ± SD (range)	$11.1 \pm 1.1 (5.8-12.0) 11.2 \pm 1.0 (7.0-12.0)$	$11.3 \pm 1.2 \\ (5.8-13.5) \\ 11.2 \pm 1.1 \\ (7.0-13.1)$	$11.5 \pm 1.2 \\ (6.8-14.2) \\ 11.5 \pm 1.1 \\ (6.1-14.0)$	$11.7 \pm 1.4 \\ (5.7-14.7) \\ 11.3 \pm 1.2 \\ (6.1-14.2)$	<0.0001 (Significant) 0.02 (Significant)

SD, Standard deviation; \*Among the 479 anaemic students reported only 445 participated as 34 students were absent on the day of assessment due to festivals/vacation.

Statistical test applied: ANOVA.

in sample group, 6 months post-intervention, had high BMI at the age 16–17 years. Among girls there was a steady increase in BMI from age 10 onwards, with a peak BMI recorded at age 16–17 years; BMI ranged from 19.9 to 20.1. The peak in BMI for boys post-intervention was at age 14–16 years; BMI ranged from 19.9 to 26.5. BMI data revealed high intake and absorption of nutrients, and corrected under-nutrition to a large extent both in girls and boys.

When boys and girls in the sample and control groups were stratified into early adolescent (10-13 years), mid

adolescent (14–17 years) and late adolescent (17–19 years), the mean increase in BMI was found to be statistically insignificant.

The elimination of  $F^-$  and consumption of nutritive diet that included iron through cereals, fruits and vegetables resulted in a rise in Hb levels. A change in perception was brought about among all stakeholders. The importance of a nutritive diet needs to be explained adequately and promoted by citing examples with do's and don'ts. Results of another study from a teaching hospital in New Delhi, which focused on the prevalence and etiology of

Table 4.	Haemoglobin status (category-wise) of the sample and control student groups at baseline and post-interventions at intervals of 1, 3 and
	6 months

			Haemoglobin (g/dl)		
Time period	Group	Non-anaemic (> 12.0)	*Moderate and mild (7.0-12.0)	Severe (<7.0)	P value
At baseline	Sample $(n = 445)$	0 (0%)	436 (98.0%)	9 (2.0%)	0.25
	Control $(n = 225)$	0 (0%)	224 (99.6%)	1 (0.4%)	(Non-significant)
Post-interventior	at intervals of:				
1 month	Sample $(n = 339)$	100 (29.5%)	238 (70.2%)	1 (0.3%)	0.003
	Control $(n = 204)$	34 (16.7%)	170 (83.3%)	0 (0%)	(Significant)
3 months	Sample $(n = 408)$	146 (35.8%)	261 (64.0%)	1 (0.2%)	0.002
	Control $(n = 164)$	34 (20.7%)	129 (78.7%)	1 (0.6%)	(Significant)
6 months	Sample $(n = 397)$	180 (45.3%)	211 (53.2%)	6 (1.5%)	< 0.0001
	Control $(n = 153)$	40 (26.1%)	113 (73.9%)	0 (0%)	(Significant)

\*Moderate and mild categories of anaemia were clubbed together for analysis. Statistical test applied: chi-square test.

Table 5. Urine fluoride level of the sample and control student groups at baseline and post-intervention at intervals of 1, 3 and 6 months

			UFL po	ost-intervention at inte	ention at intervals of			
Group	UFL level (mg/l)	UFL at baseline	1 month	3 months	6 months	<i>P</i> -value within the group		
Sample $(n = 445)$	Mean ± SD (range)	$\begin{array}{c} 1.39 \pm 0.46 \\ (1.01  3.75) \end{array}$	$1.13 \pm 0.57$ (0.119–3.86)	$0.99 \pm 0.468$ (0.136-3.30)	$\begin{array}{c} 1.02 \pm 0.66 \\ (0.171  6.23) \end{array}$	<0.0001 (Significant)		
Control $(n = 225)$	Mean ± SD (range)	$1.53 \pm 0.58$ (1.01–3.80)	$\begin{array}{c} 1.25 \pm 0.64 \\ (0.326  4.23) \end{array}$	$\begin{array}{c} 1.23 \pm 0.57 \\ (0.286  3.33) \end{array}$	$1.41 \pm 0.66$ (0.349-4.77)	<0.0001 (Significant)		

Statistical test applied: ANOVA.



**Figure 1.** Percentage rise in haemoglobin Hb (>12.0 g/dl) from four samples and two control school students. Note the rise in Hb – minimum of 20% to maximum of 39% in 1 month post-intervention in the four sample school students. Similarly in 3 months, the minimum rise recorded is 31% to a maximum of 50%. In 6 months the minimum rise recorded is 41% to a maximum of 57% in contrast to the control group.

nutritional anaemia among children, found that 68% of anaemia in school children was due to iron deficiency and 28% due to vitamin  $B_{12}$  deficiency<sup>16,17</sup>. Vitamin  $B_{12}$  is not often investigated, but it is a key constituent for Hb bio-

synthesis<sup>10</sup>. Vitamin  $B_{12}$  is produced by probiotics in the gut<sup>18</sup>. The recommended daily adherence of vitamin  $B_{12}$  is 1–1.5 µg (ref. 19). The fact that probiotics are destroyed by toxic chemicals and/or drugs containing  $F^-$  is seldom

# **RESEARCH ARTICLES**

		Urine fluoride		
Time period	Group	Above the normal limit (>1.0)	Within the normal limit (<1.0)	<i>P</i> -value
At baseline	Sample $(n = 445)$	445 (100%)	0 (0%)	1.00
	Control $(n = 225)$	225 (100%)	0 (0%)	(Non-significant)
Post-intervention	ns at intervals of			
1 month	Sample $(n = 340)$	144 (42.4%)	196 (57.6%)	0.005
	Control $(n = 204)$	118 (57.8%)	86 (42.2%)	(Significant)
3 months	Sample $(n = 408)$	180 (44.1%)	228 (55.9%)	< 0.0001
	Control $(n = 162)$	111 (68.5%)	51 (31.5%)	(Significant)
6 months	Sample $(n = 397)$	141 (35.5%)	256 (64.5%)	< 0.0001
	Control $(n = 151)$	116 (67.8%)	35 (23.2%)	(Significant)

 Table 6. Urine fluoride level status (category-wise) of the sample and control students groups at baseline and post-intervention at intervals of 1, 3 and 6 months

Normal range of UFL = 0.1–1.0 mg/l; Statistical test applied: Fischer-exact test.



**Figure 2.** Percentage reduction in urine fluoride level (UFL) from four samples and two control school students. Note the high percentage reduction in UFL in sample school students ranging from a minimum of (-) 34.5% in school 2 to a maximum of (-) 79% in school 3, in contrast to the control school students.



**Figure 3.** Body mass index (BMI) of girls and boys in control group at baseline and 6 months post-intervention. Note the BMI for boys at baseline is lower  $(13.9 \text{ kg/m}^2)$  compared to girls  $(16.2 \text{ kg/m}^2)$ . Post-intervention there is no appreciable change, except a gradual rise in BMI with age in boys, whereas in girls in the age group 14–16 years, there is a peak in BMI 6 months post-intervention.



**Figure 4.** BMI of girls and boys in sample group at baseline and 6 months post-intervention. Note the BMI peak for boys in the age group 14–16 years and girls in the age group 16–17 years.

taken into consideration; the resulting vitamin  $B_{12}$  and folic acid deficiencies may cause anaemia. Hillman *et al.*<sup>10</sup> have studied these aspects as early as 1979.

Iron absorption is enhanced by ascorbic acid (vitamin C). This was emphasized in the diet counseling session by identifying essential fruits and vegetables containing vitamin C for consumption. Phytic acid/sodium phytate in brown bread and tannin in tea inhibit iron absorption<sup>20</sup>. It is, therefore, imperative that with a wide variety of food substances available for consumption, prevalence of anaemia requires addressing the do's and don'ts for students, mothers and teachers to understand what to eat and what to avoid in the diet.

The presence of an environmental toxin such as F<sup>-</sup> and its role in non-absorption of nutrients, resulting in anaemia affects two high-risk groups, i.e. adolescent girls and pregnant women. Raising the Hb level to rectify iron deficiency anaemia in pregnant women by IFA supplementation though in vogue for the past three decades in India, has been unsatisfactory as anaemia continues to be prevalent<sup>21</sup>. By 2015 India was not on track to achieve the Millennium Development Goals 4 and 5, i.e. reduce infant mortality by 66% and maternal mortality by 75%. The nation has since then joined others to achieve the Sustainable Development Goals by 2030. To address the issue, possibly the message conveyed that F<sup>-</sup> toxicity addressed through diet editing and simultaneous diet counseling to adolescent girls and their mothers may have a bearing.

The information recorded under other factors of the families, reconfirmed that the families were from poorer sections of the society, with low income and some parents illiterate. As they are concerned about their children's education, better future, spent wisely on the market produce for feeding the children. Empowering the mothers with knowledge had an impact on preparation of diet for the children. Elimination of  $F^-$  and improved nutrients contributed to rise in haemoglobin levels among adolescent girls<sup>22</sup>.

In conclusion, this study highlighted that the consumption of F<sup>-</sup> may adversely affect adolescent girls and boys considering their baseline and post-intervention Hb levels and BMI. Withdrawal of  $F^{-}$  from consumption possibly corrected the damage caused to the gastrointenstinal mucosa/loss of microvilli, which led to the absorption of nutrients leading to rise in Hb and correction of anaemia. The nutritional intervention is simple and easy to implement in the school health programme. Urine F<sup>-</sup> measurement is an important guiding factor, as UFL >1.0 mg/l should alert all those concerned, so that F<sup>-</sup> containing items are withdrawn. The only way to know how much F<sup>-</sup> has entered the body, calls for F<sup>-</sup> assessment in body fluids. The dietary regime introduced has been practiced by families of the lower strata of society. However, the basic principles are equally valid and applicable to children with anaemia from educated, urban and rich families as well.

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