

Anaemia in adolescent girls: An intervention of diet editing and counselling

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ABSTRACT

Background. Though a major public health problem of nutritional anaemia in schoolchildren is being addressed by iron supplementation and/or fortified food, they continue to be anaemic. We aimed to study the effect of fluoride consumption on haemoglobin levels and whether elimination of fluoride from the diet would correct anaemia in children.

Methods. Two hundred and fifty adolescent girls, 10–17 years of age, from a government senior secondary girls school in East Delhi, participated in the programme. Only those girls who were dewormed in the school health programme and not on any medication particularly for malaria, were included. The investigations done were (i) haemoglobin level; (ii) fluoride content in urine; and (iii) fluoride content in drinking water both at home and in school. The anaemic students consuming safe drinking water with fluoride level < 1.0 mg/L and with urine fluoride > 1.0 mg/L were introduced to interventions, viz. diet editing and diet counselling when parents came for the monthly parent–teacher meeting. Besides the parents, their wards and class teachers also attended the counselling session. The students were monitored by re-testing haemoglobin and urine fluoride levels at 1, 3 and 6 months after the start of the intervention.

Results. There was an inverse relationship in the levels of urine fluoride and haemoglobin. Reduction in the level of urine fluoride led to a rise in the haemoglobin level. Following interventions, the haemoglobin level revealed significant improvement from the anaemic (< 12.0 g/dl) to the non-anaemic range (12.0–14.4 g/dl). At 6 months of follow-up, of the 244 girls studied, those with severe anaemia decreased from 3% to 1%, with moderate anaemia from 97% to 58% and the non-anaemic girls increased from 0% to 41%.

Conclusion. Non-toxic nutritive food and safe water with fluoride level < 1.0 mg/L are useful in improving haemoglobin levels in a high percentage of anaemic schoolchildren. A haemoglobin level of > 12.0 – 14.4 g/dL is an achievable target in children without iron supplementation.

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INTRODUCTION

Iron deficiency anaemia (IDA) is a major national as well as global

public health problem.^{1–3} As per the National Family Health Survey-3 (NFHS-3) data, 70% of children under 5 years of age, 55.3% of women and 24.2% of men in India suffer from mild, moderate or severe anaemia.⁴ A new scheme implemented by the government in 2012 is addressing the issue by weekly iron and folic acid supplementation (WIFS) to cover 120 million (12 crore) adolescents all over the country.

A major cause of anaemia is contamination of drinking water with fluoride. It leaches out minerals in the earth's crust and contaminates underground aquifers. Calcium fluorite (CaF₂), one of the minerals, has 157 ppm fluoride.⁵ Calcium fluorite, popularly known as black rock salt, *Sandhanamak* and *Vratkanamak*, is powdered and used as salt/spice in cooking. It is added to beverages to enhance their aroma and taste. Fluoride entering the biological system has possibly, over the years, contributed to the occurrence of anaemia. The public is unaware that this salt has high amounts of fluoride and is toxic. The fluoride content in various north Indian foods has been reported by Nanda;⁶ similarly, some common south Indian foods have been reported to contribute to fluorosis.⁷ A recent study documented the entry of fluoride through food sources, when drinking water was safe with fluoride levels < 1.0 mg/L.⁸ The extent of food contamination and the consequent rise in fluoride levels in the body can only be known when the urine as well as drinking water are tested to identify the source(s) of entry of fluoride.

Excess ingestion of fluoride is a major cause for anaemia during pregnancy and in low birth-weight babies due to non-absorption of nutrients including iron supplementation.⁹ Hillman *et al.*¹⁰ in 1979 showed that anaemia in cattle was the result of fluoride toxicity and fluorosis. They also showed that serum folic acid and vitamin B12, an essential constituent for haemoglobin biosynthesis, were inhibited by fluoride poisoning. Therefore, excess consumption of fluoride is a cause for concern.

We investigated the effect of decrease of fluoride consumption on correction of anaemia without iron supplementation.

METHODS

Adolescent girls studying in classes VI to X at a government senior secondary school in east Delhi were eligible to be enrolled in the study. We wrote a letter to the principals of schools in east Delhi to ascertain their willingness to participate in the project. Those who replied and sought more details were contacted. At a time convenient to the teachers and the management, we delivered a talk on 'Anaemia: Control and prevention without iron supplementation'. The project was initiated in those schools where the principal provided a 'no objection certificate'. A coordinator was nominated by the Principal to facilitate our interactions. We had two prerequisites to be fulfilled by the school. First, the school should ask the children to be dewormed

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at the school's expense and provide us a class-wise list of dewormed children so that we could commence our interaction/activities. We were involved neither in the choice of the drug nor in the dose administered. This was a decision made by the doctor of the school who attended our introductory lecture. To obtain informed consent of the parents, a note written in Hindi was provided to the class teachers, to explain to the girls and to get the consent forms to be signed/thumb impression placed by the parents. The students then returned the forms to our team. The wards, whose parents did not give their consent, were excluded from the study.

The number of students included was based on the consent received from the parents and the list of dewormed students received from the school.

Haemoglobin estimation

This was done by the investigating team of our Foundation. Estimation of haemoglobin level was done by a portable digital haemoglobinometer (Hemocue 201+ Angelholm Sweden).^{11,12}

Fluoride estimation in urine

We also tested the presence of fluoride in urine samples. Each anaemic student was provided a plastic, wide-mouth bottle, with the name of the student, class and date written in pencil on a label of adhesive plaster. Urine fluoride testing using the potentiometric method with ion-specific electrode technology was done at the Foundation. Those girls who had their menstrual period were asked not to provide urine sample.

Fluoride estimation in drinking water

Anaemic students with urine fluoride >1 mg/L were asked to get a cup of water (approximately 50 ml) in a plastic bottle directly from their domestic source of water and not from collected or stored water. Again, bottles were provided with the name of the student, class, date of collection, written in pencil. These bottles with the water sample were given by the students to the class teacher the following day. The staff of the Foundation collected the samples from the school and estimated the fluoride by an ion meter, with a fluoride-specific electrode (results were expressed as mg fluoride/L).

Nutrition education to mothers

Nutrition-related education was imparted to mothers of students in groups in the presence of their children and the class teacher during parent-teacher meetings (PTM). The nutrition education session was conducted as described below.

- First, information about the health status of the girls in terms of haemoglobin level and complaints of ill-health such as cold, cough, fever and leave of absence from the school record were explained to the parents (mothers).
- Second, information regarding the prevailing dietary habits based on the methods of cooking and food items served for breakfast, lunch and dinner in the family were collected from the mothers. The quantum of food consumed and frequency of having food at home were also ascertained. This was required to provide the requisite dietary inputs to enhance intake of iron and other essential nutrients, micronutrients, vitamins and antioxidants.
- Third, certain changes in the diet were introduced, and it was explained how these may improve the haemoglobin level of children and subsequently their general well-being.

Discussions were then held on different items of breakfast, which were simple and nutritious, and could be prepared at home. The parents were shown pictures of various raw vegetables and fruits that could be mixed as salads to serve with lunch. We suggested that they prepare steamed vegetables as salad for lunch and use lime juice and water in equal proportions as a salad dressing, adding salt and sugar to taste.

Similarly, it was suggested that before dinner they prepare mixed vegetable soup without adding cornflour (*maida*) but with a cup of milk with salt and pepper. The recipes for breakfast, lunch and dinner were discussed and suggestions offered to ensure maximum daily intake of vegetables and fruits. The items recommended were rich in nutrients, affordable and could be consumed on a regular basis. Children were advised to avoid consuming cooked food items from street vendors as those items might be unhygienically prepared and might contain toxic contaminants. Home cooked food was suggested as the best option for improving their health.

Interventions for improving the haemoglobin level

Diet editing. To avoid consumption of fluoride from all sources, mothers, students and teachers were advised to consume safe water with fluoride <1 mg/L. It was pointed out that the water samples of the school and from their home were safe for consumption. It was also suggested that street food containing black rock salt, soft drinks (*jal jeera*), water with squeezed lemon, smeared with black rock salt, salty snacks, *churans* and ready-made masala packets containing black rock salt for cooking were to be avoided. Fluoridated toothpaste was also to be avoided. Harmful effects of fluoride ingestion leading to various health problems and causing anaemia during pregnancy⁹ were also explained during the session.

Diet counselling. This was done to ensure that children received a diet with adequate proteins and carbohydrates through cereals; and essential nutrients, micronutrients, antioxidants and vitamins through dairy products, vegetables and fruits. In cooking vegetables, garlic, ginger, onion and green chillies were recommended for use as spices instead of the normally used powdered spices. To ensure adequate vitamin B12 production, consumption of probiotic-rich curd, *lassi* and/or *chaach* was suggested.

Mothers were instructed on various procedures for cooking, viz. steaming, sautéing, grilling, roasting and pressure cooking to retain the nutritive value of food. 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 used to help them understand the final dishes/meals that they should serve to their children/family. A pictorial booklet in Hindi containing 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 would read and explain to the parents (illiterate) the importance of a nutritive diet. Girls were also advised to increase their daily intake of food from 2 to 3 to 4 times.

A discussion on the then prevailing ill-health issues, which existed among other members of the family, helped to make mothers understand the importance of the problem(s). Nutrition education, i.e. diet editing and diet counselling, was tailored to the needs of the poorer sections of society and for those who were illiterate.

Monitoring and impact assessment

After introducing the two interventions, the students were monitored three times: at an interval of 1, 3 and 6 months by measuring (i) haemoglobin level; (ii) fluoride level in urine; and (iii) their health complaints, if any.

Data on other factors

Details regarding the socioeconomic status, viz. parent's literacy level, monthly income of the family, number of members in the family were obtained to understand the effect of these factors, if any.

RESULTS

We report the results of 943 adolescent girls screened for anaemia (haemoglobin level <12 g/dl) from one senior secondary school in east Delhi. Among the girls screened, 538 (57%) had anaemia with haemoglobin levels between 5.8 and 12 g/dl (Table I). When urine fluoride level was tested among 509 anaemic girls, 284 (56%) had urine fluoride levels in the range of 1.1–3.75 mg/L. Thus, 56% of the anaemic girls were consuming higher fluoride; whereas 44% of the girls were anaemic due to other reasons. Of the 238 water samples from drinking water sources at the students' home assessed for possible fluoride content, all were found safe as the fluoride content was 0.114–0.887 mg/L. Forty-six samples of groundwater sources, which were safe, were shared by the families living in the same locality. The school water was also safe with fluoride <1 mg/L.

Of the 250 girls whose baseline data were available, 162 (65%), 240 and 244, respectively, participated in the assessment of the impact of interventions at intervals of 1, 3 and 6 months (Table II). The number of students who participated at 1 month after the intervention was less because of summer holidays. At

baseline, 3% of students (8) had a haemoglobin level <7 g/dl and the rest (242) had moderate anaemia (haemoglobin 7–12 g/dl).

Assessment after the intervention related to nutrition education

Of the 162 girls who participated in the assessment at 1 month after the intervention, none was severely anaemic (haemoglobin <7 g/dl); 130 girls (80%) were moderately anaemic (haemoglobin ≥7–10 g/dl) and 32 girls were not-anaemic (haemoglobin >12 g/dl).

At 3 months after the intervention, 240 of 250 (96%) were available for evaluation. Only 1 girl remained in the severely anaemic group with a haemoglobin level of 6.8 g/dl (her mother was counselled about the diet again at this time) and 165 had haemoglobin levels in the range 7–12 g/dl. Seventy-five girls were no longer anaemic.

At 6 months after the intervention, there was a further improvement. Of the 244 girls assessed (98% of those who participated), 3 had haemoglobin level <7 g/dl (Table II).

We also assessed the urine fluoride levels at the three different intervals. While at baseline all the girls had urine fluoride levels beyond the normal limits (>1 mg/L). At the assessment after 1 month, 94 girls (58%) had urine fluoride levels <1 mg/L. There was a further increase in the number of girls with urine fluoride <1 mg/L at 3 months and 6 months (Table III).

Ten students at 3 months and 6 students at 6 months did not participate in the assessment due to their having menstrual periods at that time. Thus, the results reveal that with nutrition education the urine fluoride level decreased by 58%, 59% and 67%, respectively, at 1, 3 and 6 months.

DISCUSSION

The elimination of fluoride and the consumption of a nutritive diet that included iron through cereals, fruits and vegetables, resulted in a rise in haemoglobin levels of the students in this study. A change in perception was brought about among all the stakeholders, i.e. students, parents, teachers and school management. The importance of a nutritive diet needs to be explained adequately and promoted by providing examples with clear dos and don'ts.

The results of another study from a teaching hospital in New Delhi, which focused on the prevalence and aetiology of nutritional anaemia among schoolchildren, found that 68% of anaemia in children was due to iron deficiency and 28% due to vitamin B12 deficiency.^{13,14} Vitamin B12 is not often investigated but the observation is important as it is a key ingredient for biosynthesis of haemoglobin.¹⁰

Vitamin B12 is produced by probiotics in the gut.¹⁵ The recommended daily allowance of vitamin B12 is 1–1.5 µg.¹⁶ The

TABLE I. Distribution of students in various groups

Total number of students screened for anaemia	943
Prevalence of anaemia (haemoglobin level <12 g/dl)	538/943 (57%)
Students with anaemia and urinary fluoride >1 mg/L	284/509 (56%)
Fluoride level of drinking water of 238/284 students+ 2 school water samples (some sources were shared by families)	100% safe (Fluoride ion= 0.114–0.887 mg/L)
Consumption of drinking water contaminated by fluoride	Nil
Number of students who had consent from parents to participate in the study	250/284 (88%)

TABLE II. Haemoglobin level on the basis of severity of anemia in adolescent girls at baseline and after dietary interventions

Haemoglobin level (g/dl)		Baseline (n=250)	At 1 month (n=162)	At 3 months (n=240)	At 6 months (n=244)
<7.0 (severe)	n (%)	8 (3%)	None	1 (0.4)	3* (1)
	Mean (SD)	6.3 (0.4)		6.8 (0)	6.4 (0.6)
	Range	5.8–6.8		5.8–6.8	5.7–6.8
7.0–12.0 (moderate)	n (%)	242 (97)	130† (80)	165‡ (68.7)	141 (58)
	Mean (SD)	11.0 (0.9)	10.7 (0.9)	10.8 (1.1)	10.8 (1.2)
	Range	7.2–12.0	7.4–12.0	7.1–12.0	7.0–12.0
>12.0 (not anaemic)	n (%)	None	32‡ ((20)	75† (31)	100† (41)
	Mean (SD)		12.5 (0.3)	12.6 (0.4)	12.8 (0.5)
	Range		12.1–13.3	12.1–14.0	12.1–14.4

* p<0.01 † p<0.0001 ‡ p>0.01

TABLE III. Urine fluoride level in adolescent anaemic girls at the baseline survey and after interventions

Urine fluoride level (mg/L)		Baseline (n=250)	At 1 month (n=162)	At 3 months (n=240)	At 6 months (n=244)
<1.0 (within normal limits)	n (%)	Nil	94 (58)	142 (59)	164 (67)
	Mean (SD)		0.7 (0.2)	0.6 (0.2)	0.7 (0.2)
	Range		0.119–0.993	0.136–0.976	0.179–0.992
>1.0 (fluoride toxicity)	n (%)	250 (100)	68 (42)*	98 (41)	80 (33)*
	Mean (SD)	1.4 (0.5)	1.6 (0.5)	1.4 (0.4)	1.5 (0.7)
	Range	1.00–3.75	1.05–3.75	1.01–2.93	1.01–6.23

* p < 0.0001

fact that probiotics are destroyed by toxic chemicals and/or drugs containing fluoride is seldom taken into consideration; the resulting vitamin B12 and folic acid deficiencies can cause anaemia. Hillman *et al.*¹⁰ reported that folic acid and vitamin B12 deficiencies result in anaemia and that the production of vitamin B12 is inhibited by fluoride toxicity.

The presence of an environmental toxin such as fluoride plays a role in non-absorption of nutrients due to loss of microvilli in the intestinal mucosa. This is an important aspect in understanding anaemia^{17–19} and for introducing corrections. Thus, nutrient absorption in a biological system, besides the factor(s) responsible for haemoglobin production, need to be addressed for correction of anaemia. Iron absorption is enhanced by ascorbic acid (vitamin C)²⁰—this was emphasized in the diet counselling sessions by identifying essential fruits and vegetables, containing vitamin C for consumption. Intake of iron by an individual/population is largely a function of (i) the amount of dietary iron; (ii) the bioavailability of that iron; and (iii) the extent of iron losses. Many food items rich in iron are limited by its bioavailability, which depends on the chemical form as iron can exist in different oxidation states, viz. ferrous (+2), ferric (+3) and ferryl (+4).²¹ Further, while vitamin C promotes absorption of iron, phytic acid and tannin in tea inhibit it.^{22,23} Iron enters the lumen of the stomach as Fe⁺³; the hydrochloric acid produced in the stomach converts it to Fe⁺² for absorption. Iron is absorbed from the duodenal and jejunal regions of the small intestine.^{24,25} It has also been reported that a glycoprotein of molecular weight 160 kDa from human microvillus membrane vesicles facilitates the transport of iron.²⁶ Hence, it is possible that iron provided through supplementation should be soluble for absorption. However, if the diet contains a moiety which will precipitate the element in the intestine, it would prevent its absorption; for example sodium phytate present in brown bread will interfere with absorption of iron.²³ It is therefore imperative that with a wide variety of food substances available for consumption, prevention of anaemia requires a special emphasis on the dos 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 fluoride 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 haemoglobin level to rectify iron deficiency anaemia in pregnant women by iron and folic acid supplementation, though in vogue for the past three decades in India, has been unsatisfactory as anaemia continues to be prevalent.^{27,28} India was not on track to achieve Millennium Development Goals 4 and 5, i.e. reduce infant mortality by 66% and maternal mortality by 75% by 2015. To address the issue, possibly the messages conveyed through diet editing and counselling to adolescent girls and their

mothers may have a bearing. Adolescent girls entering wedlock should have a haemoglobin level >12 g/dl. They should be aware of how to maintain haemoglobin levels >12 g/dl, so that to a large extent, maternal and/or infant mortality, abortion, stillbirth, pre-term delivery and low birth-weight can be controlled and avoided.^{9,28,29} Adolescent girls may also derive benefit by preventing trans-generational anaemia.

The information recorded under confounding factors indicated that though the families were from poorer sections of the society with a low income, some parents though illiterate, are concerned about their children's education and future and spent wisely on the purchase of market produce for feeding children; this had an impact after empowering women with knowledge. This improved the nutrients in the diet, decreased/eliminated fluoride consumption, and possibly contributed to a rise in haemoglobin levels.

In conclusion, the consumption of fluoride may affect adolescent girls adversely considering the changes in their baseline and post-intervention haemoglobin levels. Withdrawal of fluoride from consumption possibly corrected the damage caused to the gastrointestinal mucosa/microvilli, which led to absorption of nutrients including iron from the diet leading to the correction of anaemia. The nutritional intervention is simple and easy to implement in the school health programme. If urine fluoride level is >1.0 mg/L, fluoride-containing items should be withdrawn or substituted such as fluoridated toothpastes with those containing less fluoride.

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