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Supplementation of $Na_2FeEDTA$-fortified rice improves haemoglobin status in anaemic girls

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ABSTRACT

To ensure that after fortification of rice the added nutrients are adequately bioavailable this study was planned which assessed the effect of iron-fortified rice with 35ppm of Na2FeEDTAon the blood haemoglobin levels of anemic girls. A total of 54 subjects in the age group of 18-20 years were selected and supplemented with 100g of normal raw rice and iron-fortified rice for the control group and experimental group respectively for a period of 2 months and effect of supplementation on the blood haemoglobin levels was checked on 0, 30 and 60 days. The results indicated that the haemoglobin level increased significantly ($p<0.01$) from the 0^{th} to 60^{th} day for the category of moderate and severe anaemic subjects of the experimental group. Hence the rice fortified with $Na_2FeEDTA$ can be an effective solution to prevent the enormous prevalence of anaemia.

Key words: *Iron-fortified rice, $Na_2FeEDTA$, Iron deficiency anaemia, Anaemia, Supplementation, Fortification*

At any given moment globally more individuals have Iron deficiency anaemia (IDA) than any other health problem affecting 24.8% of the total population (WHO, 2005). Women of reproductive age are most vulnerable to iron deficiency anaemia with an estimated prevalence of 44% in developing countries (Haas *et al.*, 2005). India is among the countries with high prevalence of anaemia in the world. The National Family Health Survey-3 (NFHS-3,2005-06), data suggests that anaemia is widely prevalent among all age groups and is particularly high among pregnant women with an estimation of nearly 58%, 50% among non-pregnant non-lactating women, 56% among adolescent girls (15–19 years), 30% among adolescent boys and around 80% among children under 3 years of age. Almost 7 in 10 children aged 6-59 months are anaemic, 26% are mildly anaemic, 40% are moderately anaemic and 3% are severely anaemic. More than half of young children in 24 states have anaemia, including 11 states where more than two thirds of children are anaemic. From WHO statistics, anaemia is considered as a public health problem when the prevalence of low haemoglobin concentrations in the population is greater than 5% (WHO, 2001). The high prevalence of iron deficiency in the developing world has substantial health and economic costs, including poor pregnancy outcome, impaired school performance and decreased productivity (Zimmermann and Hurrell, 2007). Physical and cognitive losses due to IDA costs developing countries up to 4.05% loss in gross domestic product (GDP) per annum, thereby stalling social and economic development. When results are expressed as a percentage of GDP, these losses are 1.18% of GDP in India (Horton and Ross, 2003). Clearly, the burden is large and the consequences are serious. According to a panel of Nobel laureates, of the top 10 priorities selected for advancing global welfare, 5 were in the area of nutrition-micronutrient supplements, micronutrient fortification, biofortification, de-worming and other nutrient programmes at school and community level (Copenhagen Consensus, 2008). The magnitude of anaemia together with the associated adverse health, development and economic consequences, highlights the need for intensified action to address this public health problem (Ezzati*et al.*, 2002 and Ezzati*et al.*, 2004). In general, three nutritional intervention strategies are currently in use to combat micronutrient malnutrition: 1) increasing the dietary intake of foods rich in micronutrients by dietary diversification, 2) periodic supplementation with target micronutrients and 3) fortification with one or more micronutrients of commonly consumed dietary items (Hurrell, 2002). While dietary modification and supplementation have offered some improvement in developing countries, economic constraints and low rates of compliance are major concerns associated with these strategies. Fortification appears to be the best long-term nutrition intervention strategy for controlling most Micronutrient Deficiencies (MND) (De Romana*et al.*, 2002). But it is also essential to ensure that after fortification of food, the added nutrients are adequately bioavailable. Thus this study was planned to investigate the effect of iron fortified rice on the blood haemoglobin levels of anaemic girls.

MATERIALS AND METHODS

To study the effect of iron fortified rice on the blood haemoglobin levels of anaemic girls between the age group of 18-20 years, a supplementation trial for 2 months was conducted in 60 anaemic girls. For the supplementation trial, initially 75 girls between age group of 18-20 years from D-Hostel of College of Home Science in Hyderabad city were screened to check the blood haemoglobin level by Cyanmethaemoglobin Test (Baker and Ramachandran, 1984). From the results of initial screening, 60 girls with depleted levels of haemoglobin were selected for the supplementation trial. After obtaining written consent from each individual to participate in the study, the selected girls were divided into control (n=26) and experimental (n=34) groups. However, during the study, due to various reasons, 4 girls from experimental group and 2 girls from control group have dropped out of the study at various stages. Hence, the supplementation trial on 60th day had a total of 54 subjects - 30 girls in experimental group and 24 girls in control group (Table 1). According to the WHO (2001) classification of anaemia, all the subjects in both the groups were further subcategorized into 3 groups: mild anaemia, moderate anaemia and severe anaemia. As per the baseline survey, experimental group consisted of 7 subjects who fall in the category of mild anaemia, 15 subjects in moderate anaemia and 8 subjects in severe anaemia; whereas in the control group it was observed that 7 subjects were in the category of mild anaemia, 10 subjects in moderate anaemia and 7 subjects in severe anaemia.

The students selected for the supplementation study were given a detailed briefing on the study protocol. Consent forms were distributed to all girls enrolled in the study and were signed by the girls and girls' parents or guardians. Fact sheets explaining the project were distributed to all study subjects. The protocol of the study was approved by the Research Review Committee and the Ethical Committee of the university.

Supplementation was carried out with 100g of raw rice and iron fortified rice to the control (n=24) and experimental (n=30) groups respectively. 100g of raw iron fortified / normal rice was cooked and vegetable *biryani* was prepared with the rice and was supplemented thrice a week to the selected subjects for a period of 2 months (60 days) to the experimental

Table 1. General profile of the subjects participated in supplementation study

Parameter	Control group			Experimental Group		
Number of subjects	24			30		
No. of subjects who dropped out of study	2			4		
Mean age (years)Range	19.20±0.83			19.54±0.52		
	18-20			19-20		
Sex	24 females			30 females		
Activity level	Sedentary	Sedentary				
	0 Day	30 Day	60 Day	0 Day	30 Day	60 Day
Mean Height (cm)	153.42±1.18	153.42±1.18	153.42±1.18	156.24±6.87	156.24±6.87	156.24±6.87
Mean Weight(Kg)	47.71±8.35	47.42±8.30	47.85±8.25	47.3±7.50	47.2±7.58	47.43±7.20

group and control group respectively.

Haemoglobin (Hb) study

Estimation of haemoglobin was done by the Cyanmethaemoglobin method (Baker and Ramachandran, 1984) on 0, 30 and 60 days to study the effect of supplementation on the blood haemoglobin levels.

$$\text{Haemoglobin in g/dl} = \frac{\text{Abs.of T}}{\text{Abs.of S.}} \times \frac{251}{1000} \times 65$$

Where, 251 = The dilution factor, 1000 = To correct mg/dl to g/dl and 65 = Concentration of haemoglobin standard.

Assessment of nutritional status

Nutritional status of the subjects chosen for the supplementation trial was evaluated by assessment through anthropometry (height and weight) and dietary assessment. Body Mass Index (BMI) was calculated using the heights and weights of all the subjects on the 0, 30 and 60 days adopting the formula reported by Lazarus *et al.*, (1996).

$$\text{Body Mass Index} = \frac{\text{Weight in kg}}{\text{Height in } m^2}$$

Of the associative factors contributing to anaemia, nutritional status is often mentioned to be crucial. Hence assessment of nutritional status was done by dietary assessment. A food frequency schedule was developed to elicit information on general meal pattern, frequency (%) of iron rich foods consumed by the subjects in different groups and mean quantity (g/day) of intake at a time.

RESULTS AND DISCUSSION

The age of the girls in experimental group was in the range of 19-20 years with mean age of 19.54±0.52 years, whereas age of girls in control group was in the range of 18-20 years with the mean age of 19.20±0.83 years. It was observed that the activity level of all the subjects in both the control and experimental groups was sedentary. The height and weight was checked periodically (0, 30 and 60 days) and BMI was calculated for all the subjects. There was no significant change in the mean height and weight of the subjects from the baseline (0^{th} day) to 60^{th} day.

Haemoglobin (Hb) study

The results of blood haemoglobin (g/dl) level of all the subjects participated in the supplementation trail is given in Table 2. The results indicate that there was no significant change in the haemoglobin level of the subjects in control group, whereas, in case of experimental group, the haemoglobin level increased significantly ($p<0.01$) from the 0^{th} day to 60^{th} day in moderate and severe anaemia subjects. The haemoglobin level also increased from 0^{th} day to 60^{th} day in the experimental subjects with mild anaemia, but the increase was not statistically significant. The

Table 2. Blood haemoglobin (g/dl) levels of subjects participated in the supplementation study

Day	Category	Mild Anaemia (Hb 11.0-11.9g/dl)		Moderate Anaemia (Hb 8.0-10.9g/dl)		Severe Anaemia (Hb<8.0g/dl)	
		C(n=7)	E(n=7)	C(n=10)	E(n=15)	C(n=7)	E(n=8)
Baseline(0^{th} Day)	Mean±SD	11.11±0.16	11.75±0.21	9.38±1.03	9.25±0.73	6.62±0.06	6.77±0.28
	Range	11.00-11.23	11.59- 11.90	8.45-10.5	8.10-10.23	6.58-6.67	6.44-6.95
	Sig. (2 tailed) 0^{th} – 30^{th} Day	0.251	0.690	0.447	0.039*	0.241	0.003**
1^{st} Month(30^{th} Day)	Mean±SD	10.9±0.09	11.34±1.20	9.95±0.55	10.21±1.15	7.06±0.36	9.8±0.77
	Range	10.83-10.97	10.49-12.20	9.57-10.59	8.95-12.47	6.80-7.32	9.14-10.60
	Sig. (2 tailed) 30^{th} – 60^{th} Day	0.740	0.649	0.128	0.294	0.417	0.564
2^{nd} Month(60^{th} Day)	Mean±SD	10.47±1.59	11.83±0.46	9.04±0.61	10.83±1.40	7.47±0.43	10.36±1.35
	Range	9.34-11.60	11.50-12.16	8.37-9.58	8.78-12.60	7.16-7.78	9.10-11.80
	Sig. (2 tailed) 0^{th} – 60^{th} Day	0.627	0.837	0.647	0.005**	0.114	0.010**

Note: *-Significant at $p < 0.05$, **-Significant at $p < 0.01$; E - Experimental group, C - Control group mean haemoglobin level in the mild anaemicanaemic experimental subjects was 11.75±0.21g/dl at baseline (0th day), which increased to 11.83±0.46g/dl on 60th day (Figure 1). The mean haemoglobin level increased from 9.25±0.73g/dl on 0th day to 10.83±1.40g/dl on 60th day and 6.77±0.28g/dl on 0th day to 10.36±1.35g/dl on 60th day respectively in cases of moderate and severe anaemic experimental subjects (Figure 2 & 3). Several studies indicate significant improvement in the haemoglobin levels in case of moderate and severe anaemic subjects in comparison to mild anaemic subjects (Beinner*et al.*, 2005; Hotz*et al.*, 2008; Haas *et al.*, 2005; Stoltzfus*et al.*, 2001). Twelve rice fortification efficacy studies from published literature,carried out in controlled environments, compared a variety of health outcomes between individuals who received fortified rice and those who received non-fortified rice (Angeles-Agdeppa*et al.*, 2008; Beinner*et al.*, 2010; Hotz*et al.*, 2008; NogueiraArcajo*et al.*, 2013a; NogueiraArcajo*et al.*, 2013b; NogueiraArcajo*et al.*, 2012; Moretti*et al.*, 2006; Radhika*et al.*, 2011; Zimmermann *et al.*, 2006; Pinkaew*et al.*, 2013; Pinkaew*et al.*, 2014; Thankachan*et al.*, 2012). Statistically significant improvements were observed in 5 out of the 8 studies that assessed serum ferritin, 6 out of 7 studies that assessed iron deficiency and in both studies that assessed iron body stores. Iron fortification of staple foods holds promise as a major intervention to deliver iron in an absorbable form to large populations on a permanent and self-sustaining basis. Fortification of staple foods and commonly used condiments is a highly promising strategy to address iron deficiency. Many countres have demonstrated both the feasibility and effectiveness of this strategy (Hurrell, 2002; Uauy*et al.*, 2002).

Rice is a major crop and primary food source for 50% of the global population (Khush, 2005). Production of iron fortified rice would improve iron nutrition in regions where such a deficiency is rampant (Meng*et al.*, 2005). Feeding trials with 192 religious sisters in the Philippines have shown that eating high-Fe rice results in an increase in serum ferritin and total body Fe, without any other changes in their diet and is efficacious in improving the Fe stores of women with Fe-poor diets in the developing world (Haas *et al.*, 2005). The results of our study are in correlation to the findings of the studies reported by Hass *et al.*, 2005.

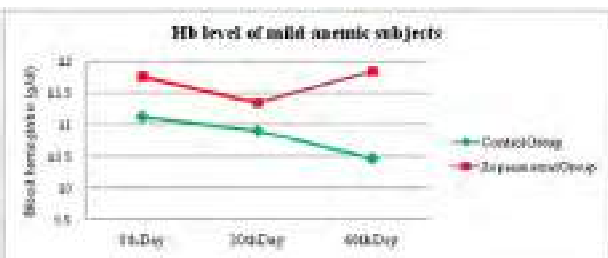

Fig. 1. Haemoglobin (g/dl) levels of mild anaemic subjects

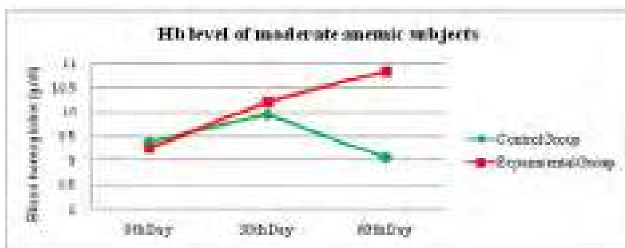

Fig. 2. Haemoglobin (g/dl) levels of moderate anaemic subjects

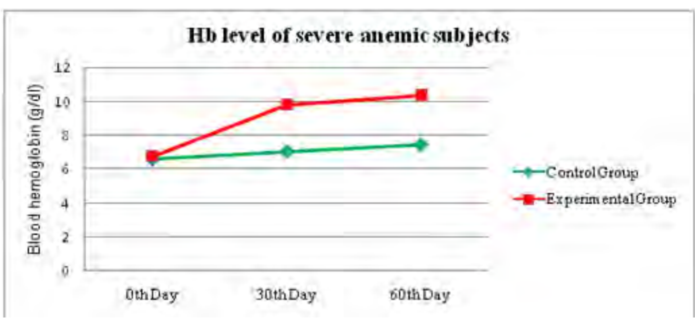

Fig. 3. Haemoglobin (g/dl) levels of severe anaemic subjects

Assessment of nutritional status by anthropometry

The results of BMI expressed as kg/m^2 indicated no significant change in BMI values of the subjects all through the supplementation period (Table 3).

Dietary assessment

From the general meal pattern of the subjects through dietary recall questionnaire, it was observed that, the subjects had their food in three main meals provided by the hostel mess. Breakfast in the early morning, usually between 8:00 a.m. to 9:00 a.m which included idly, *dosa*, *chapatti*, *puri and upma* along with tea; lunch in the afternoon included items such as rice, *sambhar*, vegetable fry or *curry*, *chutney* and curd which was usually consumed between 12:00 p.m. to 1:30 p.m; tea in the evening between 4:00 p.m.-5:00 p.m. and dinner at night between 7:30 p.m. to 8:30 p.m which was almost the similar as lunch, with rice, *sambhar*, *curry* or green leafy vegetable fry and curd. The subjects also had evening snacks between 4:00 p.m. to 5:00 p.m., which was not provided by the hostel. All the study subjects consumed tea in the evening time which was provided from the hostel kitchen. Apart from the tea that was served, some of the subjects consumed biscuits or oats or savories. An account of the distribution of the subject's food intake throughout the day is relevant to considerations of their health.

The presence of sufficient amounts of iron absorption enhancers (ascorbic acid, meat, fish, and poultry) overcomes inhibition of iron absorption from even large amounts of tea. Recommendations with respect to tea consumption included consumption of tea between meals instead of during meal. It was also reported that simultaneous consumption of ascorbic acid and/or meat, fish and poultry improves iron absorption (Zijp*et al.*, 2000). Contrary to this, the results of general meal pattern indicated that the subjects consumed tea along with breakfast rather than consuming in between meal which could be one of the reasons for low haemoglobin levels among the study subjects.

Apart from the consumption of tea along with meal, the results of food frequency data indicated low consumption of iron absorption enhancers like meat, fish, poultry and ascorbic acid food sources. The results of food frequency and mean iron rich food intake clearly indicated that both the frequency and mean intake of iron rich food was less. Consumption of haeme iron or other iron absorption enhancing foods like amla, lemon was also less both in frequency and amount, and hence it can be assumed that iron absorption in the subjects also might be low.

Iron supplementation trials in anaemic children, young adolescents etc have reported positive effects on growth (Oppenheimer, 2001; Rivera *et al.*, 2003; Venkatesh *et al.*, 2002). In our study, there was no significant effect of iron fortified rice on growth but there was significant improvement in the blood haemoglobin levels. Our findings indicate that, providing iron-fortified rice is an effective iron fortification

Table 3. BMI of subjects participated in the supplementation study

Day	Category	BMI of Mild Anaemic subjects(kg/m^2) (n=7)		BMI of Moderate Anaemic subjects(kg/m^2) (n=15)		BMI of Severe Anaemic subjects (kg/m^2) (n=8)	
		C	E	C	E	C	E
Baseline(0th Day)	Mean±SD	17.90±0.21	22.33±5.69	19.83±2.95	18.53±2.40	19.04±0.82	21.56±1.87
	Range	17.75-18.06	18.30-26.36	17.64-23.19	13.45-22.44	18.46-19.63	19.61-23.34
	Sig. (2 tailed) 0th – 30th Day	1	0.983	0.911	0.973	0.936	1
1st Month(30th Day)	Mean±SD	17.90±0.21	22.18±6.49	19.55±2.83	18.49±2.31	19.12±0.84	21.56±1.87
	Range	17.75-18.06	17.59-26.78	17.44-22.77	13.49-22.21	18.52-19.72	19.61-23.34
	Sig. (2 tailed) 30th – 60th Day	0.831	0.983	0.864	0.902	0.991	0.962
2nd Month(60th Day)	Mean±SD	17.84±0.31	22.33±5.69	19.97±2.83	18.62±2.28	19.11±0.86	21.49±1.86
	Range	17.62-18.06	18.30-26.36	17.85-23.19	13.50-22.68	18.50-19.72	19.61-23.34
	Sig. (2 tailed) 0th – 60th Day	0.831	1	0.955	0.930	0.945	0.962

Note: E - Experimental group; C – Control group strategy to reduce anaemia among the young girls. Iron fortification of staple food such as rice holds promise as a major intervention to deliver iron in an absorbable form to large populations on a permanent and self-sustaining basis. When the iron fortified rice is supplemented in school-feeding programs and mid day meal programmes, it could help reduce the large burden of IDA in Indian populations.

The results indicated that consumption of Na_2FeEDTA-fortified rice can lead to significant ($p<0.01$) improvement in the blood haemoglobin levels of the moderate and severe anaemic subjects. The results of BMI indicated no significant change in the BMI values of the subjects of both the groups all through the supplementation period which suggests that supplementation of placebo or iron fortified rice did not have any influence on the height, weight and BMI of subjects from 0^{th} day to 60^{th} day. Considering that every year millions of children, adolescent, pregnant and lactating women suffer from the physiological consequences of iron depletion, it is important to pay serious attention to the fortification of staple foods such as rice while planning nutritional health programs to reduce the burden of anaemia. The results of the study on fortification of rice point to a viable strategy for the prevention and control of anaemia.

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