IJFANS

Volume 01, Issue 01, Oct-Dec 2012, *www.ijfans.com* ISSN: 2319-1775

International Journal of Food And Nutritional Sciences

Official Journal of IIFANS

IJFANS
International Journal of Food And Nutritional Sciences

Cover and Journal Designed by cognitive.designs@gmail.com

IJFANS International Journal of Food And Nutritional Sciences

INTERNATIONAL JOURNAL OF FOOD AND NUTRITIONAL SCIENCES

ISSN 2319 – 1775 www.ijfans.com
Volume 01, Issue 01, Oct-Dec 2012

RESEARCH PAPER **OPEN ACCESS**

A COMPARISON OF IRON AND FOOD SUPPLEMENTATION STRATEGIES ON SELECTED PARAMETERS OF BEHAVIOUR IN ANAEMIC SCHOOL AGE GIRLS

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ABSTRACT

Iron deficiency anaemia (IDA) in school aged girls is an important yet overlooked issue. The present study was undertaken on 8 to 11 year old school girls (n=111) with the objective of studying the correlation between IDA and behaviour. The study was also aimed at ascertaining the impact of iron rich food supplementation vis-à-vis iron folic acid syrup supplementation. At baseline, haematological assessment included estimation of haemoglobin (Hb), red cell indices, serum iron, total iron binding capacity (TIBC), serum transferrin saturation and serum ferritin. Psychological assessment was conducted to determine school adjustment and eye-hand coordination. Anaemic subjects were divided into three groups, viz., AE1, which received twice weekly supplementation of iron folic acid syrup (53 mg iron/week); AE2, which received daily supplementation of four niger seed and defatted soy flour biscuits (45 mg iron/week) plus two lemons and AC, which remained unsupplemented to serve as control. Non anaemic group (NAC) was not intervened. Post intervention data was collected after an intervention period of 120 days. The prevalence of anaemia was 77.5% in the study population with 46.0% and 31.5% of the subjects suffering from mild and moderate anaemia respectively. Serum iron, TIBC, transferrin saturation and serum ferritin were significantly lower in anaemic girls when compared with non anaemics. There was no difference in the school adjustment of anaemic and non anaemic girls, however, it had significant, negative correlation with Hb and serum iron. Eye and hand coordination was better in non anaemic girls than that in anaemic ones. Iron supplementation enhanced educational adjustment in anaemic subjects and improved eye and hand coordination but lower than that of non anaemics.

KEY WORDS:
Anaemia, Behaviour, Iron Deficiency, Iron Deficiency Anaemia, Haemoglobin, Supplementation.

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INTRODUCTION

Iron deficiency in school age and early adolescence has been primarily studied for its detrimental effect on haematinic status. However, in iron deficiency, decreased brain iron stores may impair the activity of iron-dependent enzymes necessary for the synthesis, function, and degradation of neurotransmitters (Erikson et al, 2001) and produce scholastic under-achievement or behavioural disturbances in school children (Pollitt and Liebel, 1976; Sen and Kanani, 2006). Iron thus plays a vital role in cognitive and behavioural development of growing children and adolescents.

The importance of widespread iron deficiency and anaemia among Indian girls has to be viewed in the context of these functional consequences. Anaemia is at least one nutrient deficiency disease which can be controlled even in the current conditions. Use of appropriate iron supplementation strategies to the vulnerable groups and selection of efficient outlets of distribution of supplements need to be examined and strengthened.

The present investigation was conducted to get an insight into the correlation between IDA and eye and hand coordination as well as school adjustment in young girls. Impact of medicinal iron and iron rich food supplementation were compared with respect to haematological parameters and performance on psychological tests. Standardisation of iron enriched recipes, estimation of total iron and sensory evaluation were also a part of this endeavour.

MATERIALS AND METHODS

A pre- post- intervention trial along with a control group has been undertaken. The participants of the study were 8 to 11 year old school age girls residing in the hostels of Banasthali Vidyapith, a residential educational institution for girls. The study was delimited to the girls studying and residing in a single institution so as to have a group homogeneous with respect to living conditions, eating pattern, intellectual environment and exposure to information and knowledge; factors which can have a confounding effect on the results. After getting the consent from the authorities and the parents of the children, they were screened for haemoglobin (Hb), intelligence quotient (IQ), urinary iodine. Those having severe anaemia or IQ < 75 or having suffered a recent episode of malaria or having attained menarche were dropped. The complete data was thus obtained from 111 girls. On the basis of Hb values anaemic subjects were divided into two experimental and one control groups.

Medicinal iron and food based intervention strategies were designed next. After due consultation with medical gastroenterologist specializing in public health nutrition, the subjects in the experimental group 1 were supplemented with iron (1066.66 mg ferrous ammonium citrate /100ml; elemental iron: 213.33 mg/100ml), folic acid (3.33mg/100ml), cyanocobalamin (50 µg/100ml) syrup twice weekly. Supplementation of 25 ml of the syrup provided 53 mg of elemental iron per week. For food based intervention, two iron enriched variants each of biscuit, handwa, idli and soy chat were prepared. Iron enrichment was done by the addition of ingredients with high iron content like soybean, niger seeds, rice bran, cauliflower greens and acceptability appraisal carried out. Sensory evaluation included selection of semi trained panel using triangle test. Control and iron enriched variants were subjected to 9 point hedonic test, paired comparison test and ranking test by a panel of 17 judges. Defatted soyflour (DSF) and niger seed added biscuits not only had high iron and high acceptability but also the qualities of ease of keeping and distribution. Therefore, these were selected for supplementation. As a sequel to this phase of the research endeavour, the experimental group 2 was to be supplemented with 2 niger seed biscuits and a lemon twice daily, after the 2 major meals. This intervention with 100 percent compliance was to provide approximately 45 mg of iron in a week.

Baseline data collection included estimation of Hb (cyanmethaemoglobin method), red cell indices namely, mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC) (using automated haemo analyser), serum iron and total iron binding capacity (TIBC) (using reagent kits based on Ramsay's method) and serum ferritin in one fourth of the subjects using enzyme linked fluorescent assay (ELFA) technique. Psychological testing included assessment of IQ using General mental ability test for children, psycho motor function using the mirror drawing test (to observe eye and hand coordination) and school adjustment by using adjustment inventory for school students. On the basis of Hb at baseline, 86 subjects were found to be anaemic. Randomly, these subjects were divided into three groups, two experimental and one control. The number of subjects were 30, 31 and 25 in the experimental 1 (AE1), experimental 2 (AE2) and control (AC) groups, respectively. The experimental group 1 was supplemented iron syrup (15 ml + 10 ml), twice weekly; experimental group 2 was supplemented four niger seed biscuits and two lemons daily, for 120 days. Control group remained unsupplemented. The initially non anaemic group comprising of 25 subjects was not intervened and served as non anaemic control (NAC). After intervention phase of 120 days, post intervention data was collected which included assessment of haematological and psychological parameters measured at baseline, on all subjects.

Minitab® 15.1.0.0 (Minitab Inc.) was used for the statistical analysis of the data. A Pearson correlation coefficient was used to measure the extent to which two continuous variables are linearly related. The student's and paired t test procedure was used to make inferences about the difference between two population means, based on data from two random samples. The null hypothesis for the test was that the two population means are the same. A one-way analysis of variance (ANOVA) was used to test the hypothesis that the means of several populations are equal. The null hypothesis for the test was that all population means (level means) are the same. Significance was defined as $p<0.05$.

RESULTS

BACKGROUND INFORMATION

The 8 to 11 year old subjects (mean age 9.9 years) were from varied backgrounds with respect to state of domicile, mother tongue, family composition but they had been staying together in the hostels of the institution at least for a year. They were now exposed to same social and intellectual environment. Their eating pattern (lacto vegetarian) and lifestyles were also similar.

BASELINE CHARACTERSTICS

The prevalence of anaemia (Hb < 11.5 g/dl) was 77.5% in the study population; 46.0% subjects had mild anaemia (n=51) and 31.5% had moderate anaemia (n=35). Mean Hb of mildly and moderately anaemics was 10.4 and 9.3 g/dl respectively (table 1). Non anaemic subjects' (n=25) mean was 12.1 g/dl. Mean MCV, MCH and MCHC of anaemic subjects were 73.0 fl, 27.6 pg and 31.3% respectively. The means of same indices in non anaemic group were 88.0 fl, 28.8 pg and 32.8% respectively. Serum iron and TIBC mean values were 55.5 and 776.9 μg/dl in moderately anaemic subjects; 86.8 and 663.9 μg/dl in mildly anaemic subjects; 151.5 and 594.2 μg/dl in non anaemic subjects. Per cent transferrin saturation means were 7.2, 13.2 and 26.2 in moderately anaemic, mildly anaemic and non anaemic groups respectively. Mean serum ferritin was 17.1 μg/l in those suffering from moderate anaemia, 18.8 μg/l in mildly anaemics and 50.2 μg/l in those who were not anaemic. There was a significant difference ($p<0.05$) between anaemic and non anaemic group in all the haematological parameters.

Median IQ of non anaemic subjects was 128.0 followed by that of mildly (115) and moderately anaemic (92) subjects. The highest frequency (42) of anaemic subjects was in average IQ category whereas the highest frequency (8) of non anaemics was in genius category. Adjustment inventory for school children was used to study the level of adjustment in three areas, viz., emotional, social and educational. Maximum possible score in each area is 20. Thus, the highest score possible for total adjustment is 60. Lower score in the test is indicative of better adjustment. The range of score on total adjustment was 11 to 40 in the anaemic group where as it was from 8 to 26 in the non anaemic group. Median school adjustment scores were 21.0 for non anaemics and 19.0 for anaemics (table 2). Segregating the scores of emotional, social and educational adjustment, medians were 6.0, 7.0, 6.0, respectively in the anaemic group and 7.0 in all three categories in the non anaemic group. Majority of the anaemics (42) and non anaemics (15) had average school adjustment levels. There was no significant difference in the school adjustment of anaemic and non anaemic children ($p=0.999$). Chi square analysis suggested association between degree of anaemia and categories of IQ, and adjustment ($p=0$). Median number of errors in mirror drawing test (for eye and hand coordination) were highest in moderately anaemic group (17.0), followed by mildly anaemic (14.0) and non anaemic groups (10.0) (table 3). There was a significant difference in the mean number of errors committed by anaemics and non anaemics ($p=0$). There was a significant, negative correlation of adjustment scores with both Hb and serum iron. Psychomotor function test scores also had negative correlation with Hb as well as serum iron which was significant in both cases ($p<0.05$).

IMPACT OF INTERVENTION

While the mean Hb of anaemic intervention groups was approximately 10g/dl at baseline, it was 12.1 g/dl in the non anaemic control group. Twice weekly medicinal iron supplementation to AE1 resulted in a hike (p=0) in Hb. The mean increment in Hb was of 1.0 g (table 4). A mean increment of 0.5 g/dl of Hb was observed in AE2 group wherein iron rich biscuits and lemons were supplemented daily (p=0). Although there was an increment in mean Hb of both AE1 and AE2, at post intervention stage there existed a significant difference (p=0) in their mean Hb values. No significant changes were recorded in Hb levels of anaemic (p=0.928) and non anaemic control (p=0.079) group. Mean Hb of AE1 and AE2 which were at par with AC at baseline, were significantly different than control (p=0; 0.018) at post intervention stage. Significant difference of AE1 and AE2 with NAC persisted at the completion of intervention (p=0). MCV of AE1 increased from 73.6 to 77.7 fl and that of AE2 increased from 72.9 to 74.5 fl after supplementation. MCH of AE1 increased from 28.1 to 29.6 pg and that of AE2 increased from 27.6 to 28.2 pg at post intervention stage. MCHC of AE1 increased from 31.5 to 33.1% and that of AE2 increased from 31.5 to 32.5% at post intervention stage. All the changes were significant in the experimental groups but not in non anaemic controls. ANOVA pointed to a significant difference (p=0) in the mean serum iron and TIBC of the four groups at baseline. Tukey's test revealed that mean of non anaemic group was significantly different than the remaining 3 anaemic groups. In AE1, mean serum iron rose from 74.5 to 83.3 µg/dl, TIBC decreased from 714.1 to 673.2 µg/dl and transferrin saturation increased from 10.6 to 12.9% at post intervention stage. The change in serum iron was from 72.1 to 77. 6 µg/dl, in TIBC from 717.2 to 696.6 µg/dl and in transferrin saturation from 10.4 to 11.6% in AE2 after the completion of supplementation. The changes in AE1 and AE2 were significant and that in AC and NAC were non significant. Medicinal iron supplementation led to a rise in serum ferritin from 18.2 to 24.9 µg/l and the increase was from 15.2 to 19.1 µg/l in iron and vitamin C rich food supplemented group.

At baseline, anaemic groups did not differ significantly from non anaemic group in mean score of emotional (p=0.792), social (p=0.099), educational (p=0.707) and total (p=0.932) adjustment as assessed by ANOVA. Comparing the pre and post intervention stages, significant changes were noticed in AE2 (p=0.006) and NAC (p=0.022) for emotional adjustment, in AE1 and AE2 for social, educational and total (p=0) adjustment. Supplemented groups improved in social and educational adjustment and thus in total adjustment (figure 1). The changes in AC were non significant (p=0.341). Even in NAC the changes were non significant except in emotional adjustment. At post intervention stage, AE1 and AE2 had no significant difference (p>0.05) with NAC in emotional and social adjustment. But, in educational adjustment both the experimental groups had significantly lower scores than AC and NAC, indicating better adjustment. Anaemic experimental groups had 15.0 (median) errors in mirror drawing test (used to study eye and hand coordination) whereas the non anaemic had 10.0, at baseline. ANOVA clearly depicts that the means were significantly different at pre intervention stage. Post supplementation, there was a decrease in the number of errors in the supplemented groups (table 5). These changes were statistically significant (p=0). At this stage, non anaemics were still better than the anaemics. AE1 and AE2 had a difference of 3 and 4 median points respectively with non anaemic group.

SENSORY ANALYSIS

In hedonic test, control biscuit had a mean of 8.9. The likeability of variant 2, niger seed and DSF containing biscuit was also very high (8.7). The hedonic test scores of control and niger seed biscuits were not significantly different from each other (p=0.030). In paired comparison test all judges marked control as positive for appearance, flavour, taste and after taste when compared with variant 1 of biscuit. The difference was statistically significant. No significant difference (p>0.05) existed in either of the attributes between control biscuit and variant 2.

DISCUSSION

The present study was undertaken on 8 to 11 year old school girls of a residential institution in Rajasthan, India. All the girls under study came from all over the country and had been into this boarding since a year or more. The prevalence of anaemia in the study population was 77%. Prevalence of this magnitude has been reported in other surveys from various parts of the country. National Family Health Survey (NFHS 3, 2005-2006) reports prevalence of anaemia to be 70 to 80% in Indian children. At the baseline survey of 3000 school children in Gujarat, 84% had Hb < 12g/dl (Gopaldas, 2005). The prevalence observed in this study was similar to that observed by Gopaldas in urban children of Gujarat. Eighty per cent school children were anaemic in a study done by Leela and Shantipriya (2002). The percentage of mildly and moderately anaemics was 46.6 and 33.3 respectively.

Early stimulation, socioeconomic status home environment, nutritional status, and interactions between parent and child all influence the mental function of growing children (Vazir and Kashinath, 1999). The present research endeavour was delimited to a residential school setting so as to rule out the effect of many such confounding factors on the results of the study. Longitudinal studies consistently indicate that children who were anaemic in infancy continue to have poorer cognition, school achievement, and more behaviour problems into middle childhood (Grantham-McGregor and Ani, 2001). However, the possible confounding effects of environmental factors, particularly poor socio economic background, prevent causal inferences from being made (Gera and Sachdev, 2009). The study design in this research project was that of a pre test, post test intervention trial with random allocation of subjects into experimental and control groups. The potential confounding effect of many environmental factors was controlled by undertaking the study on subjects exposed to same living conditions of a residential school.

The performance of anaemic children was found to be poor than that of non anaemics in educational and social adjustment and eye hand coordination tests in this study. Similar has been the finding in other research works but most of the studies on children above 2 years of age have focused on preschool children. Two comprehensive critical reviews of studies involving children identified as ID and anaemia at ages >2 years (Grantham-McGregor and Ani, 2001; Watkins and Pollitt, 1998) also concluded that, in most cases, performance was poorer on tests of cognition and behaviour, at least on some tests (Bruner et al, 1996;

Soemantri et al, 1985; Soewondo et al, 1989). The mean serum ferritin levels were lower in the children with attention-deficit/ hyperactivity disorder (ADHD) than in the controls (p<0.001) (Konofal et al, 2004). Even in this study, the children with SF < 12 µg/dl had low scores on psychological tests.

From the results of this study as well as from the literature reviewed (Kanani and Poojara, 2000; Kotecha et al, 2002), it appears that a long-term supplementation programme, whether once or twice weekly, is likely to be as effective as daily IFA with regard to improvements in Hb levels. In this research work, haematological response to daily iron (6.4 mg iron/day) plus vitamin C (2 lemons providing 30 mg vitamin C approximately) rich food supplementation was also studied. This strategy also brought about a significant improvement in iron status of the subjects but it was quantitatively lower than that brought about by the pharmacologic intervention.

Iron supplementation improved motor development, but this effect was modified by baseline Hb concentrations (P=0.015 for interaction term) and was apparent only in children with baseline Hb concentrations < 90 g/l. In children with a baseline Hb concentration of 68 g/l iron treatment increased scores by 1.1 (0.1 to 2.1) points on the 18 point motor scale (Stoltzfus et al, 2001). Number of errors on mirror drawing test reduced in the supplemented anaemic subjects in this study. The test was used to judge the eye and hand coordination of the subjects which is an aspect pertaining to psychomotor functioning. Even after supplementation, the non supplemented initially non anaemics showed better eye and hand coordination than their supplemented, initially anaemic counterparts.

Although direct evidence demonstrating an effect of IDA on cognitive, behavioural, or other brain functions is limited it seems prudent to assume that a gradation of effects of iron deficiency occurs in the brain (Georgieff and Innis 2005; Lozoff 2000), with milder anaemia and IDA resulting in perhaps more subtle but still potentially adverse brain effects, particularly if they occur during sensitive periods of development.

CONCLUSION

School age children who constituted the study population are in a stage of development and changes are taking place both in the body and mind. The effects of iron deficiency, as observed through this investigation, can be manifested in bodily or mental functions, which may or may not be transitory in nature.

This study marks the importance of reducing anaemia to improve psychological test scores by showing that higher the magnitude of gain in Hb, higher the improvement in test scores. The results of this study imply that twice weekly iron supplementation could provide an effective means of raising iron stores in school age girls. By protecting them from the cognitive effects of iron deficiency iron supplementation could help girls to get the most out of school. The universality of the school setting for gaining access to children makes it highly relevant to global efforts to combat the increasing public health problems of nutrition related ill health.

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Table 1: Categorisation of study population on the basis of hemoglobin levels

Cut off values	Category	n	Haemoglobin (g/dl) (Mean±SD)
Hb < 11.5 g/dl	Total anaemics	86	10.0±0.70
Hb 10.0 to < 11.5 g/dl	Mildly anaemic	51	10.4±0.40
Hb 7.0 to < 10.0 g/dl	Moderately anaemic	35	9.3±0.55
Hb ≥ 11.5 g/dl	Total non anaemics	25	12.1±0.37
	Total subjects	111	10.4±1.09

Table 2: Inter group school adjustment scores at baseline

Groups	n	Adjustment Scores											
		Emotional			Social			Educational			Total		
		Mean	Median	SD	Mean	Median	SD	Mean	Median	SD	Mean	Median	SD
Total Subjects	111	6.5	6.0	2.22	7.3	7.0	2.32	6.1	6.0	2.11	19.9	20.0	6.17
Mildly Anaemic	51	5.3	5.0	1.06	6.3	7.0	1.68	4.8	5.0	1.70	16.4	17.0	3.91
Moderately anaemic	35	8.0	7.0	1.77	9.1	8.0	2.42	7.8	7.0	1.77	25.0	23.0	6.39
Total Anaemics	86	6.4	6.0	2.32	7.5	7.0	2.43	6.0	6.0	2.26	19.9	19.0	6.56
Total non Anaemics	25	6.9	7.0	1.46	6.6	7.0	1.75	6.3	7.0	1.46	19.9	21.0	4.74

Table 3: Inter group eye hand coordination test score at baseline

Groups	n	Number of errors in mirror drawing test		
		Mean	Median	SD
Total Subjects	111	14.3	14.0	3.44
Mildly Anaemic	51	13.8	14.0	1.31
Moderately Anaemic	35	18.0	17.0	2.78
Total Anaemics	86	15.5	15.0	2.89
Total non Anaemics	25	10.1	10.0	0.92

Table 4: Impact of intervention on haemoglobin

Groups	Haemoglobin (g/dl)			
	AE1 (n=30)	AE2 (n=31)	AC (n=25)	NAC (n=25)
	(Mean±SD)			
Pre Intervention	10.0±0.66	9.9±0.63	9.9±0.85	12.1±0.37
Post Intervention	11.0±0.54	10.4±0.58	9.9±0.82	12.1±0.37
Change (post-pre)	1.0±0.20	0.5±0.16	0.0±0.21	0.0±0.09

Table 5: Impact of intervention on psychomotor function test score

Groups	AE1 (n=30)			AE2 (n=31)			AC (n=25)			NAC (n=25)		
	Number of errors in mirror drawing test											
Intervention Stage	Mean	Median	SD	Mean	Median	SD	Mean	Median	SD	Mean	Median	SD
Pre	15.5	15.0	2.76	15.5	15.0	2.43	15.6	15.0	3.61	10.1	10.0	0.92
Post	12.6	12.0	1.93	13.8	13.0	1.75	15.2	15.0	3.57	09.2	09.0	0.91

Figure 1: Percentage of subjects in different categories of school adjustment in the four study groups at pre and post intervention stages