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Full Length Research Paper

Effect of micronutrient supplementation on the nutritional and immune status of school going children with iron deficiency anemia

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Iron deficiency anemia is a major public health problem in India widely prevalent among children. The present study was aimed to reduce the prevalence of iron deficiency anemia and also to improve the immune status, school performance through dietary supplementation with micronutrient rich foods (wheat germ, rice flakes, gingelly seeds, microwave oven dehydrated carrot powder, jaggery).The present study was conducted on children in the age group of 7 - 9 years (N: 1675) from primary corporation school located in the urban areas of Chennai district. Out of 1675 children, 1151(68.7%) children had exclusive signs and symptoms of anemia. The blood hemoglobin levels revealed that 662 children were moderately anemic and the remaining 15 and 74 children showed severe and mild signs and symptoms of anemia respectively. Out of 662 children who were moderately anemic, 150 children were randomly selected for the supplementation study. They were further divided into three groups of 50 children each to receive the food based supplement (Micronutrient rich balls), synthetic supplement (Riconia tablet, a micronutrient fortified tablet) and the third group constituted the control group. The supplementation study was for a period of six months. The impact of supplementation was studied initially and at the end of 6 months in terms of anthropometric measurements, morbidity pattern, dietary assessment and biochemical analysis. The results revealed that, supplementation in the food form resulted in significant improvement in the parameters studied. The findings of this research leads to the conclusion beyond doubt that the food based approach will serve as an effective strategy to combat deficiencies and to promote health and well being of the children and ensure global security.

Key words: Childhood malnutrition, blood hemoglobin, serum retinol, serum zinc, immune profile.

INTRODUCTION

Childhood malnutrition remains as a major public health problem worldwide and has been widely recognized as an important risk factor for child mortality. An estimated 2.8 million child deaths is reported each year in the nine low-income Asian countries including India (Rice, 2001). Attempts are currently underway to quantitatively estimate the extent to which all forms of childhood malnutrition - including deficits in protein and energy and the micronutrients namely iron, vitamin A, iodine and zinc contribute to these adverse outcomes. Among the mentioned deficiencies, Iron deficiency anemia (IDA) is an important nutritional problem affecting all segments of the population especially children. In recent years, nutrition and immunology are increasingly becoming inter-related disciplines. Micronutrient deficiency is associated with an impairment of immune responses and increased susceptibility to infections like upper and lower respiratory tract infections (URI/LRI) and skin infections especially in children (West et al., 2000).

Infectious diseases in children always worsen any deficiency present and conversely deficiency usually weakens resistance to various infections. These effects are therefore synergistic (World Health Organisation

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Table 1. Grouping of children based on blood hemoglobin levels

CPS	Degree of anemia (WHO, 1989)			Total
	Severe (<7g/dl)	Moderate (7-10 g/dl)	Mild (10-12 g/dl)	
	3	93	50	146
	-	117	69	186
	2	54	53	109
	4	86	58	148
	-	43	34	77
	3	80	67	150
	-	124	96	220
	3	65	47	115
Total	15	662	474	1151

CPS: Corporation Primary School
WHO: World Health Organisation.

(WHO), 2003). To combat the micronutrient deficiencies especially IDA several intervention programmes have been initiated. The most commonly adopted strategy is the dietary supplementation could be an effective, preventive and curative strategy, in contrast to dietary diversification and food fortification, in providing immediate relief (Parman et al., 2000). The Tenth five year plan in India has set specific nutritional goals to be achieved by 2007 (Planning Commission, 2002). The major goals are to Intensify nutrition and health education to improve infant and child feeding practices so as to bring down the prevalence of under-weight children, to reduce prevalence of anemia by 25% and that of moderate/severe anemia by 50%, to eliminate vitamin A deficiency as a public health problem and to reduce prevalence of IDD in the country to less than 10% by 2010.

In view of the goals set by the Tenth five year plan and also improve immune status of the children, nutrition-relevant interventions should be designed to make an impact at the household or community level. The present study in an effort in that direction. In this research, a micronutrient rich supplement was formulated using rice flakes, carrot and wheat germ as the major ingredients for supplementation. Studies on school going children between the age group of 7 - 9 years old are sporadic in India. It is high time that the attention is shifted towards the promotion of nutritional status and immune profile of school going age group.

Against this backdrop, the present study was aimed to reduce the prevalence of anemia and infections and also to improve the immune status of school going through supplementation with micronutrient rich food formulation.

The present study focuses on the following specific objectives to formulate a micronutrient rich supplement to combat anemia and to study the impact of supplementation of micronutrient rich balls and pharmaceutical preparation in the form of tablets in terms of nutritional status, immune profile and class performance of children in school.

METHODOLOGY

Selection of schools and grouping of children

The children in the age group of 7 - 9 years from eight Corporation primary schools located in the urban areas of Chennai city were targetted for the study. All the available 1675 children were elicited for socio-economic background, dietary details, nutrition knowledge of the mothers and children and health practices of children using a pretested questionnaire.

A health checkup was conducted with a help of a physician to detect any signs and symptoms of deficiency if any.

Blood hemoglobin was analysed for all the 1675 children as part of the health checkup using the cyanmethaemoglobin method as per the procedure by Varley (1998). Classification of the subjects based on their blood hemoglobin levels is presented in Table 1.

Out of these groups, children were randomly selected for different treatments as given in Figure 1.

Conduct of supplementation study

Formulation and preparation of micronutrient rich food supplement: The existing nutrient deficits in the diets of the children were arrived through a three day food weighment survey. The mean deficit of micronutrients in the diets of anaemic, URI/LRI, VAD and normal children were found to be 7.81 mg iron, 7.86 mg zinc and 1537 mcg of beta carotene. To bridge these existing deficits a food mix rich in iron, zinc and beta carotene was formulated with wheat germ, a rich source of zinc and iron, carrot powder rich in beta carotene and gingelly seeds, a good source of iron and zinc. An ideal composition was arrived through permutation and combinations.

Weighed quantity of wheat germ (60 g), gingelly seeds (5 g) and rice flakes (15 g) were roasted and cooled separately. Nine gramsof carrot powder equivalent to 30 g of fresh carrot (grated and microwave oven dried) was added. The mixture was ground to a fine powder. The dried powder was stored in air tight containers. Eleven grams of jaggery was mixed with water and boiled to syrup.

The powder was added to the syrup and made into balls. The

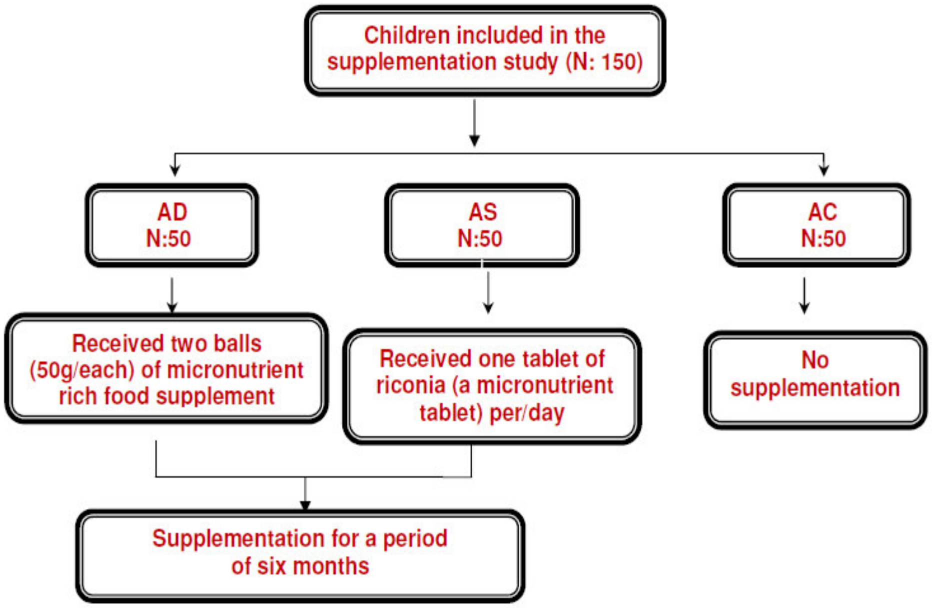

Figure 1. Grouping of children for in depth study.
AD, children receiving food based supplement; AS: Children receiving synthetic supplement in the for of tablet; AC: Control group children.

Table 2. Nutrient composition of the supplements.

Nutrients	Food supplements (per 100g)	Tablet
Protein(g)	19.5	-
Fat (g)	8.9	-
Energy(Kcal)	350	-
Calcium(mg)	150.0	162
Iron(mg)	9.29	10
Beta carotene(mcg)	2703	1500
Thiamine(mg)	1.14	1
Riboflavin(mg)	0.56	1.5
Niacin(mg)	3.76	2
Vitamin C (mg)	3.6	50
Zinc (mg)	10.64	15

synthetic supplement chosen for comparison in the study was Riconia, a micronutrient fortified tablet, a product of Best Laboratories, New Delhi, India. The nutrient composition of the two supplements is presented in Table 2.

The cost of 100 g food supplement was computed to be Rs.4.90/- and the cost of one tablet was Rs.4.75/- (one US $ = Rs.46.6) (Year 2006).

Supplementation of micronutrients

Children in AD group received 100 g per day of the micronutrient rich food supplement prepared into two balls weighing 50 g each. The balls were distributed one in the mid morning and one in the mid evening in their respective schools. The children in the AS groups received one micronutrient tablet per day. The children in the control group AC did not receive any supplement. The supplementation was carried out for a period of six months. Care was taken to see that the children consumed the balls/tablet regularly even on holidays under the supervision of the investigator. Attendance record was maintained to ensure their regular participation. Those who could participate regularly alone were included as subjects for the final evaluation.

Evaluation of the impact of supplementation: The impact of supplementation was studied in all the 150 children in terms of anthropometric measurements (weight and height), clinical picture, food and nutrient intake and morbidity pattern. Biochemical assessment was done through analysis of blood haemoglobin, total blood cell count and serum biochemistry which included total protein,

Table 3. Changes in mean height of the children.

Group	Mean height (cms) ± SD			Initial Vs Final 't' Value	Groups compared	't' Value
	Initial	Final	Difference			
Male						
AD	119.7 ± 1.51	112.9 ± 2.7	3.2 ± 0.9	15.7**	AD Vs AS	3.12**
AS	118.9 ± 2.1	121.0 ± 2.1	2.1 ± 0.4	2.3*	AS Vs AC	16.9**
AC	115.6 ± 2.5	116.5 ± 1.9	0.9 ± 0.1	1.5 NS	AD Vs AC	21.3**
Female						
AD	113.4 ± 1.2	115.7 ± 2.6	2.3 ± 0.2	13.9**	AD Vs AS	9.3**
AS	119.9 ± 2.9	120.6 ± 2.2	1.7 ± 0.7	2.0*	AS Vs AC	18.3**
AC	115.3 ± 2.1	115.7 ± 1.3	0.2 ± 0.6	0.6 NS	AD Vs AC	24.9**

** Significant at one per cent level; * Significant at five per cent level; NS: Not Significant
Std values: Male 121.7-132.2 cm, Female 120.6 - 132.2 cm (NCHS, 1987)
Number in parenthesis indicate the number of children

albumin, iron, zinc and immunoglobulin viz. IgG, IgA and IgM. All the measurements were done at the beginning of supplementation and six months after supplementation.

RESULTS AND DISCUSSION

Demographic profile of the children

Among the 150 anemic children, 50.5% of the male children were 8 years old and 42.9% of the female children were 7 years old. Fifty eight % of the anemic children were boys and 42% were girls. A higher % namely 88.6of anemic children respectively belonged to nuclear families. The results are in accordance with a study by Singh (2003) on children of urban slums where >70% of the families were nuclear. This finding also reflects on the increasing trend towards nuclear families in urban slums.

The educational status of the head of the family (mother or father) revealed that 47.3% of the parents of anemic children were educated upto high school level. Forty two point seven % and 1.3% of the parents of anemic children were educated upto primary school and graduate with the low education background, the fathers could be engaged mainly in poorly remunerative jobs. Around 43.3% of the parents of anemic were mechanics. Remainining 33.3 and 23.4% of the parents of anemic children were labourers and clerks respectively.

It was observed that 51.3% of the families of the anemic children belonged to low income category with a total monthly income less than Rs.2100 according to HUDCO classification (2000).

Impact of supplementation of micronutrients

Anthropometric measurements

(a) Mean height of the male and female children: Mean changes in the height of the children are presented in Table 3. Initially the mean height ranged from 115.6 - 119.7 cm in male children and the female children recorded 113.4 - 119.9 cm .The height of the children were lower than that of the standard values which ranged from 121.7 - 132.2 cm and 120.6 - 132.3 cm for male and female children respectively. Over a period of six months all the children registered increments in height irrespective of the type of supplements received. At the end of six months all the children who were supplemented with either food based or synthetic micronutrients had registered height nearing or above the standard values. The increments registered for the food supplemented groups were significant different from those of the tablet supplemented groups.

(b) Mean weight of the children: The mean initial weight of the male children ranged from 17.3 - 17.9 kg and female children 17.1 - 17.6 kg which was much lower than the standard values. Over a period of six months all the children receiving food based supplement recorded a significant increase ($p < 0.01$) in their weight and the children who received tablets as their supplement recorded increments at five % level of significance. The mean weight of the male and female children is shown in Figure 2.

As far as the height and weight were concerned, the children in the food supplemented group had recorded significantly greater increments when compared with their counterparts receiving micronutrients in the tablet form. Wheat germ being the major component of the food supplement is not only rich in nutrients namely iron, zinc and B-complex vitamins but also contains a considerable amount of protein (17.4 g) and the overall protein content of the food supplement was 19.6 g/100 g. Since adequate protein is essential during growth when new tissue proteins are being synthesized (ICMR, 1989), this also could have attributed to the better growth performance of the children.

(c) Dietary intake: Initially there was an inadequacy in the consumption of all the foods among the children studied. Over a period of six months the improvements in the food supplemented groups stood first followed by the

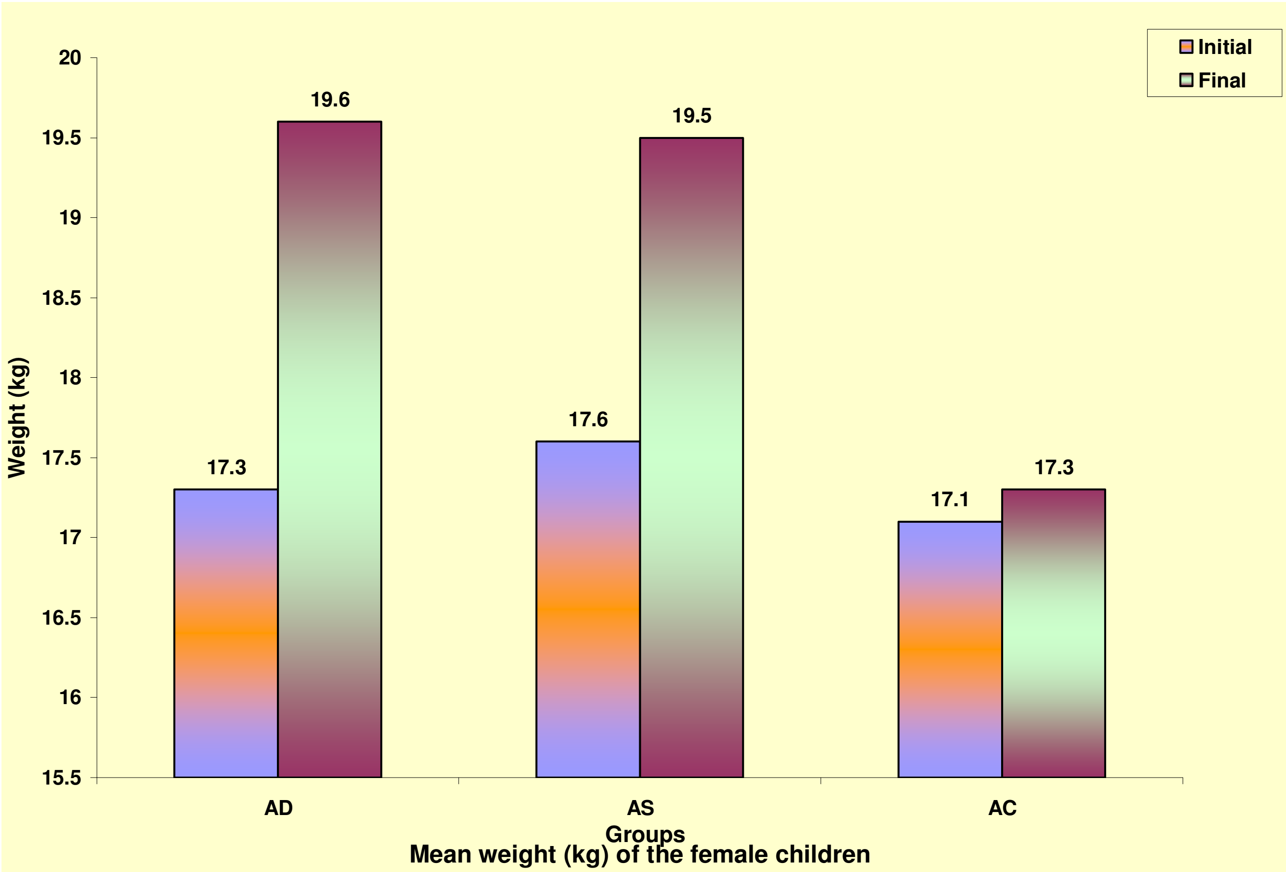

Figure 2. Mean increments in the weight of male and female children.

groups supplemented with synthetic nutrients while the control groups evinced the least changes. Inspite of these improvements, intake of all the foods were still found to be inadequate except in a few cases of cereals consumption and fat consumption.

The intake of all the nutrients was lower in the children. On supplementation with food or tablets the calorie intake alone exceeded the RDA. Though there was an improvement in the intake of other nutrients among the supplemented groups they did not meet the RDA. The children in the food supplemented group recorded greater increase than the tablet supplemented and control group children. The children in the control group recorded negligible increase in their nutrient content.

(d) Morbidity pattern: The morbidity of the anemic children in terms of the frequency of stomach discomfort, fever, diarrhoea and dysentery were more in all the three groups when compared to the other problems encountered. The present finding concur with the findings of Hunt (2000), who stated that in addition to poor dietary intake and inhibitors of absorption, increased intestinal losses following fever and parasitic infestations may be an important cause of iron deficiency anemia. The children in the food supplemented group responded well to the treatment. The reduction in the frequency of fever, diarrhoea and dysentery during the course of supplementation was statistically significant at 1% level when compared with the children in the tablet supplemented group whose frequency of occurrences reduced at 5% level of significance.

Biochemical picture

(a) The mean blood hemoglobin levels of the children: Initially, all the three groups of anemic children (AD, AS, AC) had very low mean haemoglobin values (7.7, 8.8 and 7.7 g/dl). At the end of the supplementation study, all the children who received iron supplements in the form of food recorded the highest increments (5.5 g/dl) in blood haemoglobin level which was significant at 1% level followed by the groups receiving the tablet supplementation which was significant at 5% level. The children in the control group recorded a negligible difference which was not statistically significant. At the end of the study all the children except those in the AC group had more than 12 g/dl of haemoglobin indicating normal iron nutritional status. A comparison between the two types of supplement clearly indicated that the food

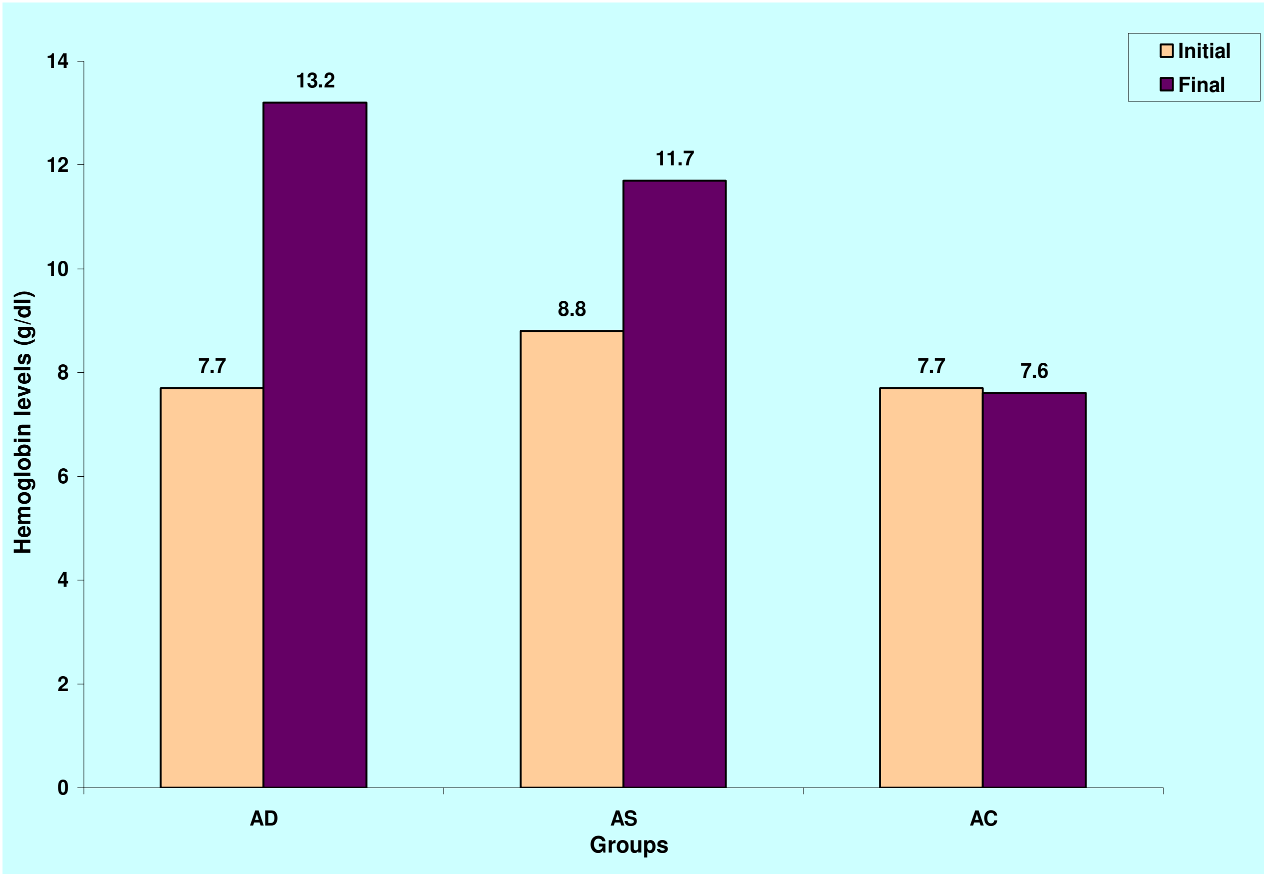

Figure 3. Mean increments in the blood hemoglobin levels of the children

supplement had more impact on blood haemoglobin levels than the tablet form of supplement. The differences were statistically significant at 1% level in the food supplemented groups. The increment received by the control group were significantly lower than those receiving the food supplements ($p < 0.01$) and those receiving the tablet supplements ($p < 0.05$).

In the present study, the anemic children in the food supplemented group had greater increments in their haemoglobin levels since the iron absorption rate is more during severe iron deficiency conditions in children. Diaz et al., (2003) studied that iron absorption rate in anemic children was as high as 60 % on supplementation with iron rich food supplement. Figure 3 represents the mean increments in the blood hemoglobin levels of the male and female children studied.

(b) The mean total blood cell count: The mean total cell count of the anemic children ranged between 3000 and 3900 in all the three groups studied. On supplementation of micronutrients, the children in the dietary group had the maximum increase of 2200 cells per ccm (from 3900 to 6100) when compared with the other two groups (AS - 2300 cells/ccm; AC - 3100 cells/ccm). However, the increments in the food supplemented group and tablet supplemented group were significant at 1% level and 5% level respectively than the control group which registered a meagre increase in the cell count. The mean increments in the total blood cell count of the children are represented in Figure 4.

(c) The mean lymphocyte, neutrophil and eosinophil levels: The mean lymphocyte, neutrophil and eosinophil levels of the children after supplementation did not show much difference irrespective of the supplement given. However, all these values were within the normal range of 40 - 65, 30 - 50 and 2.0 - 8.0% for neutrophils, lymphocyte and eosinophil, respectively as reported by Gupta (1999). Changes on supplementation were not found to be statistically significant.

(d) Mean serum total protein levels: The changes in serum total protein levels of the anaemic children belonging to AD and AS groups were found to be from 5.3 - 7.9 and 5.1 - 6.6 g/dl respectively. Statistical analysis using paired ‘t’ test indicated that the can increases in the serum total protein levels of these children were significant at one and 5% level in the AD and AS groups respectively. Leong et al. (2003) reported an increase after a period of six months in the serum total protein

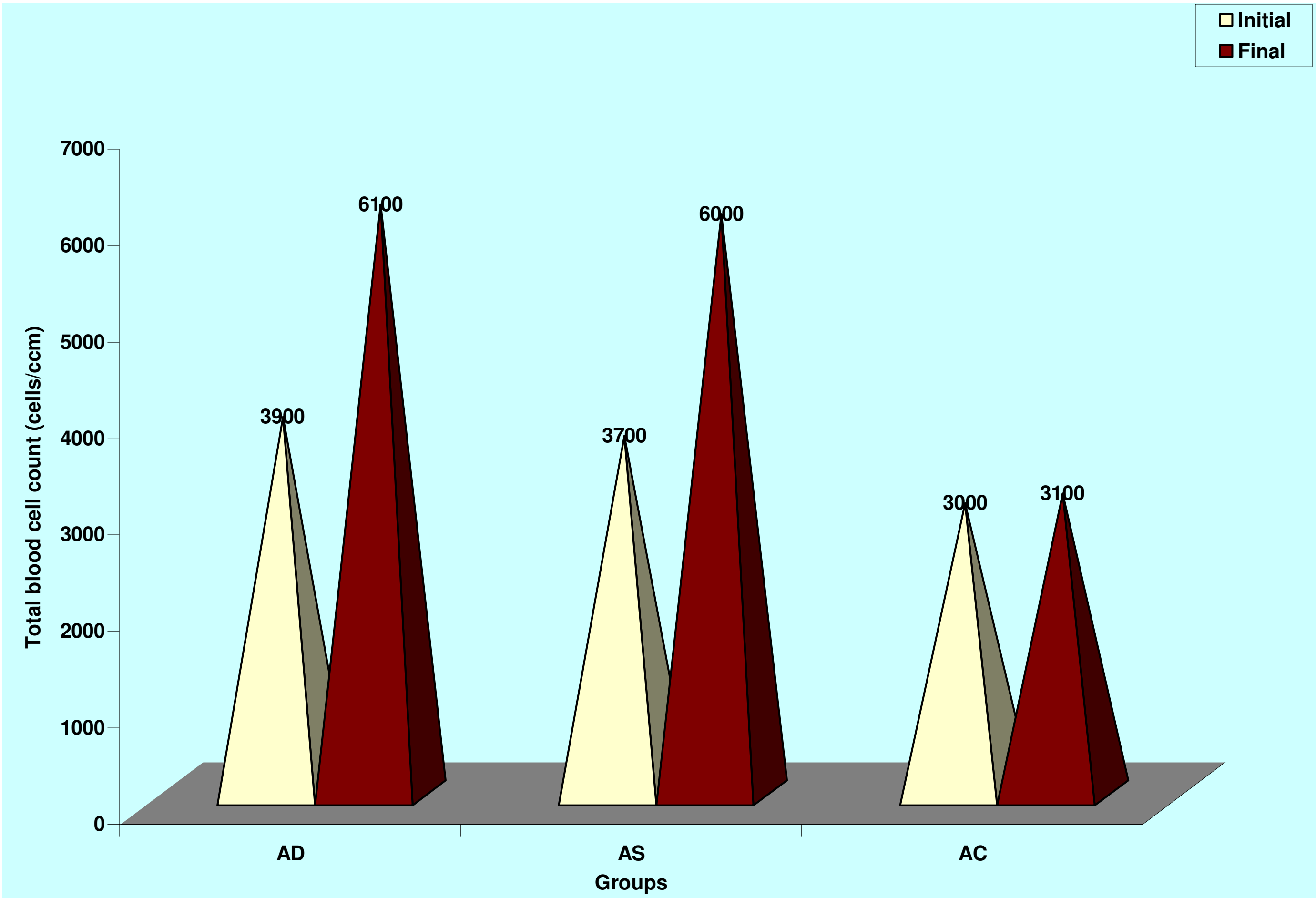

Figure 4. Mean initial and final total blood cell count of the children

levels among children on supplementation with food and tablets. The children with anemia had serum protein levels lower than the normal range (6.6 - 8.3 g/dl). The present finding agree with the finding of Agarwal et al. (1999), who stated that the serum total protein levels of children are generally lowered in cases of illness/ deficiency condition. Intervention with protein and micronutrient rich diet helps to increase the serum total protein level. On supplementation the children in AD group registered a significant increase ($p < 0.01$) in the serum total protein level which could be attributed to the high content of protein (19.56 g) in the food supplement. After six months supplementation period the children in the supplemented groups were within the normal range which indicates good nutritional status that enhanced the growth and development of children. The mean increments in the serum total protein levels of the children are represented in Figure 5.

(e) Mean serum albumin and globulin levels: Initially, the mean serum albumin levels ranged from 3.2 - 4.3 and globulin levels ranged from 2.0 - 2.8 g/dl. However no perceivable changes were observed over the six months of supplementation in the serum albumin and globulin levels and the values were within the normal range of 3.5 - 5.0 g/dl and 2.3 - 3.5 g/dl respectively.

(f) Mean serum zinc levels: The mean serum zinc level of the anemic children belonging to AD group was 49.9 mg/dl and it increased to 94.5 mg/dl after six months of supplementation. There was a significance at one per cent level as against the AS group children whose increments were significant at five percent level (from 48.3 - 89.5 mg/dl). Zinc supplementation along with other micronutrients improved the serum zinc levels in anemic children as stated by Black (2003), whose findings are similar to the findings of the present study.

The serum zinc levels in children are generally low especially in cases of infection / deficiency condition (Sivakumar et al., 2006). Their zinc intake is lower than the RDA of 8 mg /day. Since the metabolisms are altered during infection or illness, the serum zinc levels are reduced. The food supplement given to children contains adequate amount of micronutrients especially zinc and good amount of protein which might have attributed towards increasing the serum zinc levels when compared

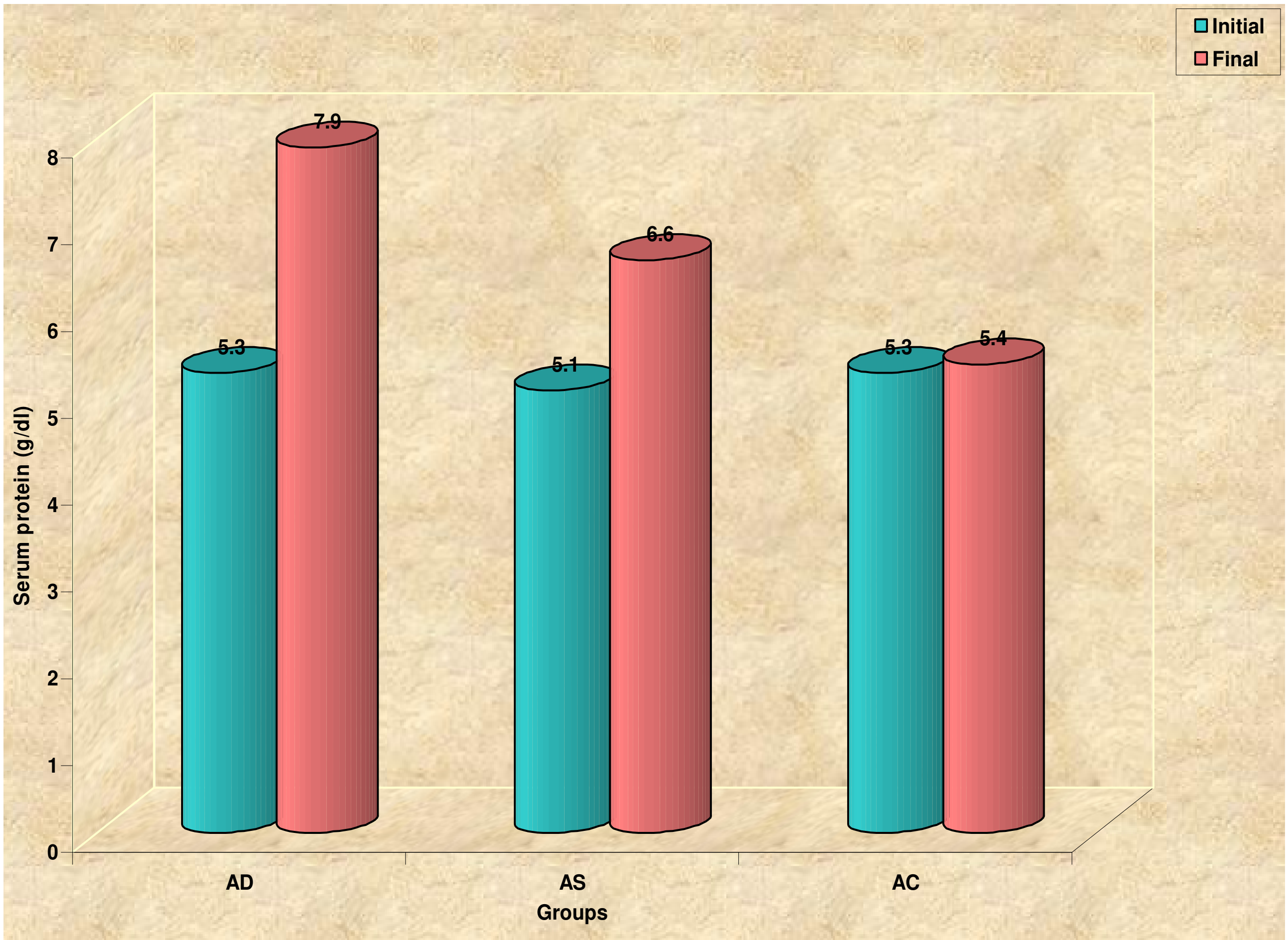

Figure 5. Mean increments in the serum total protein levels of the children.

to the children in the tablet supplemented group. The mean increment in the serum zinc levels of the children is represented in Figure 6.

(g) Changes in the serum iron levels of children with iron deficiency anemia: Mean serum iron levels of the children with iron deficiency anemia are presented in Table 4. The mean initial serum iron levels of the AD, AS and AC group children were found to be 43.7, 41.6 and 40.3 mcg/dl respectively and they were lower than the normal value range. 50 - 150 mcg/dl (ref). At the end of the supplementation study, the mean serum iron levels of the anemic children in the food supplemented group increased from 43.7 - 72.3 mcg/dl ($p < 0.01$).

The AS group children had a mean increase of serum iron level from 41.5 - 59.3 mcg/dl ($p < 0.05$). The control group children did not show any increase in their serum iron levels. The findings of the present study is in the line with the findings of Dijkhuizen et al. (2001) who reported that children who were given 10 mg of iron per day for a period of 10 months significantly helped in reducing the prevalence of anemia and in increasing the haemoglobin and serum iron levels in children.

Immune profile of the children

(a) Mean serum IgA levels of the children: The anemic children in the AD group had a maximum increase in mean serum IgA levels (from 64.9 - 129.4 mg/dl) which was highly significant ($p < 0.01$) against the AS group children whose increment levels (from 65.0 - 101.3 mg/dl) were significant at five per cent level. Iron deficiency appears to affect IgA levels and oral iron supplementation either in food or synthetic form has been associated with an improvement in the IgA levels in children as authenticated by Farthing (1989).

(b) Mean serum IgM levels of the children: The mean serum IgM levels of the anemic children in the food supplemented group improved from 26.0 - 89.7 mg/dl within six months of supplementation. Comparatively the IgM levels of children in the AS group increased from 29.3 - 77.8 mg/dl. The increments in the AD group were

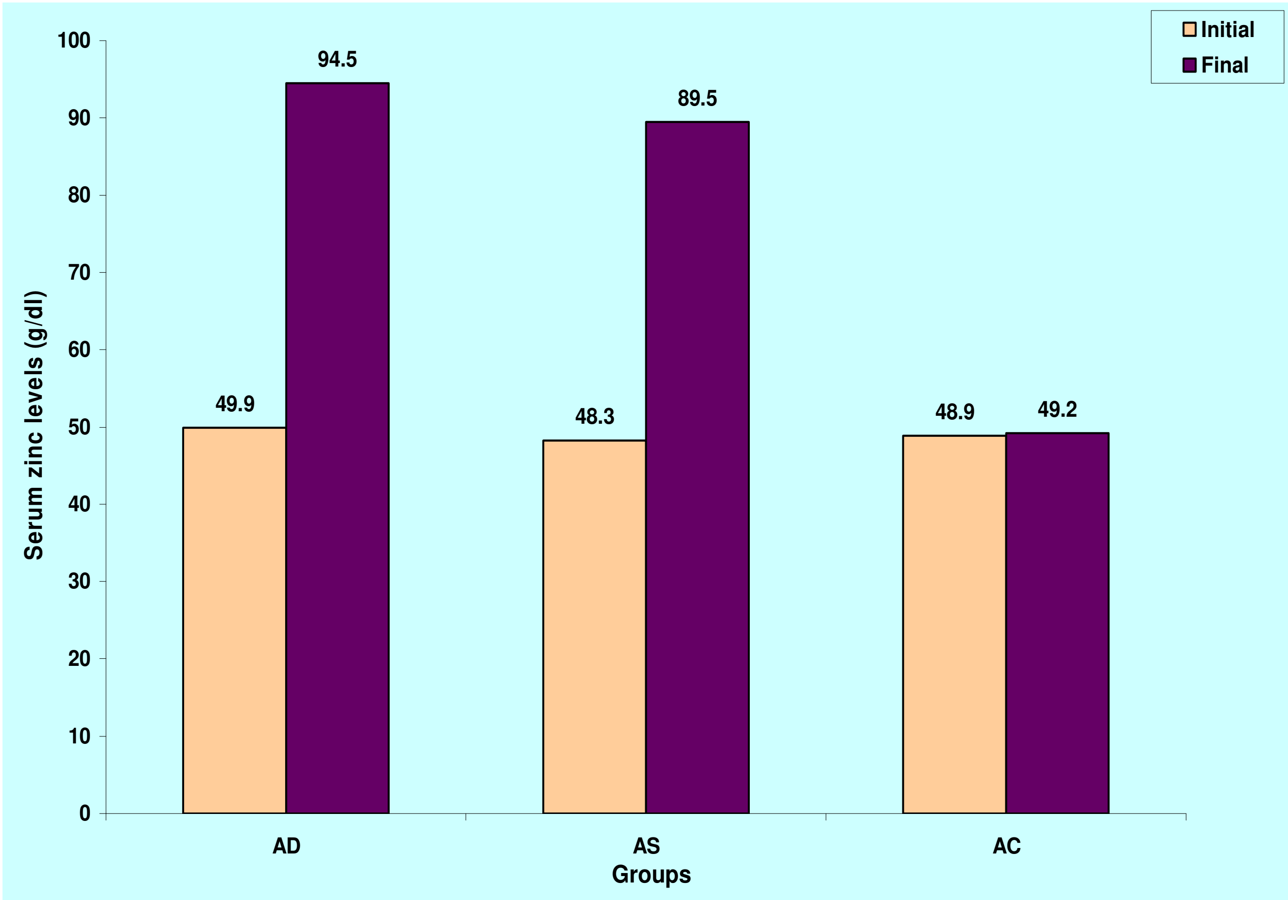

Figure 6. Mean initial and final serum zinc levels of the children.

Table 4. Mean serum iron levels of the children.

Group	Serum iron (mcg/dl) ± SD			Initial Vs Final 't' value	Groups compared	't' value
AD (20)	43.7 ± 2.7	72.3 ± 1.4	28.6 ± 2.4	39.6**	AD Vs AS	13.1**
AS (15)	41.6 ± 1.5	59.3 ± 2.0	17.7 ± 0.9	2.3*	AS Vs AC	2.7*
AC (15)	40.3 ± 1.8	41.5 ± 1.2	1.2 ± 0.5	1.7 NS	AD Vs AC	23.6**

** Significant at one per cent level; *Significant at five per cent level
NS: Not significant; Std values: 50 - 120 mcg/dl (Gupta, 1999).

significantly higher at one per cent level when compared with the AS group which was statistically significant at five per cent level. The present finding are in agreement with the findings of Bagchi et al. (1980) who reported that iron deficiency anemia in children causes an alteration in the IgM levels and oral iron supplementation improves the level of serum IgM within a period of three months.

(c) Mean serum IgG levels of the children: The anemic children in the AD group had a mean increase of 74.5 mg/dl serum IgG level and that of the AS group was 57.2 mg/dl. Iron deficiency anemia is characterised by low immune profile in children especially in the immunoglobulin levels. In a study by Sazawal, et al. (1997) 10 mg oral supplementation of iron per day improved the IgG levels in children. In the present study also improvement of serum IgG levels were registered on supplementation and it was statistically significant at 1% level in the food supplemented group and at 5% level in the group receiving synthetic supplement.

In the current study, the immune profile of children considerably increased and was within the normal range mentioned after supplementation of micronutrients. The children in the food supplemented group had greater increase in their immunoglobulin levels since the food supplement contains adequate amount of micronutrients especially zinc, iron and vitamin A. In addition, the protein and calories contributed towards a positive impact. During infection / deficiency condition immuno depression is quite common. Zinc, an immunonutrient acts as an immunomodulator which increases the IgA, IgM and IgG levels. It improves the T-cells, enhances the repair of mucous membrane of the respiratory tract and gastrointestinal (GI) tract thereby increases the defence mechanism of the respiratory tract and GI tract for eliminating the organisms and viruses. Zinc also increases phagocytosis for engulfing organisms producing infection and increases polymorphs function (Singh, 2004). The multi-roles played by zinc not only improve the serum zinc levels but also improves the immune function of the body during illness or normal condition. As a long term strategy, food based approach has proved to be beneficial in all aspects and hence in the present study zinc along with other micronutrients had helped to improve the immune status of the children irrespective of deficiency condition or illness.

Summary and Conclusion

In the present study, supplementation of micronutrients either in the food or in the tablet form resulted in significant improvement in the height, weight, lowering of morbidity, clinical picture, biochemical status, nutritional knowledge and performance in school among the children The cost of the food supplement worked out to be Rs.4.90/ child per day and that of tablet was Rs.4.75/ child per day. Though the cost of food supplement was slightly higher than that of the tablet form of supplement, the children in the food supplemented groups showed significantly greater improvements in all the parameters tested. Since the food supplement contains additional nutrients especially, protein and calories the impact was great. In addition, sound nutrition knowledge imparted to mothers and children helped to promote their home food intake. The findings of this research leads to the conclusion beyond doubt that the food based approach will serve as an effective strategy to combat multiple micronutrient deficiencies, promote health and well-being of the children and ensure global nutrition security.

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