Journal of Scientific Research & Reports

15(1): 1-13, 2017; Article no.JSRR.34470
ISSN: 2320-0227

A Comparative Study of Natural vs. Synthetic Vitamin C Supplementation to Prevent Anemia

Sukhdeep Kaur[1*] and Jasvinder Kaur Sangha[1#]

[1]Department of Food and Nutrition, Punjab Agricultural University, Ludhiana, India.

Authors' contributions

This work was carried out in collaboration between both authors. Authors SK and JKS designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Author SK managed the analyses of the study and the literature searches. Both authors read and approved the final manuscript.

Article Information

DOI: 10.9734/JSRR/2017/34470
Editor(s):
(1) Luigi Rodino, Professor of Mathematical Analysis, Dipartimento di Matematica, Università di Torino, Italy.
Reviewers:
(1) Maria Bernardita Puchulu, University of Buenos Aires, Argentina.
(2) Alicia García Falgueras, The Official College of Psychologists, Spain.
(3) Fethi Ben Slama, National Institute of Public Health, Tunisia.
(4) Dhiraj J. Trivedi, SDM College of Medical Sciences and Hospital, India.
Complete Peer review History: http://www.sciencedomain.org/review-history/20142

Original Research Article

Received 29th May 2017
Accepted 15th July 2017
Published 20th July 2017

ABSTRACT

Aim: To assess the nutritional status of anaemic girls through anthropometric, biochemical, dietary and clinical assessment; and to study the efficacy of weekly iron and vitamin C supplementation on the haematological profile of the anaemic subjects.
Study Design: An interventional school-based study.
Methodology: The study was conducted on the total sample of sixty adolescent girls aged 16-17 years. The research participants were divided into two groups of thirty each according to the supplementation provided i.e. Iron Folic Acid supplementation along with Lemon water (ILW group) and Iron Folic Acid supplementation with synthetic Vitamin C (IVC group). Both the groups were provided supplementation for three months at weekly intervals.
Results: There was no change in the anthropometric parameter of the research participants after the study. Consumption of cereals, pulses, green leafy vegetables and milk and milk products was significantly ($p \leq 0.01$) increased in both the groups after the study. Consequently, a significant increase ($p \leq 0.01$) in the intake of energy, protein, riboflavin, niacin, calcium, iron and vitamin C was observed in both the groups. After the study, reduction in the percentage of the research

**Corresponding author: Email: greenmona29@gmail.com;*
#Retired

participants with signs and symptoms of anemia was observed in all the research participants. Hematological profile of the research participants indicated that increase in the hemoglobin level was higher in the group ILW (9.95 ± 0.11 to 11.02 ± 0.09 g/dl) as compared to the group IVC (9.88 ± 0.10 to 10.80 ± 0.08g/dl).

Conclusion: Weekly iron supplementation along with natural source of vitamin C is more beneficial than synthetic vitamin C, to restore normal levels of red blood cells, hemoglobin, and iron; thus preventing iron deficiency anemia in young adolescent girls. Future research may benefit from attempting to develop, analyzed supplement vitamin C rich food products to the anemic individuals.

Keywords: Iron; anemia; supplementation; vitamin C; nutritional status; adolescent girls.

1. INTRODUCTION

Anemia is known to be a significant nutritional problem affecting people in both developed and developing countries with serious economic consequences to national development. Globally, the most significant contributor to the onset of anemia is iron deficiency; so that Iron Deficiency Anemia (IDA) and anemia are often used synonymously [1]. Anemia is a systemic condition, characterized by reduction in the red blood cell volume or decrease in the concentration of hemoglobin (Hb) in the blood, due to micronutrient deficiencies such as iron, folic acid and vitamin B_{12}; or due to malaria, hookworm infections, blood loss from chronic diseases as tuberculosis, ulcers or intestinal disorders, menstruation and child birth [2]. It occurs at all stages of the life cycle, but is more prevalent in pregnant women and young children. Globally, the prevalence of anemia among school age children is 25.4%, and about 47.7% of preschool children, and an estimated 41.8% of pregnant women suffers from this disease [3]. The main risk factors for IDA include inadequate iron intake, impaired absorption of iron from diets, and increased iron requirements especially during growth and pregnancy.

Adolescence is the transition period between childhood and adulthood; a window of opportunities to inculcate healthy eating habits. During this time, physical changes affect the body's nutritional needs, while changes in one's lifestyle may affect eating habits and food choices. Adolescents are particularly susceptible to IDA in view of their increased blood volume and muscle mass during growth and development [4]. Furthermore, a major threat to safe motherhood, IDA contributes to low birth weight, reduced immune-competence, poor cognitive development, behavioral complications, decreased work capacity and increased risk of pregnancy complication including prematurity and total growth retardation. Other signs and symptoms of iron deficiency anemia includes constipation, sleepiness, tinnitus, hair loss, feeling faint, and depression, twitching muscles, tingling, numbness, or burning sensations, glossitis, angular chelitis, missed or heavy menstrual cycle, slow social development, poor appetite, pica and koilonychias [5].

Dietary iron is available in two chemical forms: heme iron (non veg foods) and non-heme iron (plant and dairy foods). The absorption of non-heme iron from vegetarian diet is poor in comparison to heme iron. The bioavailability of non-heme iron requires acid digestion and varies by an order of magnitude depending on the concentration of enhancers (e.g., ascorbate, citrate, amino acids) and inhibitors (e.g., calcium, fiber, phytate, tannins, antacids) found in the diet. Intake of iron absorption inhibitors results in depleted iron stores. Though supplementation with iron and folic acid remains cornerstone in the treatment and prevention of anemia, addition of vitamin C has its other added advantage [6]. Ascorbic acid (vitamin C) reverses the effect of dietary inhibitors and is one of the most powerful known promoters of non-haeme iron absorption, which forms the bulk of Indian's diet [7]. Adolescent girls, due to blood loss during onset of menstruation, are at higher risk to develop anemia, especially if they don't get enough iron in their diets [8]. Considering the role of vitamin C in iron absorption, the study attempts to examine the efficacy of vitamin C supplementation on the haematological profile of the anaemic adolescent girls.

2. METHODOLOGY

2.1 Selection of the Research Participants and Location of the Study

The study was conducted in Government Senior Secondary School, Ludhiana (Punjab, India).

Initially, 150 adolescent girls in the age group of 16-17 years were randomly selected and screened for the haemoglobin values, out of which 87 girls were found to be anaemic. Finally, from these 87 anaemic girls, sixty girls were randomly selected for the study and were divided into two groups of 30 each, according to the supplementation provided to them.

2.2 Development of Questionnaire

A well-structured questionnaire-cum-interview schedule was developed to elicit the general information and socioeconomic status of the research participants. The preliminary interview schedule was pre-tested on 20 girls in the age group of 16-17 years to ensure the validity of questionnaire. Thereafter, necessary modifications were incorporated. The modified questionnaire was then used in the present study. These research participants were excluded from the final study sample. Information pertaining to food intake of the respondents was recorded by 24-hour recall method. The nutrient intake was calculated using MSU nutriguide computer programme [9]. The nutrient intakes were compared with RDA by ICMR [10] and percentage adequacy of the various foods and nutrients was calculated before and after the study.

2.3 Procurement of Lemon

PAU Baramasi variety of Lemon was procured from PAU, Ludhiana. Lemons were then washed and squeezed to make lemon water and were supplemented to the research participants along with iron and folic acid tablets (ILW group).

2.4 Supplementation of the Groups

The subject's willingness to take part in the supplementation trial was ascertained first. The research participants were then provided iron-folic acid supplements along with natural or synthetic source of vitamin C. Diet of group ILW was supplemented with iron-folic acid tablet i.e. fefole containing 150 mg of dried ferrous sulphate + 500 µg folic acid, along with a glass of lemon water (2 medium sized lemons in one glass of water, containing 50 mg of vitamin C) and diet of group IVC was supplemented with same iron-folic acid tablet along with synthetic vitamin C (tablets), i.e. citracee containing 50 mg of vitamin C. De-worming of all the research participants with single dose of albendazole (400 mg) was done in the beginning of the study. Both the groups were provided supplementation for three months at weekly intervals.

2.5 Assessment of Nutritional Status

Nutritional status of the research participants was assessed through their dietary intake, anthropometric measurements, clinical examination and hematological profile.

2.5.1 Anthropometric parameters

Two basic anthropometric parameters i.e. height and weight were measured before and after the study, using standard method given by Jellife [11]. The body mass index (BMI) was measured using standard formula and the research participants were classified on the basis of BMI classification given by WHO [12].

Body Mass Index: The body mass index was obtained using the following equation.

$$BMI = \frac{\text{Weight (Kg)}}{\text{Height } (m^2)}$$

2.5.2 Clinical parameters

Information on various symptoms of anemia like paleness of skin, pale conjunctiva, paleness and smoothness of tongue, flat or spoon shaped nails, etc. were recorded, as prescribed by Jeliffe [11]. Clinical examination for the signs/symptoms of anaemia and haemoglobin estimation of the research participants was performed by the physician.

2.5.3 Hematological profile

The biochemical parameters of the research participants i.e. hemoglobin (Hb), haematocrit and red blood cell count (RBC), were estimated using standards methods given by Dacie and Lewis [13], Raghuramulu et al. [14] and Hunter and Bomford [15], respectively. From these three analyses, the erythrocyte indices were calculated that includes mean corpuscular hemoglobin concentration (MCHC) and mean corpuscular volume (MCV). The classification of anaemia as recommended by WHO [16] and NIN [17] was followed for categorization of the research participants.

2.6 Statistical Analysis

The data on food and nutrient intake, anthropometric measurements and blood analysis of the research participants was analyzed statistically using SPSS Windows version 16.0 (SPSS Inc., USA). The mean, standard error and t-test were calculated using standard methods given by Singh et al. [18].

Paired t-test: It was used to study the statistical differences in the means of average dietary intake, anthropometric parameters and biochemical parameters before and after the study.

$$t = \frac{d}{s/\sqrt{n}}$$

$d = \overline{X}_1 - \overline{X}_2$
$\overline{X}_1$ = Mean of sample 1
$\overline{X}_2$ = Mean of sample 2
s = Standard Deviation
n = Total number of subjects in the sample

2.7 Ethical Approval

Consent to conduct the survey on the students was also ascertained from the parents through the school authorities.

3. RESULTS AND DISCUSSION

3.1 General Information

It was observed that majority of the research participants belonged to nuclear family and rest were from joint family. Over half (55%) of the research participants belonged to families having up to 4 members, followed by those (28%) belonging to families having 4–8 members; while 17% had above 8 members in their families. Regarding father's education, it was seen that most of the fathers were educated above higher secondary; while rest were either graduates or postgraduates (30 and 17%, respectively). Similarly, majority (58%) of the mothers were educated above higher secondary, whereas, 37 and 5% were graduates and postgraduates, respectively. Regarding occupation of the fathers, it was observed that most of the fathers (75%) were engaged in agriculture, followed by, service (17%) and business (8%); while, mothers were mostly housewives (86%) and few were involved in service and self-employed (7% each). The monthly family income of about 65% of the research participants ranged between Rs 5000-7000, 27% had Rs 7000-9000 and 8% had income up to Rs 5000 (Table 1).

3.2 Assessment of Nutritional Status

3.2.1 Food habits

Majority of the research participants (80%) were vegetarian and only 12% were meat eaters. Seventy percent of the research participants had three meals, whereas 26% had two meals and only 4% of the research participants consumed four meals a day. It was observed that 65% of the research participants skipped breakfast, 10% skipped dinner, and 20% skipped lunch; while 5% had regular meal.

Table 1. General information of the research participants (N = 60)

Parameters	Percentage
Age (years)	
16	70
17	30
Type of family	
Joint	20
Nuclear	80
Family size	
Up to 4	55
4-8	28.3
Above 8	16.7
Father's education	
Above higher secondary	53.3
Graduate	30
Post graduate	16.7
Mother's education	
Above higher secondary	58.3
Graduate	36.7
Post graduate	5
Father's occupation	
Agriculture	75
Service	16.7
Business	8.3
Mother's occupation	
Housewife	86
Service	6.7
Self-employment	6.7
Family income	
< 5000	8.3
5000 to 7000	65
7000 to 9000	26.7

3.2.2 Food intake

The mean daily foods intake of the research participants is presented in Table 2(a). Per cent adequacy of food intake by the research participants before and after the study is given in Table 2(b).

Cereals: The mean daily intake of cereals was reported to be less than the recommended intake range of 300 g as suggested by ICMR (1999). The percent adequacy of cereal consumption before and after the study was 84, 82.66 and 92.66, 89% in the group ILW and IVC, respectively. It was observed that the consumption of cereals was statistically significant ($p \leq 0.01$) in both the groups.

Pulses: The percent adequacy of pulses consumption by the research participants of group ILW and IVC before the study was 98.33 and 95%, respectively. After the study, it increased to 106.66 and 103.33%, respectively of the suggested intakes of ICMR (1999). It was found that after the study, the mean intake of pulses was statistically significant ($p \leq 0.01$) in both the groups. Similar results have been reported by Singh et al. [19].

Green Leafy Vegetables (GLVs): It was observed that the mean daily intake of green leafy vegetables was statistically significant ($p \leq 0.01$) in all the groups. The percent adequacy of green leafy vegetables before and after the study increased from 37 and 35% to 47 and 45%, respectively in ILW and IVC group. The data revealed that there was inadequate consumption of GLVs, before and after the study, when compared to the recommended intake range of 100 g as suggested by ICMR (1999). Inadequate consumption of green leafy vegetables was also reported in other studies [20,21,22].

Roots and tubers: The consumption of roots and tubers was statistically significant in both the groups ($p \leq 0.01$). The percent adequacy of roots and tubers intake by the research participants of group ILW and IVC, beforehand after the study was 86, 85% and 94 and 93%, respectively of the recommended intake of 100 g as given by ICMR (1999), indicating the inadequate consumption of roots and tubers by the research participants. Bala [20] also reported inadequate consumption of Roots and Tubers.

Other vegetables: The percent adequacy of other vegetables before and after the study was 132, 130% and 142 and 140%, respectively in the group ILW and IVC. Above data indicated that consumption of other vegetables was more than the recommended intake of 100 g as suggested by ICMR (1999) both before and after the study by the research participants of both the groups. Similar results have been reported by Agrahar and Pal [23].

Fruits: The percent adequacy of fruit consumption by the research participants of group ILW and IVC before and after the study was 53, 51% and 59 and 56%, respectively. The increase in the intake of fruits in both the group ($p \leq 0.01$) was statistically significant. Similar results have been reported by Mittal and Srivastava [24].

Milk and milk products: The percent adequacy of milk and milk products before and after the study was 77, 76% and 88 and 87%, respectively in the group ILW and IVC. From the data it was found that the consumption of milk and milk products was lower than the suggested intake of 500 g (ICMR, 1999).

Fats and oils: The consumption of fats and oils in the group ILW & IVC ($p \leq 0.01$) was statistically significant. After the study, the percent adequacy increased from 111.2, 105.2% to 114.8 and 109.6%in the group ILW and IVC, respectively. Similar results have been reported by Malhotra and Passi [25].

Sugar and jaggery: Statistically, no significant improvement in the consumption of sugar and jaggery was observed in the group ILW and IVC. The percent adequacy of sugar and jaggery in the group ILW and IVC before and after the study was 83.33, 83.33% and 80 and 80%, respectively of the ICMR recommendations (1999). Inadequate intake of Sugar and jaggery was also reported by Bala [20] and Rao et al. [26].

Meat, egg and fish: It was observed that the consumption of egg was at least thrice a week and of meat once a week, whereas the consumption of fish was negligible in almost 90% of the research participants. The percent adequacy, before and after the study was 40, 46.66% and 43.33 and 56.66% in the group ILW and IVC, respectively. It was observed that the intake of meat, egg and fish before and after the study was below the standard value of 30 g as suggested by ICMR (1999). The consumption of meat, egg and fish was statistically non-significant in ILW group. However, in the group IVC, the consumption of meat, egg and fish was statistically significant ($p \leq 0.05$).

As shown in Table 2(b), although there was slight increase in the intake of all the food groups after the study, the percent adequacy of cereal, roots and tubers, fruits, milk and milk products sugar and jaggery was still lower than that suggested by ICMR (1999); whereas, the percent adequacy of pulses, other vegetables, fats and oils consumption of both the groups was more than the recommended intake range suggested by ICMR (1999). The percent adequacy of green leafy vegetables, meat, egg and fish was far lower than the recommended intake range of ICMR (1999).

3.2.3 Nutrient intake

The mean daily nutrient intake of the research participants is presented in Table 3(a). Per cent adequacy of nutrient intake by the research participants before and after the study is given in Table 3(b). The percent contribution of carbohydrates, protein and fat to the total energy intake is presented in Table 4.

Energy, protein and total fat: The percent adequacy of energy intake among the research participants of group ILW, and IVC before and after the study was 81.94, 79.80% and 91.01, 89.17%, respectively. Inadequate energy intake was also reported by Singh et al. [19]. The percent adequacy of protein intake before the study was 98.41 and 96.82%, respectively. After the study, the percent adequacy increased to 109.52 and 107.93%, respectively in the group ILW, and IVC. This increase was due to increased consumption of milk and milk products, cereals and pulses. Similar results have been reported by Gautam et al. [27] and Srimani et al. [22]. The initial and final percent adequacy of fat intake was 141.75, 136% and 155 and 152.75%, respectively in the group ILW and IVC.

Vitamins: Initially, the percent adequacy of thiamine was 166 and 159%, respectively in the group ILW and IVC. The final percent adequacy in the group ILW and IVC increased to 189 and 181%, respectively, indicating increase in the consumption of thiamine intake due to higher intake of cereals and pulses by all the research participants after the study. The percent adequacy of riboflavin intake before the study was 130, 116.6%, while after the study, it was 145.83 and 141.66% in the group ILW and IVC, respectively. As compared to ICMR (1999) recommendations, the intake of riboflavin was higher in both the groups before and after the study. The percent adequacy of niacin intake by the research participants of group ILW and IVC before and after the study was 80, 77.85% and 104.28 and 97.14%5, respectively. There was higher intake of niacin after the study. Similar results have been reported by Laxmaiah et al. [28]. The percent adequacy before the study was 133.6 and 127.0%, while after the study; it was 154 and 147%, respectively in the group ILW and IVC. The percent adequacy before and after the study, was found to be 131, 107% and 155.5 and 136% in the group ILW and IVC, respectively. After the study, the percent adequacy was higher than the ICMR (1999) recommendations of 40 mg vitamin C. The percent adequacy of vitamin B_{12} intake by the research participants in the group ILW and IVC before and after the study was 65, 47% and 111, 96%, respectively.

Minerals: Increase in the intake of iron was due to increased consumption of green leafy vegetables, root tops of other leafy vegetables, cereals, pulses and non-veg food. The initial and final percent adequacy of iron intake was 57.33, 53.33%and 61.66 and 58.33%, respectively in the group ILW, and IVC. It was observed that the intake of iron was lower than the recommended value of 30 mg as suggested by ICMR (1999). Grossly inadequate intake of iron was also reported by Rao et al. [26]. The percent adequacy before the study was 143.04 and 138.26%, while after the study it was 160.64 and 154.36%, respectively in the group ILW and IVC indicating that the intake of calcium was higher both before and after the study by all the research participants as compared to that suggested by ICMR (1999). The increased intake of calcium was due to more consumption of milk and milk products, pulses and GLVs. Similar results have been reported by Agrahar and Pal [23].

From the data recorded on the nutrient intake of the research participants, it can be concluded that, the mean daily intake of energy, protein, iron, niacin and vitamin B_{12} was below the RDAs in both the groups; whereas the intake of riboflavin, folic acid and calcium was above the RDAs in both the groups before and after the study. The intake of vitamin C was above the RDA in the group ILW and IVC before and after the study. The intake of energy, protein, thiamine, riboflavin, niacin, folic acid, vitamin C, vitamin B_{12}, calcium and iron was higher in the group ILW as compared to the group IVC. Similarly, the dietary profile of rural preschool children and school girls (13 – 15 years) of Punjab was assessed and it was revealed that the intake of macro and micronutrient rich foods such as cereals, pulses and green leafy vegetables, milk and milk products and fats and oils were lower than the RDAs among preschool children. Except for protein, calcium and thiamine, the mean intake of all the nutrients was lower than the recommended levels [28].

Table 2(a). Mean daily food intake of the subjects before and after the study (Mean ± SD)

Food group (g)	Group	Before	After	t value	ICMR (1999)
Cereals	ILW	252 ± 6.5	278 ± 6.6	4.738**	300 g
	IVC	248 ± 5.5	267 ± 4.7	3.266**	
Pulses	ILW	59 ±0.8	64 ± 0.7	4.738**	60 g
	IVC	57 ± 0.6	62± 0.9	4.174**	
Green leafy vegetables	ILW	37 ± 1.3	47 ± 1.3	6.509**	100 g
	IVC	35 ± 1.2	45 ± 1.7	7.860**	
Root & tubers	ILW	86 ± 2.5	94 ± 1.9	4.020**	100 g
	IVC	85 ± 2.5	93 ± 1.7	3.889**	
Other vegetables	ILW	132 ± 1.7	142 ± 1.4	4.703**	100 g
	IVC	130 ± 2.4	140 ± 3.0	3.485**	
Fruits	ILW	53 ± 2.2	59 ± 1.7	3.900**	100 g
	IVC	51 ± 1.4	56 ± 1.1	3.089**	
Milk and milk products	ILW	385 ±11.3	440 ± 6.7	5.100**	500 g
	IVC	380 ± 8.8	435 ± 8.3	6.670**	
Fats & oils	ILW	27.8 ± 0.6	28.7 ± 0.5	3.164**	25 g
	IVC	26.3 ± 0.3	27.4 ± 0.3	4.364**	
Sugar & jaggery	ILW	25± 0.46	24 ± 0.4	1.000	30 g
	IVC	25 ± 0.48	24 ± 0.3	1.409	
Meat, egg and fish	ILW	12 ± 2.6	13 ± 2.5	1.439	30 g
	IVC	14 ± 3.0	17 ± 2.8	2.164*	

*** Significant at p<0.001*
ICMR (Indian Council of Medical Research)
ILW (iron-folic acid tablet + lemon water)
IVC (iron-folic acid tablet + vitamin C tablet)
n=30 each (ILW and IVC)

Table 2(b). Percent adequacy of food intake by the research participants before and after the study (%)

Food group	ILW		IVC	
	Before	After	Before	After
Cereals (g)	84	93	83	89
Pulses (g)	98	107	95	103
GLVs (g)	37	47	35	45
Root & tubers (g)	86	94	85	93
Other vegetables (g)	132	142	130	140
Fruits (g)	53	59	51	56
Milk and milk products(g)	77	88	76	87
Fats &oils (g)	111	115	105	110
Sugar & Jaggery (g)	83	80	83	80
Meat, fish and egg (g)	40	43	47	57

ILW (iron-folic acid tablet + lemon water)
IVC (iron-folic acid tablet + vitamin C tablet)
n=30 each (ILW and IVC)

As shown in Table 3(b), the percent adequacy of energy was lower as compared to the standard value of ICMR (1999); whereas, percent adequacy of protein and fat was higher than that suggested by ICMR (1999). The percent adequacy of thiamine, riboflavin, folic acid, vitamin C and calcium was higher in both the groups after the study. The per cent adequacy of niacin and vitamin B_{12} was marginally lower than the ICMR recommendations; whereas, the intake of iron was far lower than the recommended value suggested by ICMR (1999). Nutritional iron deficiency arises when physiological requirements cannot be met by iron absorption from the diet. Dietary iron bioavailability is low in populations consuming monotonous plant-based diets [29].

Table 3(a). Mean daily nutrient intake of the subjects before and after the study (Mean ± SD)

Nutrient	Group	Before	After	t value	ICMR (1999)
Energy (kcal)	ILW	1688 ± 13.8	1875 ± 13.9	8.826**	2060 kcal
	IVC	1644 ± 20. 1	1837 ± 22.2	6.797**	
Protein (g)	ILW	62.0 ± 1.3	69 ± 1.2	5.461**	63 g
	IVC	61.0 ± 1.8	68 ± 1.7	4.634**	
Total fat (g)	ILW	56.7 ± 1.3	62 ± 0.9	2.851**	40 g
	IVC	54.4 ± 0.9	61.1 ± 1.0	5.277**	
Thiamine (mg)	ILW	1.66 ± 0.0	1.89 ± 0.1	4.458**	1 mg
	IVC	1.59 ± 0.0	1.81 ± 0.1	4.164**	
Riboflavin(mg)	ILW	1.56 ± 0.0	1.75 ± 0.1	3.697**	1.2 mg
	IVC	1.44 ± 0.0	1.70 ± 0.1	4.370**	
Niacin (mg)	ILW	11.2 ± 0.1	14.6 ± 0.1	13.66**	14 mg
	IVC	10.9±0.1	13.6 ± 0.2	8.695**	
Folic acid (µg)	ILW	133.6 ± 2.4	154.2 ± 2.8	6.600**	100 µg
	IVC	127.0 ± 1.7	147.6 ± 3.4	6.214**	
Vitamin C (mg)	ILW	52.4 ± 1.2	62.2 ± 1.4	5.768**	40 mg
	IVC	42.8 ± 1.5	54.4 ± 1.9	8.338**	
Vitamin B_{12} (µg)	ILW	0.65 ±0.1	1.11± 0.1	6.171**	1 µg
	IVC	0.58±0.1	0.96 ± 0.1	3.744**	
Calcium (mg)	ILW	715.2 ± 8.7	803.2 ± 8.3	7.273**	500 mg
	IVC	691.3 ± 16.6	771.8 ± 12.3	5.022**	
Iron (mg)	ILW	17.2 ± 0.4	18.5 ± 0.4	6.740**	30 mg
	IVC	16.0 ± 0.4	17.5 ± 0.4	6.934**	

*** Significant at p<0.01*
ICMR (Indian Council of Medical Research)
ILW (iron-folic acid tablet + lemon water)
IVC (iron-folic acid tablet + vitamin C tablet)
n=30 each (ILW and IVC)

Table 3(b). Percent adequacy of nutrient intake by the research participants before and after the study (%)

Nutrient	ILW		IVC	
	Before	After	Before	After
Energy (kcal)	82	91	80	89
Protein (g)	98	110	97	108
Total fat (g)	142	155	136	153
Thiamine (mg)	166	189	159	181
Riboflavin (mg)	130	146	117	142
Niacin (mg)	80	104	78	97
Folic acid (µg)	134	154	127	147
Vitamin C (mg)	131	156	107	136
Vitamin B_{12} (µg)	65	111	47	96
Calcium (mg)	143	161	138	154
Iron (mg)	57	62	53	58

ILW (iron-folic acid tablet + lemon water)
IVC (iron-folic acid tablet + vitamin C tablet)
n=30 each (ILW and IVC)

3.2.4 Anthropometric profile

The overall mean height, weight and BMI of the research participants are presented in Table 5. Distribution of the research participants according to WHO BMI index [12] is presented in Table 6. No significant difference was found in the mean height, weight of the research participants after the study in both the groups. There was no change in the mean BMI of the research participants after the study in the group IVC. However, a significant difference was found ($p \leq 0.05$) in the mean BMI of the research participants in the group ILW after the study.

Thus, it can be concluded that no significant improvement was observed in the anthropometric parameter of the research participants after the study. Ghosh and Bala [30] also reported a significantly ($p < 0.05$) greater proportion of females in the lowest BMI group. Almost similar results have been reported in the present study.

Table 4. Per cent contribution of carbohydrates, protein and fat to the total energy intake

Nutrient (g)	ILW		IVC	
	Percentage			
	Before	After	Before	After
Carbohydrates	54.7	55.4	55.4	55.3
Protein	14.6	14.7	14.8	14.8
Total Fat	30.2	29.7	29.7	29.9

ILW (iron-folic acid tablet + lemon water)
IVC (iron-folic acid tablet + vitamin C tablet)
n=30 each (ILW and IVC)

Table 5. Anthropometric profile of the subjects before and after the study (Mean ± SD)

Group	Variable	Before	After	t value	Jellife (1966)
Height (cm)	**Age**				
ILW	16	150.7± 1.6	150.8 ± 1.6	1.250	162 cm
	17	154 ± 4.4	154 ± 4.4	-	163 cm
IVC	16	156.9± 1.3	157 ± 1.2	1.000	162 cm
	17	154.2± 2.1	154.2 ± 2.1	-	163 cm
Weight (kgs)					
ILW	16	41.7± 1.1	41.9± 1.0	0.382	53 kg
	17	43.5± 1.6	43.6± 1.5	1.000	54 kg
IVC	16	45.7± 1.2	45.8 ± 1.3	1.000	53 kg
	17	42.3± 1.3	42.7 ± 1.3	1.491	54 kg
BMI (Kg/m^2)	ILW	18.5± 0.4	18.6 ± 0.4	2.675*	18.5-24.99 @
	IVC	18.2± 0.3	18.2 ± 0.3	0.754	

** Significant at p<0.05*
@ WHO (2005)
ILW (iron-folic acid tablet + lemon water)
IVC (iron-folic acid tablet + vitamin C tablet)
n=30 each (ILW and IVC)

Table 6. Distribution of the subjects according to BMI index (WHO, 2005)

Categories of body mass index (kg/m^2)	Risk of co-morbidity	ILW		IVC	
		Frequency			
		Before	After	Before	After
Underweight (< 18.5)	Low (but risk of other clinical problems)	17 (56.6)	16 (53.3)	18 (60)	18 (60)
Normal (18.5-24.99)	-	13 (43.3)	14 (46.6)	12 (40)	12 (40)
Overweight (≥ 25.00)	Average	-	-	-	-
Pre obese (25-29.99)	Increased	-	-	-	-
Obese I (30-34.99)	Moderate	-	-	-	-
Obese II (35-39.99)	Severe	-	-	-	-
Obese III (≥40)	Very severe	-	-	-	-

Figures in parentheses represent percentages.
ILW (iron-folic acid tablet + lemon water)
IVC (iron-folic acid tablet + vitamin C tablet)
n=30 each (ILW and IVC)

3.2.5 Clinical symptoms of anemia

The clinical symptoms of anemia among the research participants are presented in Table 7. All the research participants showed clinical symptoms of anemia before the study. In ILW group, before the study, higher percentage of research participants were found with symptoms like headache (77%) followed by lethargy (43%), pale skin (40%), breathlessness (27%), loss of appetite (57%) and pale conjunctiva (17%); whereas, after the study, reduction in percentage of the research participants with the signs and symptoms of anemia was observed and the respective percentages were 43, 23, 27, 10, 13 and 7%. On the other hand, in IVC group, before the study higher percentage of research participants were found with symptoms like lethargy (80%) followed by headache (63%), breathlessness (43%), pale skin (30%), pale conjunctiva (23%) and loss of appetite (13%); whereas, after the study, these signs and symptoms of anemia reduced to 36, 33, 17, 13, 10 and 0%, respectively. Jarrah et al. [31] also identified daily symptoms like dizziness, fatigue, depression and headaches in more than 50% of anemic female students. The single most powerful independent explanatory factor of fatigue was anaemia, implicating a need for interventional studies.

3.2.6 Hematological profile

The mean hemoglobin (Hb), haematocrit, red blood cell (RBC) count, mean corpuscular hemoglobin concentration (MCHC) and mean corpuscular volume (MCV) of the research participants are presented in Table 8. Distribution of the research participants according to NIN (1986) classification is presented in Table 9. According to WHO (1972) and NIN (1986) classification, the mean Hb level of all the research participants in the group ILW and IVC was below the standards (≥ 12), or were anemic; while after the study 6.66% of the research participants in the group ILW were found non anemic. A statistically significant ($p \leq 0.01$) difference was observed in the mean Hb levels of all the research participants after the study. Non-significant difference was found after the study in the mean values of MCHC and MCV of the research participants in both the groups. The mean values for the Hb, haematocrit and RBC of the research participants were higher in the group ILW as compared to IVC. Akramipour et al. [32] also reported lower than normal blood hemoglobin levels in the adolescent girls in Western Iran. Similarly, Deshmukh et al. [33] studied the effectiveness of weekly supplementation of iron among adolescent girls and concluded that the overall prevalence of anaemia came down significantly to 54.3% from 65.3%; and a significant rise in the mean haemoglobin levels was seen among tribal and rural girls. Ansari et al. [34] suggested that women of childbearing age should be provided nutritional education regarding food sources of iron, especially prior to becoming pregnant, and taught how food choices can either enhance or interfere with iron absorption. Furthermore, there is a need for routine iron supplementation for all groups of children to decrease the wide prevalence of iron deficiency.

Table 7. Clinical symptoms of anemia among the subjects before and after the study

Clinical symptoms	ILW		IVC	
	Percentage			
	Before	After	Before	After
Pale conjunctiva	17	7	23	10
Pale skin	40	27	30	13
Flat nails in fingers	-	-	3	3
Loss of appetite	57	13	13	-
Headache	77	4	63	33
Lethargy	43	2	80	37
Breathlessness	27	10	43	17

ILW (iron-folic acid tablet + lemon water)
IVC (iron-folic acid tablet + vitamin C tablet)
n=30 each (ILW and IVC)

Table 8. Hematological profile of the subjects before and after the study (Mean ± SE)

Parameter	Group	Before	After	t value	Reference
Hb (g/dl)	ILW	9.9 ± 0.1	11.0 ± 0.1	15.715**	≥ 12 @
	IVC	9.8 ± 0.1	10.8 ± 0.1	10.737**	
Haematocrit (%)	ILW	32± 0.4	35.4 ± 0.4	5.870**	37 – 48 #
	IVC	32.6± 0.6	35.5 ± 0.3	5.591**	
RBC (million/m^3)	ILW	3.7 ± 0.1	4.1 ± 0.1	4.841**	3.8 – 5.8 #
	IVC	3.6 ± 0.1	4.1 ± 0.1	4.098**	
MCHC (%)	ILW	31.2± 0.5	31.1 ± 0.3	0.155	32 – 36 % #
	IVC	30.6 ± 0.6	30.4 ± 0.3	0.318	
MCV(fl)	ILW	86.9 ± 3.1	85.5 ± 1.7	0.415	76 – 100 #
	IVC	90.4± 2.3	87.6 ± 1.7	0.879	

*** Significant at p<0.01*
@ WHO (1972) # Chugh (2007) [35]
ILW (iron-folic acid tablet + lemon water)
IVC (iron-folic acid tablet + vitamin C tablet)
n=30 each (ILW and IVC)

Table 9. Distribution of subjects according to their Hemoglobin level (NIN, 1986)

Categories of anemia with hemoglobin level (g/dl)	ILW		IVC	
	Percentage			
	Before	After	Before	After
Severe (≤ 7 g/dl)	-	-	-	-
Moderate (8.0 – 9.9 g/dl)	36.6	-	43.3	3.3
Mild (10.0 – 10.9 g/dl)	56.6	36.6	56.6	53.3
Marginal (11.0 – 11.9 g/dl)	6.6	56.6	-	43.3
Non-anaemic (≥ 12 g/dl)	-	6.6	-	-

ILW (iron-folic acid tablet + lemon water)
IVC (iron-folic acid tablet + vitamin C tablet)
n=30 each (ILW and IVC)

4. CONCLUSION

The results of the study concluded that there was inadequate consumption of cereals, pulses, green leafy vegetables, milk and milk products, roots and tubers, fruits and non veg food (meat, egg and fish) in the diets of the adolescent girls. Correspondingly, the intake of energy, protein, iron, niacin, vitamin C and vitamin B_{12} was below RDAs which was reflected from their low weights. Majority of the research participants were vegetarian which was the major reason that the research participants were falling in the category of mild anemia even after supplementation. As a result, the blood hemoglobin, RBC and haematocrit levels of the research participants were lower than the normal range. Although after the study, there was a significant improvement in the hematological profile of the research participants in the group ILW followed by IVC. Therefore, providing vitamin C supplements in the form of lemon water and synthetic vitamin C (tablet) had additional advantage; although more significant improvement was observed in the hematological profile of the research participants receiving iron supplements along with natural vitamin C than in the group receiving synthetic vitamin C (tablet). Furthermore, to prevent anemia, varied and diverse diet should be consumed, along with foods rich in iron such as seafood, clams, cocoa, spinach, beef liver, pork liver, lamb liver, chicken liver, egg yolks, pistachios, pine nuts, hazelnuts, dates, almonds, legumes, dried figs, rabbit, beef, corn, lettuce, parsley, etc.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Benoist B, McLean E, Cogswell M, Egli I, Wojdyla D. Worldwide prevalence of anemia 1993–2005. WHO Global Database on Anemia, Geneva. 2008;7-13.
2. Osazuwa F, Ehigie F. Prevalence of anemia in preschool and school aged

children in Nigeria. New York Science J. 2010;3(12):150–153.
3. WHO. Global database on iron deficiency anaemia. Micronutrient Deficiency Information System. WHO, Geneva; 2008.
4. Larson DR. American Dietetic Association Complete Food and Nutrition Guide. New York: Wiley; 2002.
5. Siti-Noor AS, Wan-Mazin WM, Narazah MY, Quah BS. Prevalence and risk factor for iron deficiency in Kelantanese preschool children. Singapore Medl J. 2006;47(11):935-939.
6. Anderson L, Dibble MV, Turkki PR, Mitchell HS, Rynbergen HJ. Nutrition in health and disease. 17th ed. JB Lippincott Co. Philadelphia-Toronto; 1982.
7. Mehnaz S, Afzal S, Khalil S, Khan Z. Impact of iron, folate and vitamin C supplementation on the prevalence of iron deficiency anaemia in non-pregnant females of peri urban areas of Aligarh. IJCM. 2006;31(3):201-203.
8. Srilakshmi B. Nutritional anaemia. Dietetics, 5th ed, New Age International (P) Ltd. 2005;126-136.
9. Song WO, Moerr S, Bond J, Korkarale M, Mann SK, Seghal S, et al. Nutriguide Asian Indian foods. Nutritional Analysis Computer Programme. Michigan State University, USA. 1992;7-38.
10. ICMR. Nutrient requirements and recommended dietary allowances for Indians. A Report of the Expert Group of Indian Council of Medical Research. National Institute of Nutrition. Hyderabad; 1999.
11. Jellife DB. The assessment of nutritional status of the community. World Health Organization Monograph Series No. 53. Geneva. 1966;50-84.
12. World Health Organization. BMI classification. Global database on body mass index. An Interactive Surveillance Tool for Monitoring Nutrition Transition; 2005.
13. Dacie JB, Lewis SM. Practical hematology. English Language Book Society and Churchill Ltd. London. 1989;214-217.
14. Raghuramular N, Madhavan WK, Kalyansundran S. A manual of laboratory techniques. National Institute of Nutrition. Indian Council of Medical Research. Hyderabad. 1983;254-256.
15. Hunter I, Bomford VN. Clinical haematology. Academic Press. New York. 1967;11.
16. WHO. Nutritional anaemia. Tech Rep Ser No. 503, World Health Organization, Geneva. 1972;21.
17. NIN. Anaemia and Endurance Capacity (Physical performance). Annual Report, National Institute of Nutrition. Indian Council of Medical Research. Hyderabad. 1986;164-165.
18. Singh S, Bansal ML, Singh TP, Kumar R. Statistical methods for research worker. Kalyani Publishers. Ludhiana, New Delhi. 1991;212-264.
19. Singh MB, Fotedar R, Lakshminarayana J. Micronutrient deficiency status among women of desert areas of western Rajasthan, India. Public Health Nutrition. 2008;12(5):624-629.
20. Bala. Anthropometry and nutrient adequacy of well-to-do school girls of 13-15 years from Punjab. M.Sc. Thesis. Punjab Agricultural University, Ludhiana, India; 2000.
21. Tupe R, Kundu NK. The dietary patterns of iron and vitamin C rich foods in adolescent girls: implications for community based nutrition education programme. In: Abstr IX Asian Congress of Nutrition. Nutrition foundation of India, New Delhi. 2003;240-41.
22. Srimani S, Mahasweta B, Tapati S, Chaudhari D. A comparative study on the prevalence of anaemia among rural and urban school going adolescents (16 to 18 years) girls and its relationship with selected factors in West Bengal. J Indian Diet Assoc. 2008;33(1):42-50.
23. Agrahar MD, Pal PP. Intake of nutrients and food sources of nutrients among the Khasi tribal women of India. Nutrition. 2004;20(3):268-273.
24. Mittal PC, Srivastava S. Diet, nutritional status and food related traditions of Oraon tribes of New Mal (West Bengal), India. Rural Remote Health. 2006;6(1):385.
25. Malhotra A, Passi SJ. Diet quality and nutritional status of rural adolescent girl beneficiaries of ICDS in north India. Asia Pacific J Clin Nutr. 2007;16(1):8-16.
26. Rao KM, Balakrishna N, Laxmaiah A, Venkaiah K, Brahmam GN. Diet and nutritional status of adolescent tribal population in nine states of India. Asia Pacific J Clin Nutr. 2006;15(1):64-71.
27. Gautam VP, Taneja DK, Sharma N, Gupta VK, Ingle GK. Dietary aspects of pregnant women in rural areas of Northern India. Matern Child Nutr. 2008;4(2):86-94.

28. Laxmaiah A, Rao KM, Brahmam GN, Kumar S, Ravindranath M, Kashinath K, et al. Diet and nutritional status of rural preschool children in Punjab. Indian J Pediatr. 2002;39(4):331-338.
29. Larocque R, Casapia M, Gotuzzo E, Gyorkos TW. Relationship between intensity of soil-transmitted helminth infections and anemia during pregnancy. Am J Trop Med Hyg. 2005;73:783–789.
30. Ghosh A, Bala SK. Anthropometric characteristics and nutritional status of Kandh: A tribal population of Kandhmal District, Orissa, India. Anatomy Human Biol. 2006;33(5-6):641-647.
31. Jarrah SS, Halabi JO, Bond AE, Abegglen J. Iron deficiency anaemia (IDA) perceptions and dietary iron intake among young women and pregnant women in Jordan. J Transcultural Nursing. 2007; 18(1):19-27.
32. Akramipour R, Rezaei M, Rahimi Z. Prevalence of iron deficiency anaemia among adolescent schoolgirls from Kermanshah, Western Iran. Hematology. 2008;13(6):352-355.
33. Deshmukh PR, Garg BS, Bharambe MS. Effectiveness of weekly supplementation of iron to control anaemia among adolescent girls of Nashik, Maharashtra, India. J Hlth Pop Nutr. 2008;26(1):74-78.
34. Ansari BN, Badruddin SH, Karmaliani R, Harris H, Jehan I, Pasha O et al. Anaemia prevalence and risk factors in pregnant women in an urban area of Pakistan. Food Nutr Bulletin. 2009;29(2):132-139.
35. Chugh SN. Textbook of clinical medicine. 2nd Ed, Arya Publications, New Delhi; 2007.

Peer-review history:
The peer review history for this paper can be accessed here:
http://sciencedomain.org/review-history/20142