ORIGINAL ARTICLE

DIETARY ASCORBIC ACID SUPPLEMENTATION AND ITS EFFECTS ON IRON EFFICACY AND HEMATOLOGICAL RESPONSES AMONG ADOLESCENT GIRLS

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ABSTRACT

Objectives: To assess the effects of vitamin C supplementation on the blood iron indices from composite diets.

Material and Methods: This randomized experimental trial followed a pre-test post-test design. Vitamin C supplementation (100mg / day) from dietary sources was done for three months while the sample consumed almost uniform diets.

Results: Based on written consents, a group of 60 young girls was equally divided into experimental and controlled groups. The diets of the experimental group were supplemented with citrus sources. Of the sample, anthropometry of majority girls fell well within the WHO standards. Dietary intake record showed major dietary energy sources were carbohydrates and fats in both of the groups. Dietary intake of cereal based diet was common while iron and Vitamin C of both the groups were quite low. Anemia was prevalent in this age group. Upon supplementation blood iron indices improved gradually and significantly for all indices.

Conclusion: The study concludes that adding a Vitamin C source with a meal can improve anemia situation in adolescent girls over an extended period of time.

Key Words: anthropometry, dietary intake, hematocrit, hemoglobin, serum iron, vitamin C supplementation

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INTRODUCTION

A continuous supply of nutritious, safe and appealing food is necessary to sustain life. Adolescence is a period of great change, both physically and psychologically. Nutritional requirements are greater during this time than at any other phase of life due to increased demands of growth, hormonal changes, and menarche. Being a future mother a female adolescent need a healthy and well balanced diet. One of the main consideration along with protein, carbohydrate, fats and vitamins is iron[1]. Early Egyptians, Greeks, Hindus, and Romans have identified the beneficial effects of iron in health[2-4]. In contrast to other minerals iron is an abundant mineral on earth and is considered a biologically essential component of every living cell. Iron is an essential mineral involved in oxygen transport and metabolism. Almost two-third of the iron in the body is found in hemoglobin and smaller amounts are found in myoglobin, a protein that helps supply oxygen in muscles and assist in cellular biochemical reactions as a part of enzyme systems[5-9].

Iron deficiency is the most common nutritional deficiency in the world. It is estimated that around two billion global population is anemic out of which 50% is due to iron deficiency[10]. In addition to iron vitamin B12, vitamin A, folic acid, riboflavin, and copper are required for the proper production of hemoglobin[11]. About 10-25% of world population mostly women are deficient in iron and are most prevalent in women of lower income households and developing countries[12,13]. Young women absorb 1.4mg/day of iron while men absorb only 0.9mg/day of iron daily. The main reason for this difference is due to menstrual losses in pre menopausal women which make them at higher risk of iron deficiency anemia[14]. Vitamin C, also known as ascorbic acid ($C_6H_8O_6$) is one of the most important nutrients

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needed for boosting iron absorption in the body[15,16]. A number of studies have shown that 25-1000 milligrams of ascorbic acid when taken with a meal can increase the absorption of iron from 0.8 to 7.1%[17,19]. Vitamin C acts as a reducing agent that is able to keep iron ions in the most readily absorbed ferrous (Fe2+) form. It facilitate the absorption of non-heme iron by maintaining it in the most soluble (Fe2+) form or by forming soluble and readily absorbed complexes chelates[20,21]. Ascorbic acid also overcome the negative effects of inhibitors such as phytates, polyphenols, calcium from milk products and enhances the absorption of both native and for tification iron in the diet. Iron absorption form a vegetarian diet can best be optimized by the addition of ascorbic acid vegetables in the diet[22,23]. In contrast to the striking effects of vitamin C on iron absorption the effects of vitamin C supplementation effects on iron status had been very minimal. There were no significant alterations in the biochemical indices with supplementation as high as 2000mg of vitamin C[24]. The current investigation was undertaken to reexamine the effects of dietary vitamin C supplementation over an extended period of time on the blood iron status of adolescent girls.

MATERIAL AND METHODS

A sample of 60 healthy college girls aging 16-19 years were selected at Benazir Tribal Girls Hostel, University of Peshawar. Selection of the subjects was made on the basis of anthropometry and medical history. Girls with recent history of infections, febrile conditions, heavy menstrual records, and prolonged history of drugs for ulcers, epilepsy, antidepressants and heavy caffeine intake were omitted. Based on written consent the sample was randomly divided into two equal groups i.e. experimental and control

The data was collected through a self-constructed questionnaire to record dietary intake, anthropometry and biochemical blood intake.

Anthropometric Measurements

The subjects were examined for their height (cm), weight (kg), BMI (kg/m^2), and triceps skin fold thickness (cm) as per WHO standard[26].

Food Intake Record

In order to minimize the effects of dietary diversity the experiment was performed in the hostel where uniform major meals were served. Daily food intake record was taken by means of seven days dietary intake served in the hostel mess and outside it. Weighed paper plates, plastic cups and spoons of uniform size were given to the subjects. Meals were weighed and served in these plates and cups and left over were weighed later with kitchen weighing scales for portion size estimation. Energy, carbohydrates, proteins, fats, vitamin C and Iron contents of the foods consumed were calculated with the help of Food consumption tables for Pakistan.

Vitamin C estimation and supplementation

In order to estimate percent vitamin C of the fruits titrometric method was used. This estimation helped in the portion size determination of the fruits. On the basis of this estimation one to one and a half orange and guava along the major meals i.e. lunch and dinner were given (subject to the cost and availability of the fruit). In the pre test blood samples of all the 60 girls were tested for hemoglobin, hematocrit and serum iron. Later the experimental group was served with a citrus/vitamin C rich source along major meals (lunch and dinner) for a period of 90 days. Participants in the control group consumed their routine diet.

Assessment of Blood Iron Status

i.Hemoglobin

Hemoglobin was assessed by Drabkin method in which 0.2μg of blood was added to 5ml of the Drabkin solution and absorbance was done at 546 nm on UV spectrophotometer 20.

ii. Hematocrit Value

Hematocrit values were measured against a scale in wintrobes tubes with the help of EBAIII (Hettich) centrifuge at 3000 rpm for 5 minutes.

iii. Serum Ferritin

3mL of the blood samples was poured in centrifuge tubes to separate serum. The slightly yellowish liquid serum was separated by desture and used for serum iron estimation. Ferric Iron was dissolved from its carrier protein, transferrin, in the presence of acetate buffer (pH 4.5) and reduced to ferrous form with the help of ascorbic acid Ferrous Ion chelate with ferens – S produced blue complex reagents. A standard and a reagent blank for each series of determination were prepared. The solutions were brought to 15-25 °C before use. To 200μg of the samples 1000μL of solution 1 and 2 were added and absorbance of sample blank (ABS) against reagent blank and absorbance of sample (AS) against standard was observed and estimated by the following formula.

$$\frac{AS - Abs}{Ast} x\ 100 = \mu g\ /\ dL$$

STATISTICAL ANALYSIS

Data was calculated for percentages while results from the triplicate analyses were subjected to mean, standard deviation, and one way ANOVA for the determination of difference of significance.

RESULTS

Results of the anthropometric measurement (Table – 1) showed that mean age of the sample was 16.73 years with a mean height being -1.82% of the reference value for the height–for–age as per WHO standards. Mean weight (Kg) of the sample was +0.6% as per the weight-for-height standard. Mean BMI was +0.5% while mean triceps skin fold thickness of the sample was +8.4 of the recommended standard for this age group.

Weight-wise distribution of the sample (Figure 1) in the study showed that 16.66% of the subjects were under weight, 60 % of the subjects had healthy or normal weight while 21% of were overweight followed by 3% of obese subjects against the WHO classification.

Data regarding nutrient intake of the sample showed that mean intake for carbohydrate of the sample (Table 2) was as per DRIs in both groups while the daily mean intake for protein was quite low against Recommended Daily Allowance of 66g/d for this age group. The average daily iron intake for both of the groups was 46.95% of the reference against the RDA of 18mg/day. Mean intake of fats was much closer to the RDA for this age group. Upon addition of fruit for vitamin C a significant increase occurred in the vitamin C, fiber and less significant increase in calcium occurred in the experimental group.

Data regarding the prevalence of anemia among the sampled teenage girls (Figure 2) showed that only 23.3% of the sample had normal hemoglobin level while 63.33% girls had hemoglobin below the normal level of 12gm/dl of blood. Percent hematocrit and serum ferritin levels also followed the same pattern.

Results of the supplementation indicated a positive progressive improvement in all of the blood indices for iron. In pretest hemoglobin in gm/dl (Table-3) of both the groups revealed an overall anemia prone tendency in both experimental and controlled group. As analyzed against supplementation for first month there had been a modest increase in the hemoglobin level of the sample. This increase in the hemoglobin levels of both the groups might be due to Eid-Ul-Azha in the first month of supplementation when the students went to their homes for a week and their meat intake increased significantly. However this effect was counteracted in the second month of supplementation when the hemoglobin level of the control group declined. The situation was further improved in the third month when compared to the control group. Results of the serum iron profile also exhibited similar pattern (Table – 4) where serum Iron gradually increased. Results of the controlled group showed varied but subnormal levels. Effect of vitamin C on the percent hematocrit indices (Table-5) of the experimental group was more obvious where mean level raised up to normal values against pre test values.

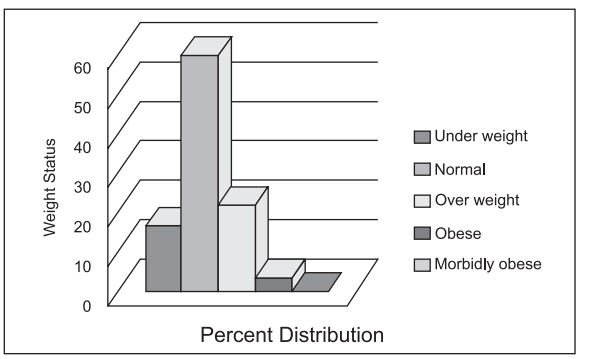

Figure 1: Prevalence of Anemia among the Sample

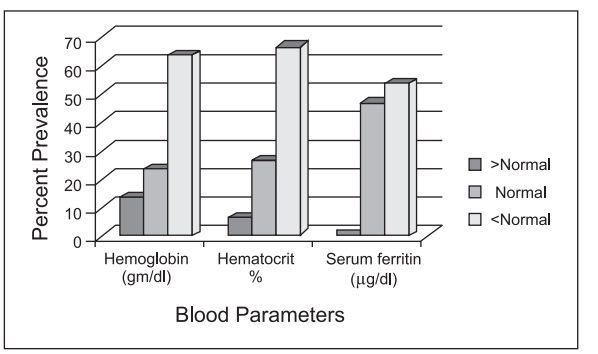

Figure 2: Weight Wise Distribution of the Sample

DISCUSSION

Iron deficiency anemia is prevalent throughout the world mainly due to the insufficient absorption of nonheme iron which makes bulk of iron from the diet[21]. Ascorbic acid can reverse the effects of dietary inhibitors and is a known powerful nonheme iron absorption promoter. The present study assessed the crucial aspect of the effects of dietary vitamin C supplementation on the absorption of iron from diet and consequently its

Table 1: Anthropometric Measurement of the Sample

Variables	Ranges	Mean +SD	%Ref. Value*
Age (years)	15–19	16.73±12.76	
Height (cm)	152.–170.18	160.+4.247	-1.82%
Weight (Kg)	40–70	53.46+9.148	+0.6%
BMI	19.23–30.72	21.74+8.120	+0.5%
Skinfold Thickness (cm)	1.00–2.78	1.581+0.439	+8.4%

*WHO[26]

Table 2: Daily Nutrient Intake of the control and Additional Dietary Vitamin C Intake by the Experimental Group

Nutrients	Experimental Group		Control Group		P-Level	RDA
	Range	Mean +SD	Range	Mean +SD		
Carbohydrates (gm)	139.62–452.88	288.80 + 54.9	170 – 435.13	268.77+47.66	1.24	ND
Protein (gm)	20 – 42.22	42.30+ 5.78	23 – 51.7	41.76+6.938	0.29	66 gm
Iron (mg)	6.45 – 14.488	11.258 + 2.235	7.67 – 12.9	10.825+1.667	0.23	18 mg
Fats (gm)	49 – 62.22	60.76 + 8.508	51.73- 74.25	64.57+10.79	1.03	60-90g/d
Energy (Kcal)	1440.81–2813.15	2078.9 + 23.55	1440.8-2900.7	2057.498+71.9	0.28	2150 Kcal
Vitamin C (mg)	150 – 235	125.117 + 4.388	15.67- 67. 37	45.06+7.513	0.002*	60– 65 mg
Calcium(mg)	372-897	623±26.62	324-919	565.9±23.7	0.07	1200mg
Fiber (g)	13.4-32.2	22.5±3.52	5.8-10.7	7.2±5.47	0.031*	25g
Tea (ml)	0-714	323±41.6	0-750	340±34.2	0.92	

Table 3: Effect of vitamin C supplementation on the hemoglobin profile of the adolescent girls

Parameters	Pre-test[1]	Post- Supplementation		
		30 Days	60 Days	90 Days
Experimental group	9.8 + 14.04	10.6 + 1.6	10.9+11.4	11.04+ 18.36
%Difference from Pre-Test		+0.8(+8.163) p=0.961	+1.1(+11.22) p =0.077	1.24(+12.653) p =0.040*
Controlled group	9.7+ 11.06	10.3 + 2.3	9.6 + 2.5	9.82+26.35
%Difference from the Pre-test	P= 0.606	+0.5(5.452) p = 0.826	-0.1(1.090) p = 0.961	+0.12(1.24) p =0.782
Difference between the two groups	0.1	0.3	1.3 (13.26)	1.04
P-level	1.25	0.074	0.053*	0.027*

1 Pre-supplementation values % of the reference values are calculated for the terminal values.*

Blood values are means of three replications

Values within the parentheses are percentage difference from the pre-test means

* Values are significant at P=<0.05 level

Table 4: Effect of vitamin C supplementation on the serum ferritin profile of the adolescent girls

Parameters	Pre-Test[1]	Post-Supplementation		
		30 Days	60 Days	90 Days
Experimental group	29.78 + 19.34	30.01 + 4.7	36.7 + 31.7	42.45 + 12.45
%Difference from Pre-test		0.23(0.772) p =0.782	6.69(22.47) p =0.035*	5.75(19.31) p =0.025*
Controlled group	29.45 + 16.45	30.34 + 4.6	29.6 + 4.9	30.6 + 4.6
%Difference from Pre-test		0.89(3.022) p = 0.941	-0.15(-0.509) p = 0.721	1.15(3.905) p = 0.906
Difference	+0.33	-0.33	7.1	11.85
P-Level	0.981	1.801	0.043*	0.022*

1 Pre-supplementation values
Blood values are means of three replications
Values within the parentheses are percentage difference from the pre-test means
* Values are significant at P=<0.05 level

Table 5: Effect of vitamin C supplementation on the hematocrit profile of the adolescent girls

Parameters	Pre- Test[1]	Post-Supplementation		
		30 Days	60 Days	90 Days
Experimental group	29.08 + 19.34	31.83 + 18.90	31.4 + 75.81	41.78 + 4.94
%Difference from Pre-test		2.75(2.837) p =0.825	0.43(1.479) p =0.050*	9.7(33.37) p =0.0261*
Controlled group	30.76 + 6.00	31.66 + 1.35	31.05 + 6.45	30.5 + 7.55
%Difference from Pre-test		0.9(2.926) p =0.092	0.61(1.983) p=0.58	0.26(0.845) p =0.907
Difference	-1.68	+0.17	+ 0.35	+12.28
	0.930	0.508	0.634	0.0391*

1 Pre-supplementation values
Blood values are means of three replications
Values within the parentheses are percentage difference from the pre-test means
* values are significant at P=<0.05 level

effect on the anemia status among teenage girls over an extended period of time.

Results of the anthropometric indices of the sample (Table-1) were comparable to the WHO standards for this age group indicating a greater percentage of girls in good weight –to –height[26]. However a tendency towards weight gain (Table-2) was observed and was similar to the findings of Ogden et al[27,28]. Results of the mean nutrient intake per day (Table-2) followed similar pattern as reported by other investigators and when compared against the recommended dietary allowances for Pakistani population[29,32]. The diet of sampled adolescents was sufficient in energy, carbohydrates, and fats. The daily low intake of dietary iron, calcium, fiber, and vitamin C and increased intake of tea were similar to the findings of other investigators[33,34]. The mean results for iron intake were quite comparable to the results of Jaworowska and Bazylak who reported average daily iron intake being 8.4 mg daily iron intake for this age group[35]. They also found that the intake of energy, total protein and vitamin C from the diets of about 50% women were below the recommended intakes[35]. Carbohydrate intake in the current study was similar to the finding of another investigation which reported that major share of carbohydrates and fats intake peaked during adolescent (10-19 years)[30]. The higher vitamin C intake of the experimental groups was due to the addition of 100mg/day vitamin C in their diets. Anemia among the sampled adolescent girls was quite common and coincided with the findings of other studies[36,37]. The effect of vitamin C on the blood iron status of the experimental group followed a modest significant increase over a period of 90 days as tested against the pre test values. Fisherman also observed enhanced

hematologic responses in a population based study[38]. Siegenberg et al proposed the need for utilizing the twofold effects of vitamin C for more absorption of iron per unit energy from the diet[21]. The current study showed a similar modest effect on iron status as that of other studies from a complete meal[38,39]. The significant increase in the hematologic indices in the current study was observed mostly in girls with moderate to severe anemia.

LIMITATIONS

The study was delimited to female students only consuming uniform diets. The time span of the study was 90 days.

CONCLUSION

The current study supports the enhancing effect of ascorbic acid on iron status from a composite diet.

RECOMMENDATIONS

The challenge remain to educate and encourage population for an increased dietary vitamin C intake both with composite diet and alone to help eradicate the highly prevalent anemia in this region.Further cohort studies are needed to assess the effect of supplementation on absorption of both heme and nonheme iron from Pakistani diets among different population age groups over an extended periods of time. Studies are also needed to analyze the inhibitory effects of dietary phytates and oxalates as Pakistani diets are at large are composed of rich plant based iron foods.

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AUTHOR'S CONTRIBUTION

Following authors have made substantial contributions to the manuscript as under:

Ghaffar F: conceptualize the project ,data collection, data analyses.

Bibi T: Data collection, lab analysis.

Din ZU: Data analysis

Authors agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.