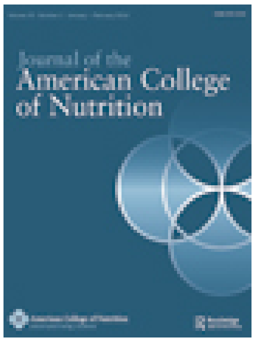

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Fruit and Vegetable Consumption and Their Association With the Indicators of Iron and Inflammation Status Among Adolescent Girls

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ABSTRACT
Background: The objective of this study was to identify an association among dietary components, iron, and inflammatory status among adolescent girls.
Method: Dietary information for 85 adolescent girls was collected through food frequency questionnaires. Biomarkers of iron and inflammatory status were analyzed.
Results: We found that 28.2% of adolescent girls had anemia and 65.9% girls were iron-deficient. Girls who did not consume guava had 3.8-fold (95% confidence interval $=1.1$–9.4; $p=0.020$) increased the risk of having low serum iron levels. Girls who consumed amaranth had significantly ($p=0.024$) higher serum hepcidin levels ($n=44$; 129.7 ± 81.40 pg/mL vs $n=41$; 94.6 ± 55.8 pg/mL) as well as ferritin levels ($n=44$; 19.7 ± 16.4 µg/L vs $n=41$; 14.0 ± 10.2 µg/L). Overall consumption of fruits and green leafy vegetables among girls significantly affects their iron status.
Conclusions: Regular consumption of vitamin C–rich fruits and green leafy vegetable intake are imperative for improvement of iron status among adolescent girls.

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KEYWORDS
Anemia; vitamin C; green leafy vegetables; hepcidin; iron deficiency

Introduction

According to the World Health Organization, anemia is a major health problem worldwide and around 1.6 billion people are anemic (1). In India, 50% of women, 58% of pregnant women, and 56% of adolescent girls are reported to be anemic (2). Persistent anemia causes impaired cognitive function, poor memory, attention deficit, poor academic achievement, and decrement in problem-solving skills (3, 4).

Nutritional as well as non-nutritional factors are among the major causes of anemia. Among nutritional factors, deficiencies of iron, folate, vitamin B12, and vitamin C lead to development of anemia (5). Iron-deficiency anemia (IDA) is the most common type of nutritional anemia and is responsible for approximately 50% of all anemia cases (6). Poor diet quality and low dietary iron bioavailability are the major contributors to development of IDA (7).

Gastrointestinal infestations and infections (including Helicobacter pylori) inhibit vitamin B12 absorption that leads to the development of vitamin B12 deficiency (8). Along with c, vitamin B12 deficiency is also responsible for the development of anemia. A variety of cells including macrophages produce tumor necrosis factor alpha (TNF-α) in response to inflammation or infection (9). TNF-α is thought to be responsible for the inhibition of iron release from the macrophage system leading to the development of hypoferremia and inhibition of erythropoiesis (10).

Hepcidin is a liver-derived peptide that plays a significant role in the regulation of iron homeostasis (11). Under inflammatory conditions or infections, hepcidin levels are increased, which causes iron dysregulation with hypoferremia and anemia of inflammation (12). While in iron deficiency, hepcidin production is suppressed to ensure the appropriate physiological concentration of plasma iron levels (13). Previous studies in children reported a correlation between serum ferritin and hepcidin levels which indicated that the absorption of dietary iron is facilitated by hepcidin to maintain demands of erythropoiesis and repletion of iron stores (14, 15). Pasricha et al. (2014) reported that hepcidin levels can effectively discriminate IDA and anemia of inflammation among African children, suggesting that hepcidin can be used as a reliable biomarker of iron status (16).

In most developing countries, like India, the main source of iron is non-heme iron from plant-based diets. Non-heme iron constitutes 91% of the total iron present in the Indian diet (17). According to the current estimates, the intake of iron is less than 50% of the recommended dietary allowance (18). Fruits and green leafy vegetables (GLV) contain high levels of vitamin A, vitamin C, folic acid, and minerals like calcium, iron, and phosphorus. It has been reported that total iron content of selected GLV ranged from 22.3 to 84.4 mg/100g (19). In accordance with a National Nutrition Monitoring Bureau (NNMB) report, there was a decline in intake of fruits, vegetables, nuts, oil seeds among 13 to 17-year-old girls (20). A significant increase in hemoglobin (Hb) concentration (6.4–11.1 gm/dL) was reported among Indian nonanemic adults after consuming GLV for only 3 weeks (21).

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In our previous study in adolescent girls, we found that vitamin B12 showed a negative association with TNF-α and positive association with serum ferritin (22). We also observed an increased risk of high TNF-α level among girls who had vitamin B12 deficiency as well as anemia. Therefore, to identify whether food components contribute to the positive association between iron status and inflammation, we studied dietary habits, iron, and inflammatory status of adolescent girls. We collected their dietary information through food frequency questionnaires and measured levels of biomarkers that are related to iron and inflammatory status.

Participants and methods

Participants

A total of 85 adolescent girls aged between 11 and 16 years (mean age, 13.7 ± 1.1 years) studying in a school in Pune participated in the study. None of the participants received iron or vitamin supplements during the study and none of them experienced any other hematological diseases (such as thalassemia, sickle cell anemia, and iron overload), infectious diseases (such as acute infectious enteritis, intestinal tuberculosis, intestinal amebic dysentery, and inflammatory bowel disease), or malignant diseases.

Ethical consideration

The ethical committee of King Edward Memorial Hospital Research Centre, Pune, India, approved the study. Written informed consent was obtained from guardians of all participants.

Anthropometric measurements

Body weight (kg) was measured using an electronic weighing balance to the nearest 0.02 kg. Height was measured using stadiometer to nearest 0.1 cm. Percentage body fat was measured using an Omron body fat monitor (Japan), which works on the bioimpedance analysis principle. All readings were taken in duplicate by a trained investigator, and the average was used for analysis. Body mass index (BMI; kg/m^2) was calculated as weight (kg) divided by the square of height (m).

Biochemical estimations

Blood samples were collected by a trained technician in 5-mL anticoagulant tubes (K2EDTA Labtech Disposables). Hb (gm/dL) levels were measured using a Lablife Noble III automated haematology analyzer (Diagnova, India). Blood samples were allowed to clot at room temperature (25 °C) after which the serum was separated by centrifugation and stored at −80 °C and later used for various biochemical estimation Indicators for iron status viz. serum iron and serum ferritin were measured. Serum iron (µg/dL) was measured using the ferrozine colorimetric method, and serum ferritin (µg/L) was measured using an ELISA kit (Diagnostic Biochem, Canada). Serum hepcidin (pg/mL; USCN Life Sciences, China) and TNF-α (pg/mL) were estimated using ELISA kits (KrishgenBiosystems, India). Total serum vitamin B12 (pg/mL) was estimated using chemiluminescent enzyme immunoassay by automated analyzer (Axsym, Abbott, US). Current assay for total serum B12 measures all forms of cobalamin, i.e., hydroxocobalamin, adenosylcobalamin, and methylcobalamin.

Dietary assessment

Participants were asked to complete a demographic and health background questionnaire and food frequency questionnaire. The 55 food items were categorized into four groups: ‘fruits’, ‘GLV’, ‘nuts, dried fruits and oilseeds’, and ‘nonvegetarian’ items. Respondents indicated how frequently they had consumed these food items during the previous month. Eight-point response categories ranged from “never” to “several times a day” (23). Food items that were consumed once in a month by more than 50% of the participants were categorized in a “frequently consumed” group and others are in “rarely consumed.” Categories of food group as shown in Table 1.

Statistical analysis

All statistical analyses were performed by using Statistical Package for Social Sciences (SPSS for Windows version 17.0, SPSS Inc., Chicago, US) and MedCalc for Windows (MedCalc Software, Ostend, Belgium). Biochemical parameters were expressed in terms of mean ± standard deviation for continuous variables and in terms of absolute frequencies and percentages for categorical variables. All

Table 1. Classification of Food Frequency Questionnaire Items into Food Groups.

Food Group	Food Items
Fruits: Frequently consumed	Amla, guava, papaya, orange, lemon, tomato, pomegranate
Fruits: Rarely consumed	Cashew fruit, grapes, sweet lime, watermelon, muskmelon, pineapple, custard apple, strawberry, zizipus, mango
GLV: Frequently consumed	Amaranth, colocasia leaves, fenugreek leaves, spinach, shepu, safflower leaves, fetid cassia, coriander leaves
GLV: Rarely consumed	Bathua leaves, beet greens, Bengal gram leaves, cabbage, cauliflower greens, cowpea leaves, drumstick leaves, betel leaves, radish leaves, agathi, mustard leaves, mint, mayalu, knol-khol greens, fenugreek leaves
Nuts, dried fruits, and oilseeds: Frequently consumed	Coconut, sesame seeds, almond, cashew nuts, pistachio nuts, dates, black currants, and raisins
Nuts, dried fruits, and oilseeds: Rarely consumed	Niger seeds, garden crest seeds
Nonvegetarian items	Meat, fish, egg

Note. GLV: = Green leafy vegetables.

biochemical markers were adjusted for age except serum ferritin and serum hepcidin. When comparing ordinal data across categorical variables, independent t test was used. Logistic regression analysis was used to estimate the association between foods consumption and biochemical markers.

Results

The basic anthropometric characteristics, iron status, and inflammatory status of 85 girls are given in Table 2. Their mean age was 13.7 ± 1.1 years and mean weight was 45.0 ± 11.0 kg. Mean BMI was 18.8 ± 3.6 kg/m2. In this study, 28.2% of girls were anemic (Hb <12 gm/dL), 65.9% of girls were iron-deficient (serum ferritin <15 µg/L), and 21.2% of girls had IDA (Hb <12 gm/dL and serum ferritin <15 µg/L) (24). Along with this, 13.0% of girls were vitamin B12-deficient (< 200 pg/mL) and 48.2% of girls had low serum iron levels, i.e., < 60 µg/dL (25).

Biochemical parameters

Pearson correlation (r) analyses showed a significant positive correlation between hepcidin and ferritin ($r = 0.258$, $p = 0.017$). We did not find a significant correlation between Hb and hepcidin, as girls participating in our study did not have severe anemia (mean Hb $= 12.4 \pm 0.8$ gm/dL). We observed a significant negative correlation ($r = -0.255$, $p = 0.019$) between vitamin B12 and TNF-α, indicating an inverse relationship between vitamin B12 deficiency and inflammation.

Effect of fruit consumption on iron and inflammatory status

Overall, 45% of girls consumed amla, 73% of girls consumed guava, 50% of girls consumed orange, 52% of girls consumed papaya, 80% of girls consumed tomato, and 83% of girls consumed lemon more than once in a month. To study the role of these foods, we carried out logistic regression analysis and computed unadjusted odds ratios to evaluate the risk for participants to have lower serum iron. Girls who did not consume guava had 3.8-fold (95% confidence interval [CI] = 1.1–9.4; $p = 0.020$) increased risk of having low serum iron levels. Similarly, girls who did not consume tomato and lemon had 4.6-fold (95% CI = 1.3–15.7, $p = 0.009$) and 4.8-fold (95% CI = 1.2–19.0, $p = 0.015$) increased risk of having low serum iron levels. Hence, overall low fruit consumption was associated with a high risk of low serum iron levels in our study population.

A significant positive correlation ($r = 0.364$, $p = 0.025$) between hepcidin and ferritin was observed among girls who consumed amla (at least once in a month). Girls who consumed amla had significantly ($p = 0.042$) higher serum iron levels (n = 38; 70.3 ± 30.4 µg/dL vs n = 47; 55.7 ± 34.1 µg/dL; Figure 1A). However, there was no significant difference in the levels of biochemical markers among high amla consumers (at least once a week) and nonconsumers. We also found that girls who consumed lemon had significantly higher serum ferritin levels, as shown in Figure 1D. Similar findings were observed while comparing data between high lemon consumers and nonconsumers. These results indicate that amla and lemon consumption had an effect on iron status. We also observed lower hepcidin levels among girls who consumed amla.

Similar to what was seen with amla, we found a significant positive correlation ($r = 0.366$, $p = 0.002$) between hepcidin and ferritin among girls who consumed tomato (at least once in a month). Girls who consumed tomato had higher serum iron levels (Figure 1C). We also found that girls who consumed tomato at least once in a week had significantly ($p = 0.034$) higher serum iron levels than nonconsumers. Overall results showed that tomato consumption had a positive effect on iron status.

Further, we found a significant positive correlation ($r = 0.273$, $p = 0.032$) between vitamin B12 and ferritin among girls who consumed guava. Girls who consumed guava had significantly higher ($p = 0.022$) serum iron levels (n = 62; 67.2 ± 31.6 vs n = 23; 48.8 ± 34.0 µg/dL; Figure 1B). We did not find a significant difference in biochemical markers associated with iron and inflammatory status among high guava consumer and nonconsumers.

Overall results suggested that consumption of vitamin C–rich fruits had a significant effect on iron status among girls.

Table 2. Anthropometric Characteristics, Iron Status, and Inflammatory Status in Adolescent Girls (n = 85).

Parameters	Mean (± SD)
Anthropometric parameters:	
Age (years)	13.7 (1.1)
Height (cm)	150 (10)
Weight (kg)	45.0 (11.0)
BF (%)	23.2 (7.0)
BMI (kg/m2)	18.8 (3.6)
Iron status:	
Hb (gm/dL)	12.4 (0.8)
SI (µg/dL)	62.3 (33.1)
SF (µg/L)	16.9 (14.0)
Vitamin B12 (pg/mL)	314.9 (125.6)
Inflammatory status:	
Hepcidin (pg/mL)	112.8 (72.0)
TNF-α (pg/mL)	16.3 (16.7)
Prevalence of:	
Anemia (%)	28.2
ID (%)	65.9
IDA (%)	21.2
Low SI (%)	48.2
Vitamin B12 deficiency (%)	13.0

Note. BF: body fat; BMI: body mass index; Hb: hemoglobin; ID: iron deficiency; IDA: iron-deficiency anemia; SD: standard deviation; SI: serum iron; SF: serum ferritin; TNF-a: tumor necrosis factor alpha.

Effect of GLV consumption on iron and inflammatory status

GLV are a rich source of carotenoids as well as iron, calcium, vitamin C, riboflavin, and folic acid (19). Hence, we studied the effect of GLV consumption on iron as well as inflammatory status among girls. Approximately 51% of girls consumed amaranth, 68% consumed colocasia leaves, 37% consumed fetid cassia, 40% consumed safflower leaves, and 31% consumed

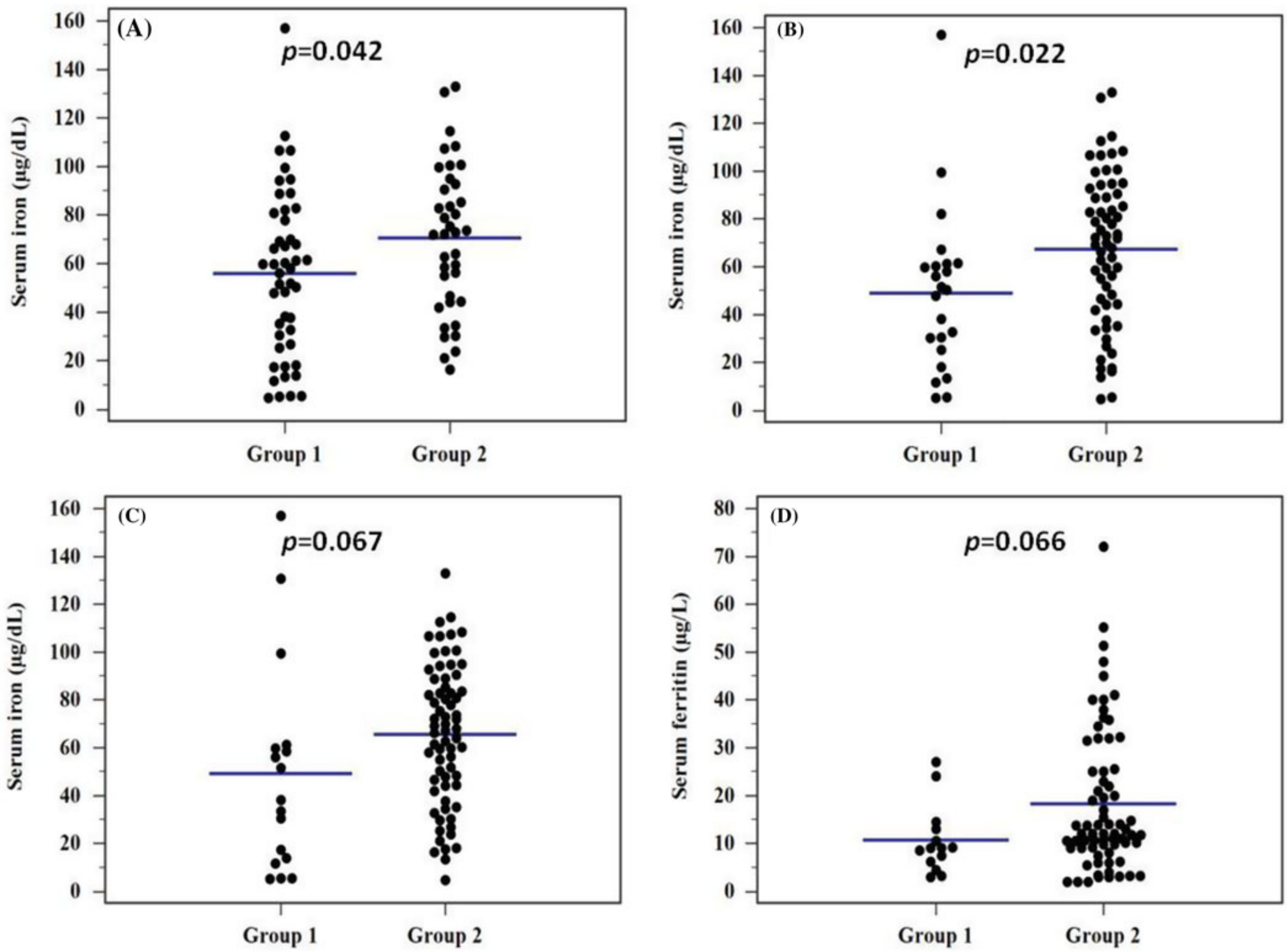

Figure 1. Mean serum iron (µg/dL) and ferritin (µg/L) levels among group 1 and group 2 girls. Girls who did not consume amla (A), guava (B), tomato (C), and lemon (D) were categorized in group 1. Girls who consumed amla (A), guava (B), tomato (C), and lemon (D) at least once in a month were categorized in group 2. Blue horizontal lines indicate the mean value, and p values indicate the difference in mean values between two groups by independent t test.

radish leaves at least once in a month. We observed a significant positive correlation ($r = 0.313$, $p = 0.039$) between vitamin B12 and ferritin among girls who consumed amaranth. Girls who consumed amaranth had significantly ($p = 0.024$) higher serum hepcidin levels ($n = 44$; 129.7 ± 81.40 pg/mL vs $n = 41$; 94.6 ± 55.8 pg/mL; Figure 2A). We also found that girls who consumed amaranth had higher ferritin levels ($n = 44$; 19.7 ± 16.4 µg/L vs $n = 41$; 14.0 ± 10.2 µg/L; Figure 2E). Girls who consumed amaranth at least once in a week (high consumers) had significantly ($p = 0.041$) higher serum iron levels than nonconsumers, indicating the positive effect of amaranth consumption on iron status in girls.

Similar to what was seen with amaranth, girls who consumed colocasia leaves had significantly ($p = 0.05$) higher serum hepcidin levels ($n = 58$; 123.2 ± 76.7 pg/mL vs $n = 27$; 55.6 ± 43.0 pg/mL; Figure 2B) and higher serum iron levels ($n = 58$; 65.37 ± 27.24 µg/dL vs $n = 27$; 55.60 ± 43.02 µg/dL). No difference was observed in serum ferritin and Hb levels. However, we did not find a significant difference in biochemical markers levels among high colocasia leaf consumers and nonconsumers.

A significant negative correlation ($r = -0.388$, $p = 0.028$) between serum iron and hepcidin was observed among girls who consumed fetid cassia. We observed significantly higher ($p = 0.033$) serum iron levels in girls who consumed fetid cassia ($n = 32$; 72.1 ± 26.2 µg/dL vs $n = 53$; 56.3 ± 35.6 µg/dL; Figure 2C). Further logistic regression analysis showed that girls with a low intake of fetid cassia had 3.9 times higher risk of having low serum iron levels (odds ratio = 3.9; 95% CI = 1.5–10.0; $p = 0.004$). However, no difference in biochemical markers levels was observed between high fetid cassia consumers and nonconsumers.

Girls who consumed safflower leaves showed a significant positive correlation ($r = 0.34$, $p = 0.047$) between ferritin and hepcidin. Girls who consumed safflower leaves had higher serum iron levels ($n = 34$; 70.5 ± 30.0 vs $n = 51$; 56.7 ± 34.26 µg/dL; Figure 2D). In addition, girls who consumed safflower leaves at least once in a week (high consumers) had significantly ($p = 0.020$) higher serum ferritin levels than nonconsumers. There was a significant positive correlation of ferritin with hepcidin ($r = 0.34$, $p = 0.007$) and vitamin B12 ($r = 0.284$, $p = 0.029$) among girls who consumed radish leaves. Girls who consumed radish leaves had significantly ($p = 0.004$) higher serum ferritin levels ($n = 26$; 23.4 ± 17.0 vs $n = 59$; 14.0 ± 11.5 µg/L). In addition, girls who consumed radish leaves at least once in a week (high consumers) had significantly ($p = 0.032$) higher serum hepcidin levels than nonconsumers. These results suggest that girls who consumed GLV had improved iron status over those who did not consume GLV.

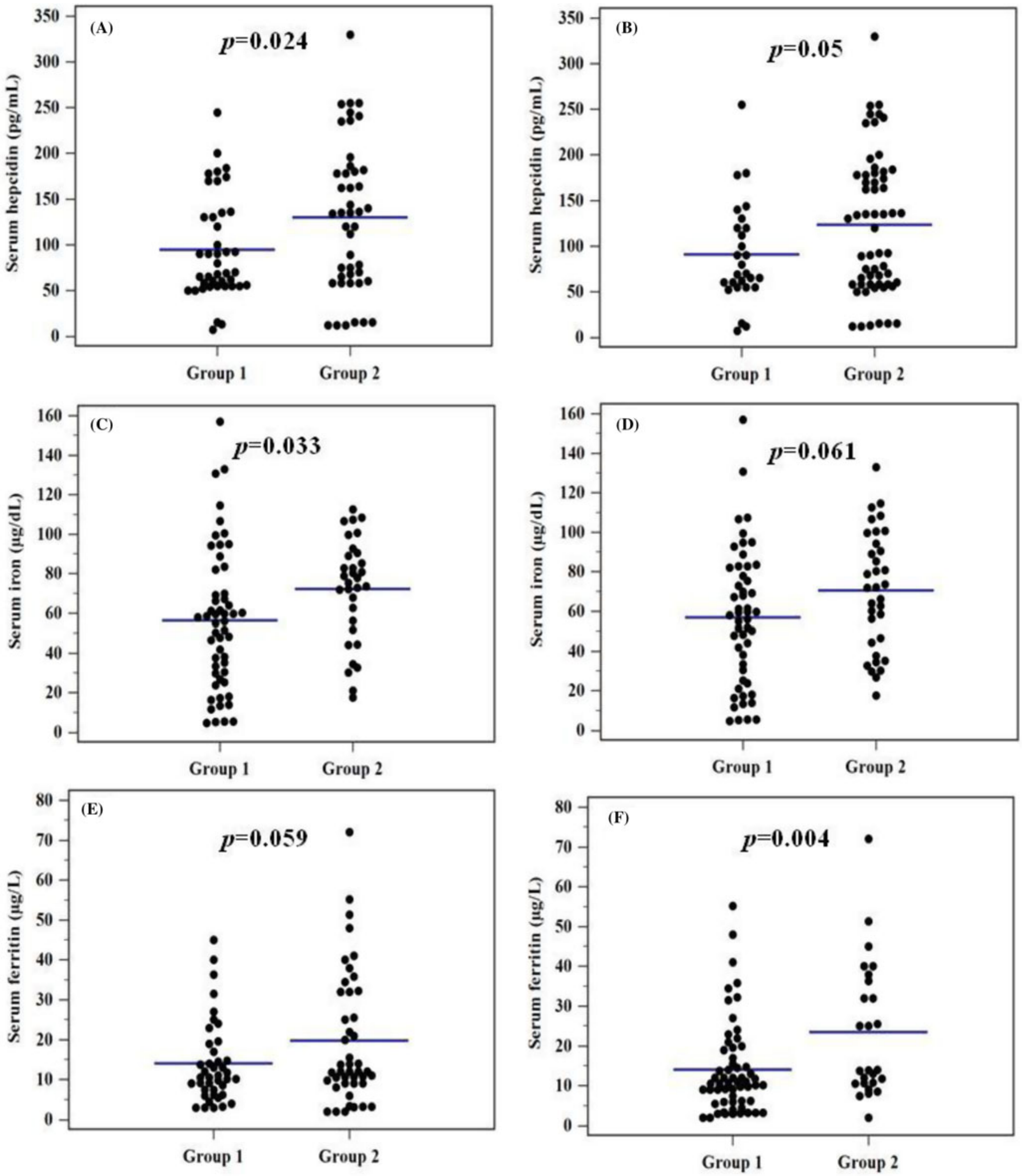

Figure 2. Mean serum hepcidin (pg/mL), iron (µg/dL), and ferritin (µg/L) levels among group 1 and group 2 girls. Girls in group 1 did not consume amaranth (A and E), colocasia leaves (B), fetid cassia (C), safflower leaves (D), and radish leaves (F). Girls in group 2 consumed amaranth (A and E), colocasia leaves (B), fetid cassia (C), safflower leaves (D), and radish leaves (F) once in a month. Blue horizontal lines indicate the mean value, and p values indicate the difference in mean values between two groups by independent t test.

Effect of nut and oilseed consumption on iron and inflammatory status

Nuts and dried fruits are rich sources of various minerals and micronutrients, dietary fibers, and several vitamins (26). A recent International Nut and Dried Fruit Council report suggested that there is an increase in consumption of nuts and dried fruits among middle-income countries including India (27).

In the present study, we found that overall 88% of girls consumed dried coconuts, 66% consumed sesame seeds,

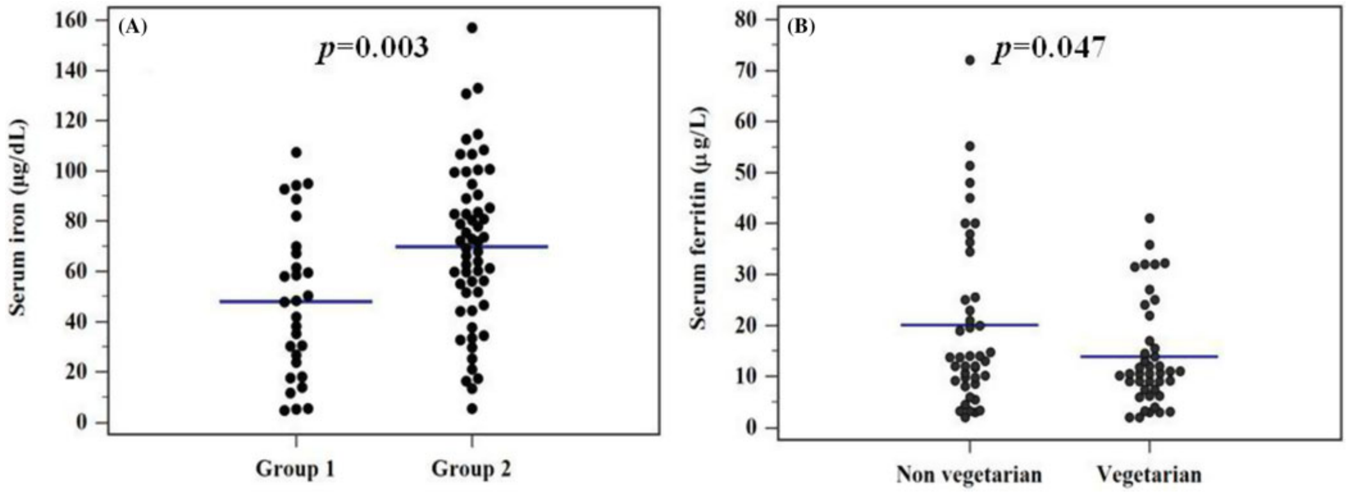

Figure 3. Mean serum iron levels (μg/dL) among group 1 and group 2 girls. (A) Girls in group 1 did not consume sesame seeds and girls in group 2 consumed sesame seeds once in a month. (B) Mean serum ferritin levels (μg/L) among group 1 and group 2 girls. Blue horizontal lines indicate the mean value, and *p* values indicate the difference in mean values between two groups by independent t test.

92% consumed almonds, 88% consumed cashews, 71% consumed pistachio nuts, 74% consumed dates, and 72% consumed raisins at least once in a month. Girls who consumed sesame seeds showed a significant positive correlation ($r = 0.313$, $p = 0.019$) between ferritin and hepcidin. Girls who consumed sesame seeds had significantly ($p = 0.003$) higher serum iron levels ($n = 56$; 69.8 ± 32.2 μg/dL vs $n = 29$; 47.7 ± 30.0 μg/dL), as shown in Figure 3A. In addition, we also found that girls who consumed sesame seeds had significantly higher ($p = 0.049$) serum ferritin levels ($n = 56$; 21.13 ± 16.20 vs $n = 29$; 14.75 ± 12.33 μg/L). However, we did not find a significant difference in biochemical markers levels among high sesame seed consumers (at least once in a week) and nonconsumers.

Iron status among vegetarian and nonvegetarian girls

In the present study, we found that 50% of girls had consumed meat, eggs, or chicken at least once in a month. We observed a significant positive correlation of ferritin with hepcidin ($r = 0.323$, $p = 0.037$) and vitamin B12 ($r = 0.321$, $p = 0.038$) and a significant negative correlation ($r = -0.392$, $p = 0.010$) with serum iron among nonvegetarian girls. As shown in Figure 3B, serum ferritin levels were significantly higher ($p = 0.047$) among nonvegetarian girls ($n = 42$; 20.0 ± 16.6 vs $n = 42$; 13.9 ± 10.2 μg/L). These findings are in support of previous studies indicating that consumption of nonvegetarian food items showed improvement in iron status (28, 29).

Discussion

The current study examined associations between consumption of food items with iron and inflammatory status in adolescent girls. The iron status is mainly dependent upon the bioavailability of the iron rather than the total intake of iron (7). Along with this, inflammatory cytokines also regulate iron metabolism (30).

Adolescent girls are at the highest risk for iron shortage due to a higher requirement of micronutrients and macronutrients including iron for their growth and development. Persistent iron deficiency results in the development of anemia. In our study, we found that approximately 28% of girls had anemia and about 66% of girls were iron-deficient. We observed a significant positive correlation between hepcidin and ferritin, and a similar correlation was reported in other studies (14, 31). TNF-α is a hallmark of chronic inflammation (9) and is also involved in the inhibition of iron release from the macrophageal system (32) as well as erythropoiesis (10). In our study, inflammation, as reflected by elevated serum TNF-α, was not significantly associated with Hb levels in girls. One possible explanation is that the girls who participated in this study were relatively healthy. We found that vitamin B12 showed a significant negative association with TNF-α. Several lines of evidence have suggested the association of vitamin B12 deficiency with an increased incidence of inflammation (33, 34). The main reason we preferred TNF-α in our study over other markers of inflammation is that TNF-α regulates iron metabolism via inhibition of iron release from the macrophage and inhibition of erythropoiesis. In this study, we have not measured other acute-phase response proteins, such as C-reactive protein or α-1-acid glycoprotein, and it is possible that a combination of elevated α-1-acid glycoprotein (AGP) and/or elevated C-reactive protein would have detected a greater proportion of girls with inflammation than TNF-α alone.

We studied the associations among fruit consumption, iron status, and inflammatory status among girls. We found that girls who consumed amla had higher serum iron levels (Figure 1A) and showed a significant positive correlation between hepcidin and ferritin. Amla is a rich source of vitamin C, and it has been known to improve nutritional iron uptake and iron utilization in humans (35). A recent study showed that vitamin C alters the hepcidin mRNA expression in HepG2 cells (36). In the present study, we found that girls who consumed amla had lower hepcidin levels, which may be due to the combined effect of adequate vitamin C levels as well as improved iron status among these girls.

Tomatoes are a rich source of lycopene, a potent antioxidant, and also contain a high amount of vitamin C (37). In this study, girls who consumed tomatoes had higher serum iron levels (Figure 1C) and showed a significant positive correlation between hepcidin and ferritin. A recent study in overweight and obese women showed that tomato consumption for 20 days significantly reduces interleukin 8 and TNF-α levels (38). However, in our study, we did not find a significant difference in TNF-α levels among girls who consumed tomato or those who did not consume it.

Further, we found that girls who consumed guava had significantly higher serum iron levels (Figure 1B) and showed a significant positive correlation between vitamin B12 and ferritin. Guava is a rich source of vitamin C and it contains approximately 260 mg vitamin C/100 g fruit (39). A recent study showed that the consumption of mungbean based meal along with the consumption of guava enhances Hb level among Indian school children by 3.7 gm/dL and it also helps in the improvement of serum ferritin levels (40). In another study, the inclusion of 100 g of fresh guava fruit along with rice-based meals showed a 2-times enhancement in iron absorption in Indian adolescent girls (41). In accordance with these studies, our study also showed that girls who consumed guava had a better iron status compared to those who did not consume guava.

Overall results showed that consumption of vitamin C–rich fruits such as amla, tomato, guava, and lemon had a significant effect on iron status. However, various factors affect the vitamin C content of fruit. The type of cultivar, growing conditions, and stage of ripeness significantly affect the vitamin C content of fruit (42, 43). Furthermore, the vitamin C is destroyed due to heat, light, exposure to air (44) and also during the processing of fruits during pureeing, juicing and cooking. Therefore, consumption of fruit in its raw form would be more beneficial in order to improve iron status.

India is home for a variety of green leafy vegetables suited with the sub-tropical and temperate climates through out the year. Amaranth is one of the commonly consumed GLV in India. In addition to this, colocasia leaves, spinach, radish leaves, etc. are used locally in different parts of the country. We studied the impact of GLV consumption on iron as well as inflammatory status among girls.

Girls who consumed amaranth had higher serum hepcidin (Figure 2A) as well as hepcidin levels (Figure 2E). In addition, there was a significant positive correlation between vitamin B12 and ferritin among girls who consumed amaranth. Elevated serum iron levels trigger hepcidin secretion in liver which reduces iron absorption as well as inhibits release of stored iron (45). Hence, high hepcidin levels among girls who consumed amaranth may be due to higher ferritin levels.

Further, girls who consumed fetid cassia had higher serum iron levels (Figure 2C). Logistic regression analysis showed that girls who did not consume fetid cassia had a 3.9-times risk of having low serum iron levels. Fetid cassia is a rich source of vitamin C and also contains approximately 12.4 mg/100 g of iron (46). Hence, we found that girls who consumed fetid cassia had higher serum iron levels.

GLV are a rich source of beta-carotene, ascorbic acid, iron, zinc, folate, and dietary fiber (47). A recent NNMB report suggested that the average consumption of GLV among 13- to 15-year-old Indian girls was 16 g/d, which was far below the recommended dietary allowance of 40 g/d (20). However, bioavailability of iron from GLV may depend upon the content of promoters (vitamin C) and inhibitors (phytates, tannins, polyphenols) of the iron absorption. Studies of dietary iron intake in Indian populations have revealed that the intake was lower than the requirement (17), as the diets were mostly plant-based (rice, pulses, and vegetables), which contains high phytic acid levels (18). Inclusion of iron absorption enhancers such as vitamin C could overcome the negative effect on iron absorption of all inhibitors (which include phytate, polyphenols, and calcium) (48). However, various processes like cooking, industrial processing, and storage can degrade vitamin C and remove its enhancing effect on iron absorption (49). Hence, modifications in cooking practices of GLV (e.g., cooking in iron utensils) and inclusion of vitamin C–rich fruits and vegetables in daily meals could improve the bioavailability of iron.

Recent studies have suggested the beneficial effects of nut (50), dried fruit (51), and oilseed (52) consumption on human health. In our study, we found that girls who consumed sesame seeds had higher serum iron as well as ferritin levels. Sesame seed supplementation has reportedly increased serum iron as well as tissue iron levels among iron-deficient rats (53). Overall results showed that sesame seed consumption significantly improves iron status among girls.

Bioavailability is one of the factors that affects the absorption of iron in the body (48). Heme iron has a greater bioavailability than non-heme. Individuals consuming only a plant-based diet can expect to absorb approximately 5% to 12% of dietary iron, and a mixed diet, including heme iron, will absorb ~14% to 18% (48). Several cross-sectional studies and narrative reviews suggested that the iron status of vegetarians is poor due to the absence of highly bioavailable heme-iron in vegetarians' diets and the inhibiting effect of certain components present in plant foods on non-heme iron bioavailability (54, 55). Studies have also reported a higher prevalence of iron deficiency or IDA in vegetarians, especially among females, compared to nonvegetarians (56, 57). In our study, serum ferritin levels were found to be significantly higher among nonvegetarian girls than vegetarian girls, indicating a better iron status among nonvegetarian girls.

Conclusions

The present study highlights the burden of iron deficiency among adolescent girls. Low consumption of vitamin C–rich fruits has been significantly associated with lower levels of circulating as well as stored iron. Similarly, lower GLV consumption is also responsible for the poor iron status of these girls. The iron status of the individual depends on various factors including iron bioavailability and other host-related factors. Inflammation may also play an important role. Therefore, it is necessary to have new and innovative strategies to improve the nutritional status of adolescent girls well before they enter into their reproductive years.

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Disclosure statement

The authors report no conflicts of interest.

Abbreviations

BMI	Body mass index
BF	body fat
CI	confidence interval
GLV	green leafy vegetables
Hb	hemoglobin
ID	iron deficiency
IDA	iron-deficiency anemia
SI	serum iron
TNF-α	tumor necrosis factor-alpha

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