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Plant-Based Diet and Iron Deficiency Anemia in Sundanese Adolescent Girls from Islamic Boarding Schools in Indonesia

VIEWING AN OLDER VERSION

ID 6469883

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> Abstract

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2. Editorial Comments Reviewer 1

— Editorial Comments

Recommendation Tatiana Emanuelli AE 29.06.2021
Revision requested

Message For Author
ID 6469883

Dear Dr Mohammad Zen Rahfiludin,

Reviewer's comments on your paper are appended below. You will see that they raised some issues that must be addressed before publication. I recommend that you revise the manuscript taking into account the changes requested by reviewers.

When submitting the revised manuscript you must include line numbers and highlight all changes performed using red font.

Include a point-by-point response letter that explains all changes made or provide a rebuttal to the points that have not been changed. A revised manuscript should be submitted by July 29, 2021.

To submit your revised manuscript, please access "Author Activities" in your account and upload the PDF file of your revised manuscript. Also, please submit your point-by-point response letter to the comments of the reviewer(s) as an additional PDF file.

Sincerely,
Tatiana Emanuelli

Reviewer 1:

The authors have performed a nice study with an appropriate sample size to correlate nutritional status with Iron deficiency anemia in adolescent girls residing in boarding schools. However, in the current form, manuscript requires major changes to be addressed. I have few comments:

1. Please go through thoroughly since the paper is grammatically not sound. Use article 'an' and 'the' where ever appropriate.
2. Line 32- It should be 'more due to' instead of 'due more to"; Line 34 – boarding schools instead of boarding school; second- growth instead of second growth, line 38- affects; line 40 – 'suffering from anemia or were anemic'
3. Line 40 – please clarify iron deficient Or iron deficient anemia?
4. Line 43-44 – Statement is not clear. Please rewrite sentence for clarity.
5. Line 51- it should be "is found"
6. Line 52- Sentence not clear.
7. Line 86- Indonesian or Indonesia?
8. Line 111- 24-h or 24- hour
9. Line 117 – verb is missing
10. Line 119-123 – sentence is grammatically incorrect.
11. Page 4 – clearly define criteria used for anemia and iron deficient anemia
12. Line 132-133 - Sentence not clear.
13. Line 149- Move this sentence to methodology
14. Line 160 – Which categories? Please clarify
15. Line 160 – Please mention proportion of subjects with iron intake lower than RDA and their correlation with IDA
16. Table 1 – Please mention clearly in table (what does values inside and outside bracket signifies) median (IQR)? Moreover authors have given values of range for nutritional intake variables but have not mentioned proportion of cases with sufficient or insufficient levels and their proportion among IDA and non- anemic cases.
17. Line 166- It would be better to include serum ferritin levels in the study as it represents a good marker for iron storage.
18. Please mention proportions for all parameters with and without anemia
19. Line 171- Authors are advised to correlate variables of nutritional/iron intake with IDA as authors did for tea and coffee.
20. Line 192 – females instead of female
21. Line 214- Please follow uniform writing style for anemia (either anemia or anaemia) throughout paper
22. Line 218 – Another instead of other
23. Line 243- cafeterias instead of cafeteria
24. Line 181- Please mention proportion given in national report for comparison
25. Line 187-188 – rewrite sentence since prevalence in Bangladesh (32%) is lower than your study
26. Line 198-200 – Incomplete sentence (in comparison to what?)
27. Line 208-210 – Exact no. of cases with higher or lower ratios among anemic and non anemic cases
28. Line 231 – correlation of what with MCV? Mean corpuscular values or MCV values?
29. Line 236-238 – Meaning not clear. Please rewrite.
30. Authors did not find any significant correlation among variables of nutritional/iron intake and haematological parameters and similarly for tea coffee consumption. Please suggest possible reasons for this outcome in your study in discussion.

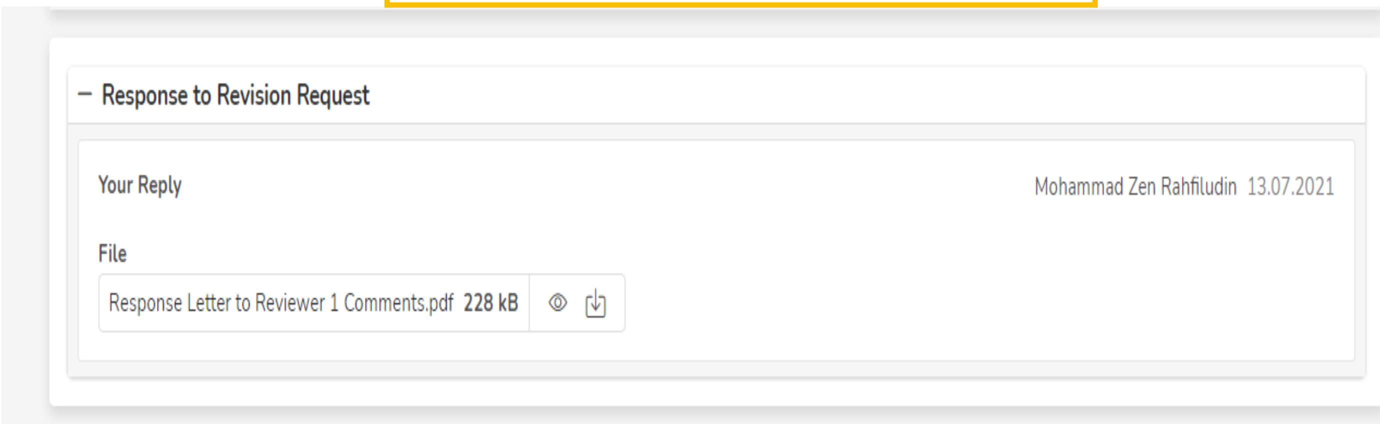
— Reviewer Reports 1 submitted

Reviewer 1 17.06.2021

File

comments.docx 15 kB

3. Upload Revisi 1



Dear Tatiana Emanuelli,

We have revised our manuscript based on the reviewer's recommendations as follows:

1. Prior to submission to your journal, we have sent our manuscript to Bioscience Editing Solution in New York, USA for language editing
2. All unsuitable vocabularies have been replaced
3. We have re-written sentences that grammatically incorrect or unclear
4. Iron intake based on Indonesia Recommended Dietary Allowance (RDA) and its correlation to iron deficiency anemia (IDA) have been added in Table 4
5. We did not include serum ferritin levels analysis in our manuscript since it could be influenced by infections, thus it might not reflect the actual iron stores in the body. Moreover, it is unlikely to obtain more blood samples for serum ferritin level analysis currently as our country imposes lockdown early this month
6. We have been using uniform term ‘anemia’ throughout the manuscript
7. Line 181 – we have mentioned the proportion of anemia given in national report for comparison
8. Line 187-188 – Indonesia IDA prevalence in this study, which was 22.2% (mentioned in line 185), was indeed lower compared to Bangladesh (32%)
9. We have suggested possible reasons for variables that have no correlations with each other

Please be informed that all changes explained above were highlighted using red font.

Thank you.

Best regards,
Mohammad Zen Rahfiludin

Plant-Based Diet and Iron Deficiency Anemia in Sundanese Adolescent Girls from Islamic Boarding Schools in Indonesia

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Abstract

Background. Adolescent girls are at risk for iron deficiency anemia (IDA) due to the higher demand of iron for growth and the loss of a fair amount of blood during menstruation. Consumption of higher bioavailable iron can reduce the risk, however Sundanese who eats mostly plant-based food may not meet this requirement. We investigated the correlation between plant based-diet and IDA in adolescent girls of Sundanese. *Methods.* A total of 176 girls from seven Islamic boarding schools in Tasikmalaya were recruited. Nutritional intake data were obtained using 24-h dietary recall. Blood samples were analyzed with Sysmex-XNL and IDA was measured with several parameters included hemoglobin, mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentration (MCHC). *Result.* The prevalence of IDA was 22.2%. Iron intake was 6.13 mg/day which was lower than recommended. The molar ratio of phytic acid to iron was 7.79 while molar ratio of vitamin C to iron was 0.02. There were correlation between heme iron and hemoglobin ($p = 0.009$) as well as hematocrit ($p = 0.018$). Iron from meat, fish, and poultry was correlated with hemoglobin ($p = 0.009$) and hematocrit ($p = 0.011$). *Conclusion.* Sundanese plant-based diet did not affect IDA status. IDA was more due to the less consumption of animal-based food which had higher bioavailability. Hence, providing animal-based menu at the school cafeteria more frequently is crucial to prevent IDA in students at boarding schools.

Introduction

Adolescence is a period of second- growth spurt after infancy between the ages of 10 and 19 years. In this stage, adequate nutritional status is necessary since it does not only determine their quality of life but also indirectly affects the nutritional status of their future children and their ability to care for and nourish them adequately [1]. In our previous study, more than half of pregnant mothers were anemic (50.7%) and iron deficient (69.6%), hence appropriate measure was urgently needed since they were young to prevent further implications [2].

An increase in lean body mass, blood volume, and red cell mass during the rapid growth in adolescents ought to deplete iron stores in their bodies, making them at greater risk for iron deficiency [3]. In low- and middle-income countries, many adolescents even have iron deficiency anemia (IDA) as a result of malnutrition from childhood [4]. The prevalence of iron deficiency and IDA is higher among adolescent girls than boys [5] since they lost a fair amount of blood due to menstruation in addition to increased requirements of nutrition for growth [6].

Higher demand for iron requires increase consumption of iron-rich food. There are two types of dietary iron, namely, heme and non-heme. Heme iron, which is mainly found in meat, fish and poultry, has better bioavailability than non-heme iron which is found mostly in plant-based foods [7]. Heme iron is estimated to contribute 10–15% of total iron intake in meat-eating populations, but, because of its higher and more uniform absorption (estimated at 15–35%), it could contribute ≥ 40% of total absorbed iron. In opposite, although non-heme iron constitutes a greater portion of the total iron in foods, its absorption is low and is affected by the presence of soluble enhancers and inhibitors consumed prior to or with the meal [7, 8].

In plant-based diets, phytate is the main inhibitor of iron absorption [8]. Phytate occurs when phytic acid, a negatively charged molecule, binds to mono- and divalent dietary mineral cations, forming very stable phytate complexes at neutral pH. As a divalent cation, iron bioavailability in the gastrointestinal decreases, and the small intestine pH increases the dissociation and formation of phytate-divalent cation complexes that precipitate, making them less available for absorption into the human body [9]. Polyphenols that widely present in coffee and tea also strongly inhibit dietary non-heme iron absorption. Consumption of a cup of tea with the meal decreased iron absorption by 59% in people with IDA and 49% in normal people [10]. In contrast, the presence of vitamin C (both synthetic and dietary) is the most significant enhancer of iron absorption, which can enhance absorption up to sixfold in those who have low iron stores, overcoming the inhibitor effects of phytic acid. Vitamin C facilitates non-heme iron absorption by reducing ferric ($Fe3+$) to ferrous ($Fe2+$) form which is more easily absorbed [11].

The adequacy of iron in food is determined by the amount and quality of iron in the food consumed. The quality of iron is affected by the bioavailability of iron which is the proportion of iron consumed compared to absorbed iron and iron used for physiological functions and storage and influenced by food and body condition [12]. The bioavailability of iron can be estimated by calculating its molar ratio in the diet. The molar ratio of phytic acid to iron > 1 indicates inhibition of iron absorption [13]. Meanwhile, consumption of vitamin C with a molar ratio of 2:1 can overcome the inhibition of iron absorption caused by the presence of phytic acid [14].

As an excellent source of iron, meat consumption is an important factor that must be considered. However, its consumption in developing countries is still low. In Indonesia, per capita beef consumption is only 2.7 kg per year. It is very low compared to other countries in Southeast Asia, such as Malaysia (15 kg) and the Philippines (7 kg), and much inferior when compared to other large countries such as Australia (90.2 kg), United States (90 kg), Argentina (86.5 kg), and Brazil (78 kg). Meanwhile, chicken and fish consumption are higher with per capita consumption around 15 kg and 32.4 kg per capita per year respectively [15].

Generally, the food consumption pattern in Indonesia is still dominated by plant-based foods [16]. National vegetable consumption in 2018 is approximately 54 kg per capita per year [17], which is higher than other Asian countries such as Malaysia (46.9 kg), Thailand (37.6 kg), Sri Lanka (31.6 kg), and Bangladesh (20.5 kg) [18].

As the largest archipelagic country in the world, the territory of Indonesia consists of 17 thousand islands inhabited by more than a thousand ethnicities with different food cultures. As many as 15.5% of Indonesia's population are Sundanese, who originate from West Java, making them the second largest ethnic group in the country [19]. Sundanese food is widely known to include many vegetables, such as karedok (raw vegetable salad in peanut sauce), lalapan (a variety of raw vegetables served with chili sauce), and sayur asem (vegetable tamarind soup). Hence, we want to find out whether plant-based dietary habit significantly affects their health. This study aims to prove the correlation between iron consumption from plant based-diet by considering the molar ratio of vitamin C and phytic acid to iron with iron status in Sundanese adolescent girls.

Materials and Methods

Study Design and Subject

This was a quantitative study with an analytical design and a cross-sectional approach. The subjects were 176 girls randomly selected from seven Islamic boarding schools in Tasikmalaya, West Java province, Indonesia. The sample size was calculated based on the minimum sample size formula of n = and using the related article [20]. It was estimated to be 171. Considering the probability of its reduction, it increased to 176. We included female students who were willing to participate and who were living as well as eating at school.

Measurement

Data on subject characteristics were obtained through face-to-face interviews with subjects. Nutritional intake data were obtained using 24- hour dietary recall method for three non-consecutive days. Dietary pattern for sources of iron and enhancers and inhibitors of iron absorption was also assessed. Although students lived in the school dormitory and were provided with three meals a day, some students still bought food outside the school compound, thus we did not only record the daily meal they got at the school cafeteria but also food or snack they bought from outside of school. The food intake was recorded in the form of household portions (e.g. tablespoons, teaspoons, cups, etc.). We used food pictures as a visual aid to determine the right amount of food they consumed. The food intake is then converted into grams and analyzed using Nutrisoft software to calculate the nutrition intake.

The moles of phytic acid, vitamin C, and iron were determined by dividing the weight per 100 grams of food by their atomic weight (phytic acid: 660 g/mol; vitamin C: 176.12 g/mol; iron: 56 g/mol). The molar ratio between phytic acid to iron was obtained after dividing the mole of phytic acid with the mole of iron. This method is also applied to calculate the ratio of vitamin C to iron [13, 21].

A total of 3 mL of venous blood sample were drawn from each subject in the morning for hematological analyses. The samples were analyzed in the laboratory using the hematology analyzer Sysmex-XNL. IDA status was measured with four parameters included hemoglobin, mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentration (MCHC). MCV was used to define the size of red blood cells, while MCH and MCHC were used to determine their hemoglobin content [22]. Subjects were identified as anemic if their hemoglobin level was below 12 g/dL, while IDA was determined with the following criteria: hemoglobin < 12 g/dL, MCV below normal value (< 82 fL for age group 11 – 14 years; < 85 fL for age group 15 – 75 years), MCH < 27 pg, and MCHC < 32 g/dL [23, 24]. Hematocrit was low if the value was < 36% for children aged between 12 – 14 years and girls aged > 15 years [25].

All participants obtained written informed consent after they were given a thorough explanation about aims, procedures, and associated risks. The study protocol was approved by the Health Research Ethics Committee Faculty of Public Health Diponegoro University (No. 29/EA/KEPK-FKM/2020).

Statistical Analysis

Data were analyzed using SPSS software version 23. The normality of the data was assessed using the Kolmogorov–Smirnov test and the Rank Spearman test was used to assess the correlation of variables. The data were considered statistically significant if p-value < 0.05.

Results

A total of 176 female students were enrolled in this study. Mean age of subjects was 15.5 years. Majority of subjects was junior high school graduate (86.4%), which mean they were in senior high school at the time of study. The subjects' parents had low education levels with mostly elementary school graduates. Father was the main breadwinner of the family (99.4%) while most mothers did not work (85.2%) (Table 1).

Most subjects did not drink tea (76.7%), coffee (94.3%), or tea and/or coffee (72.2%). Daily intake of iron was 6.13 mg, in which non-heme iron was higher (4.86 mg) than heme iron (1.05 mg) and iron from meat, fish, and poultry (MFP) (0.30 mg). Molar ratio of phytic acid to iron was 7.79 while molar ratio of vitamin C to iron was 0.02 (Table 1).

Table 1. Sociodemographic characteristics of the study population

Variables	Value
Age (years)	15.50 (12 – 20)
Education level, n (%)	
No Education	10 (5.7)
Elementary	14 (8.0)
Junior High	152 (86.4)
Father's education level, n (%)	
No Education	2 (1.1)
Elementary	108 (61.4)
Junior High	45 (25.6)
Senior High	17 (9.7)
College	4 (2.3)
Mother's education level, n (%)	
No Education	2 (1.1)
Elementary	101 (57.4)

Junior High	53 (30.1)
Senior High	18 (10.2)
College	2 (1.1)
Father's employment status, n (%)	
Unemployed	1 (0.6)
Employed	175 (99.4)
Mother's employment status, n (%)	
Unemployed	150 (85.2)
Employed	26 (14.8)
Drinking tea, n (%)	
No	135 (76.7)
Once a day	30 (17.0)
Twice a day	11 (6.3)
Drinking coffee, n (%)	
No	166 (94.3)
Once a day	7 (4.0)
Twice a day	2 (1.1)
Three times a day	1 (6.0)
Drinking tea and/or coffee, n (%)	
Yes	49 (27.8)
No	127 (72.2)
Nutritional intake	
Energy (kcal)	1283.3 (420.0 – 2945.8)
Protein (g)	30.2 ± 12.1
Iron (mg)	6.13 (0.6 – 14.7)
Heme-iron (mg)	1.05 (0.0 – 9.0)
Meat, fish, poultry (MFP) (mg)	0.3 (0.0 – 9.0)
Non-heme iron (mg)	4.86 (0.6 – 12.6)
Phytic acid (mg)	567.0 (144.0 – 1676.0)
Vitamin C (mg)	2.93 (0.0 – 117.6)
Molar ratio	
Phytic acid:iron	7.79 (2.2 – 25.9)
Vitamin C:iron	0.02 (0.00 – 0.41)

Table 2 showed median value of hematological parameters. Based on hemoglobin concentration, most subjects did not have anemia (67.6%). There were 75.0% of subjects with abnormal hematocrit status. The majority of subjects had low MCV (61.4%) and high MCHC (71.6%). As much as 50.6% of subjects had MCH value of < 27 pg and 49.4% of subjects had MCH value of ≥ 27 pg. A total of 57 subjects had anemia (32.4%) and 39 subjects had iron-deficiency anemia (22.2%).

Table 2. Hematological parameter and characteristics of iron status

Variables	Value
Hemoglobin (g/dL)	12.6 (6.5 – 14.7)
Hematocrit (%)	38.0 (24.0 – 43.5)
Erythrocyte (10^6/µL)	4.71 (3.79 – 6.14)
MCV (fL)	81.9 (56.5 – 92.3)
MCH (pg)	26.9 (15.3 – 31.1)
MCHC (g/dL)	32.8 (27.1 – 36.1)
Hemoglobin status, n (%)	
Anemia	57 (32.4)
Normal	119 (67.6)
Hematocrit status, n (%)	
≤ 36	44 (25.0)
> 36	132 (75.0)
MCV status, n (%)	
Low	108 (61.4)
Normal	68 (38.6)
MCH status, n (%)	
< 27	89 (50.6)
≥ 27	87 (49.4)
MCHC status, n (%)	
< 32	50 (28.4)
≥ 32	126 (71.6)
IDA status, n (%)	
IDA	39 (22.2)
Non-IDA	137 (77.8)

Our analysis showed that there was a significant correlation between hemoglobin and hematocrit with heme iron and MFP. Non-heme iron, vitamin C, and molar ratio of phytic acid to iron and vitamin C to iron were found to not correlate with all hematological parameters (Table 3). Few students had adequate iron intake based on Indonesia RDA, but this was not significantly related to iron deficiency anemia. Iron deficiency anemia was also not correlated with drinking tea and drinking tea and/or coffee (Table 4).

Table 3. Correlation between nutritional intake and iron status in adolescent girls of Indonesian Sundanese

Variables	(*r* ; *p*) value				
	Hemoglobin	Hematocrit	MCV	MCH	MCHC
Heme iron	0.195; 0.009	0.179; 0.018	-0.014; 0.850	0.038; 0.617	0.095; 0.210
MFP	0.195; 0.009	0.190; 0.011	0.003; 0.965;	0.040; 0.601	0.077; 0.308
Non-heme iron	0.075; 0.323	0.086; 0.254	0.103; 0.174	0.073; 0.334	0.032; 0.670
Phytic acid	0.074; 0.331	0.070; 0.358	0.073; 0.338	0.072; 0.344	0.078; 0.305
Vitamin C	0.020; 0.796	0.088; 0.245	-0.013; 0.869	-0.076; 0.315	-0.114; 0.132
Molar ratio of vitamin C:iron	-0.027; 0.724	0.039; 0.603	-0.060; 0.427	-0.118; 0.118	-0.127; 0.094
Molar ratio of phytic acid:iron	-0.116; 0.126	-0.097; 0.199	-0.031; 0.686	-0.057; 0.449	-0.049; 0.519

Table 4. Correlation between iron intake and drinking tea and coffee with iron deficiency anemia

Variables	Iron deficiency anemia		*p*-value
	Yes (%)	No (%)	
Iron intake based on RDA			
Inadequate	46 (26.6)	127 (73.4)	0.568
Adequate	0 (0.0)	3 (100.0)	
Drinking tea			
Yes	9 (22.0)	32 (78.0)	0.971
No	30 (22.2)	105 (77.8)	
Drinking tea and/or coffee			
Yes	11 (22.4)	38 (77.6)	0.954
No	28 (22.0)	99 (78.0)	

Discussion

This study found an overall prevalence of anemia (32.4%) was slightly higher than that in the national report, which was 26.8% in children aged 5 – 14 years old and 32.0% in young adults aged 15 – 24 years old [26]. WHO stated that prevalence of anemia between 20.0 – 39.9% indicated moderate public health significance [27]. This showed that anemia was indeed a public health problem among the Sundanese adolescent girls in the area. Among all anemic subjects, the proportion of subjects with IDA was 22.2%, which was considerably higher than other developing countries such as Iran (13.9%) [28], Ethiopia (11%) [6], and Thailand (5.7%) [29]. Nevertheless, the prevalence was lower compared to other Asian countries such as Malaysia (34%) [30] and Bangladesh (32%) [31].

Low iron intake might lead to a high prevalence of IDA in the present study. Median iron intake of the subjects was only 6.13 mg/day which was lower than recommended. According to Indonesian recommended dietary allowance (RDA), daily intake of iron should be 8 mg for females aged 10 – 12 years and 15 mg for females aged 13 – 15 years [32]. Furthermore, the subjects' iron intake mainly came from non-heme iron and thus had lower bioavailability. However, it did not significantly affect iron status in this study of Sundanese plant-based diet. On the other hand, heme iron and MFP showed a positive correlation with some hematological parameters which were hemoglobin and hematocrit value. A study in Korea supported this finding in which anemic adolescents girls, indicated by low hemoglobin concentration, consumed less red meat than those without anemia [33]. Girls who consumed meat (beef, mutton, pork) $<$ 4 times/week were more than twice as likely to suffer from iron deficiency compared to those who consume meat $\geq$ 4 times/week [34]. Low hemoglobin concentration was also associated with infrequent consumption of fish, poultry, as well as milk and dairy products [35]. Hematocrit value was likely affected by socioeconomic status. People with low socioeconomic status tended to have low hematocrit value which indicated poor intake and absorption of iron [36]. Moreover, boarding schools' menus in developing countries generally contained a little animal-based food and lacked quality of dietary diversity, thus increasing risks of poor iron status [37].

All hematological parameters were not related to the intake of inhibitor and enhancer of iron. This result proved that the presence of phytic acid and vitamin C in the diet did not significantly affect iron status in Sundanese girls. It might be because food sources of vitamin C and iron were not consumed together in the same meal so that the function of vitamin C as an iron absorption enhancer was not optimal. In addition, the amount of vitamin C consumed by the students was much lower (only 2.93 mg/day) than recommended, which were 50 mg/day for females aged 10 – 12 years, 65 mg/day for females aged 13 – 15 years, and 75 mg/day for females aged 16 years and above. A study in the Philippines showed that vitamin C would have an effect on hemoglobin concentration if its intake is more than 24 mg/day [38]. The molar ratio of phytate to iron and molar ratio of vitamin C to iron were used as a determinant of iron absorption, the higher the ratio the more the phytate intake and the lower the absorption of minerals. In this study, the molar ratio of phytate to iron and the molar ratio of vitamin C to iron were low. It means that the intake of phytate and vitamin C is low, resulting in no relationship between the molar ratio of phytate to iron and the molar ratio of vitamin C to iron with iron status [39].

We also found that there was no significant relationship between consuming tea and consuming tea and/or coffee with iron deficiency anemia. Several studies showed that coffee and tea were not associated with iron deficiency in healthy people with no risk of iron deficiency [40]. Hogenkamp, et al. found that Iron deficiency and iron deficiency anemia were not significantly explained by black tea consumption in a black adult population in South Africa [41]. Sung, et al. stated that green tea intake was not related to serum ferritin levels, but coffee consumption was associated with lower serum ferritin levels in Korean adults [42]. Another study found that the mean serum-ferritin concentration was not related to black, green and herbal tea consumption in men, pre- or postmenopausal women [40]. Evidence suggested that the type of food you ate had a greater influence on iron absorption than the effect of drinking coffee or tea. Thus, coffee and tea were more likely to inhibit the absorption of non-heme iron from plant-based foods but have very little effect on heme iron from animal foods [8]. Similarly, tea consumption in 2573 French men (n = 954) and women (n = 1639) had no influence on iron status [43]. Another cross-sectional study with 157 Indian participants did not find differences in anemia prevalence between men and women who consumed diets that contained high and low tannin amounts [40]. Hence, consuming tea and coffee could inhibit iron absorption which will cause anemia, but iron deficiency anemia, on the other hand, was influenced by various factors such as the type of food consumed (heme or non-heme iron), when to consume tea and coffee (preferably 1 hour before eating so that it will not affect the absorption of iron), and the level consumption of substances that increase the absorption of iron from food. In the present study, most students only drank coffee and tea occasionally, for instance, after waking up in the morning and during late-night study time to help them stay awake, and thus it was unlikely to affect iron absorption that leads to iron deficiency anemia.

This study also showed no significant relationship between red cell indices (MCV, MCH, and MCHC) and all variables analyzed. Nevertheless, MCV and MCH values were a little below normal, implying the presence of iron deficiency anemia. Considering that Sundanese food was similar to a vegetarian diet, we compared the MCV and MCH values in those two groups. It showed that MCV in our study was above MCV value in vegetarians (81.9 fL vs 78.4 fL), while MCH in both groups was much the same (26.9 pg vs 27.2) [44]. Furthermore, MCV and MCH values in Sundanese girls were similar with high school girls who suffer iron deficiency without anemia in Nakhon Si Thammarat, Thailand (MCV = 80.0 fL; MCH = 26.9 pg) [29].

Conclusions

The prevalence of IDA was considered high in Sundanese adolescent girls. However, the Sundanese diet which consisted mostly of plant-based food was not a factor that caused IDA. Instead, it was due more to the less consumption of animal-based food which had higher bioavailability. Hence, it is important to improve quality of food in boarding school cafeterias by providing animal-based menus more often to prevent IDA.

Data Availability

The data used to support the findings of this study are available from the corresponding author upon request.

Conflicts of Interest

The authors declare that there is no conflict of interest regarding the publication of this paper.

Funding Statement

This study was receiving a grant from Faculty of Public Health, Diponegoro University, with grant number 010/UN7.5.9/KS/2020.

References

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4. Editorial Comments Revised 2

— Editorial Comments

Recommendation Tatiana Emanuelli AE 02.08.2021

Minor Revision Requested

Message For Author

ID 6469883

Dear Dr Mohammad Zen Rahfiludin,

I invite you to revise your manuscript again to address some points that were not adequately modified in the first revision.
I noticed that the following questions from reviewer 1 were not addressed:

1. Table 1 – Please mention clearly in table (what does values inside and outside bracket signifies) median (IQR)? Moreover, authors have given values of range for nutritional intake variables but have not mentioned proportion of cases with sufficient or insufficient levels and their proportion among IDA and non- anemic cases.
2. Table 2 - It would be better to include serum ferritin levels in the study as it represents a good marker for iron storage.
3. Please mention proportions for all parameters with and without anemia

I strongly advise that Tables 1 and 2 must separately show data from the IDA and non-anemic cases, in addition to the average of the whole population studied. In addition, the manuscript must be carefully revised by a native English speaker.
When submitting the revised manuscript, you must include line numbers and highlight all changes performed using red font.
Include a point-by-point response letter that explains all changes made or provide a rebuttal to the points that have not been changed. A revised manuscript should be submitted by September 1, 2021.
To submit your revised manuscript, please access "Author Activities" in your account and upload the PDF file of your revised manuscript. Also, please submit your point-by-point response letter to the comments of the reviewer(s) as an additional PDF file.

Sincerily,
Tatiana Emanuelli

— Response to Revision Request

Your Reply Mohammad Zen Rahfiludin 13.07.2021

File

Response Letter to Reviewer 1 Comments.pdf 228 kB

5.Upload Revisi 2

— Response to Revision Request

Your Reply

Mohammad Zen Rahfiludin 25.08.2021

Dear Tatiana Emanuelli, Thank you for giving me the opportunity to submit a revised draft of my manuscript titled "Plant-Based Diet and Iron Deficiency Anemia in Sundanese Adolescent Girls at Islamic Boarding Schools in Indonesia" to Journal of Nutrition and Metabolism. We appreciate the time and effort that you and the reviewers have dedicated to providing your valuable feedback on my manuscript. We have been able to incorporate changes to reflect all suggestions provided by the reviewers. We have highlighted the changes within the manuscript with red font. Here is a point-by-point response to the reviewers' comments and concerns. • Comment 1: Table 1 – Please mention clearly in table (what does values inside and outside bracket signifies) median (IQR)? Moreover, authors have given values of range for nutritional intake variables but have not mentioned proportion of cases with sufficient or insufficient levels and their proportion among IDA and non- anemic cases. Response: The meaning of values in Table 1 has mentioned in the column head (Mean ± SD). We also separately show data from the IDA and non-IDA cases with the proportion of sufficient and insufficient levels in all variables, including nutritional intake (see Table 3). • Comment 2: Table 2 - It would be better to include serum ferritin levels in the study as it represents a good marker for iron storage. Response: You have raised an important point here. However, as infections could affect serum ferritin levels, they might not reflect the actual iron stores in the body. Hence, we did not include this variable in our study. Moreover, we could not obtain more blood samples for serum ferritin level analysis as our country imposes community activities restrictions enforcement due to the COVID-19 pandemic. • Comment 3: Please mention proportions for all parameters with and without anemia Response: Thank you for pointing this out. We agree with this comment. Therefore, we have mentioned the proportion for all parameters with and without IDA in a new table (Table 3) In addition to the above comments, the manuscript has been carefully revised by a native English speaker. We look forward to hearing from you in due time regarding our submission. Thank you. Best regards, Mohammad Zen Rahfiludin

File

Response Letter_2.pdf 552 kB

Dear Tatiana Emanuelli,

Thank you for giving me the opportunity to submit a revised draft of my manuscript titled "Plant-Based Diet and Iron Deficiency Anemia in Sundanese Adolescent Girls at Islamic Boarding Schools in Indonesia" to *Journal of Nutrition and Metabolism*. We appreciate the time and effort that you and the reviewers have dedicated to providing your valuable feedback on my manuscript. We have been able to incorporate changes to reflect all suggestions provided by the reviewers. We have highlighted the changes within the manuscript with red font.

Here is a point-by-point response to the reviewers' comments and concerns.

- **Comment 1:** *Table 1 – Please mention clearly in table (what does values inside and outside bracket signifies) median (IQR)? Moreover, authors have given values of range for nutritional intake variables but have not mentioned proportion of cases with sufficient or insufficient levels and their proportion among IDA and non- anemic cases.*
  Response: The meaning of values in Table 1 has mentioned in the column head (Mean ± SD). We also separately show data from the IDA and non-IDA cases with the proportion of sufficient and insufficient levels in all variables, including nutritional intake (see Table 3).
- **Comment 2:** *Table 2 - It would be better to include serum ferritin levels in the study as it represents a good marker for iron storage.*
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In addition to the above comments, the manuscript has been carefully revised by a native English speaker. We look forward to hearing from you in due time regarding our submission.

Thank you.

Best regards,
Mohammad Zen Rahfiludin

Plant-Based Diet and Iron Deficiency Anemia in Sundanese Adolescent Girls at Islamic Boarding Schools in Indonesia

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Abstract

Background. Adolescent girls are at risk for iron deficiency anemia (IDA) due to the higher demand of iron for growth and the loss of blood during menstruation. Consumption of foods containing iron that have higher bioavailability can reduce the risk of IDA, although diets that are largely plant-based, like those consumed by many Sudanese, may not contain sufficient bioavailable iron. Here we investigated the correlation between plant based-diets and IDA in adolescent Sudanese girls who were students at Islamic boarding schools in Indonesia. *Methods.* A total of 176 girls from seven Islamic boarding schools in Tasikmalaya were recruited. Nutritional intake data were obtained using 24-hr dietary recall. Blood samples were analyzed with a Sysmex-XNL instrument to measure several parameters including hemoglobin, mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentration (MCHC). *Results.* The prevalence of IDA in the study population was 22.2%. Iron intake was 6.59 mg/day, which was lower than recommended amount. The molar ratio of phytic acid to iron and vitamin C to iron was 8.72 and 0.03, respectively. There was a correlation between heme iron and both hemoglobin ($p = 0.009$) and hematocrit ($p = 0.018$). Iron from meat, fish, and poultry was correlated with hemoglobin ($p = 0.009$) and hematocrit ($p = 0.011$). *Conclusion.* The Sundanese plant-based diet did not affect IDA status. Instead, IDA was associated with consumption of less animal-based foods that have iron with higher bioavailability. Increased access to an animal-based menu at the school cafeteria could be an approach to prevent IDA in students at Islamic boarding schools in Indonesia.

Introduction

Adolescence spans the ages between 10 and 19 years and is a period of marked physical growth. During this stage, adequate nutritional status of females in particular can determine not only current quality of life but can also indirectly affect the nutritional status of future children and the ability to care for and nourish them adequately [1]. In our previous study, we found that more than half of pregnant mothers in the study population were anemic (50.7%) and iron deficient (69.6%), indicating that appropriate measures were urgently needed to avoid health issues associated with anemia and iron deficiency [2].

An increase in lean body mass, blood volume, and red cell mass during the rapid growth in adolescence can deplete iron stores and increase the risk of iron deficiency [3]. In low- and middle-income countries, many adolescents have iron deficiency anemia (IDA) that results from malnutrition during childhood [4]. The prevalence of iron deficiency and IDA is higher among adolescent girls than boys [5] in part due to blood loss during menstruation in addition to increased nutritional requirements for growth [6].

Higher demand for iron requires increased consumption of iron-rich foods. Dietary iron exists as heme and non-heme iron. Heme iron, which is mainly found in meat, fish and poultry, has better bioavailability than non-heme iron, which is found mostly in plant-based foods [7]. Although heme iron is estimated to contribute 10-15% of total iron intake in meat-eating populations due to its higher and more uniform absorption (estimated at 15-35%), heme iron could contribute $\geq$ 40% of total absorbed iron. Meanwhile, non-heme iron constitutes a greater portion of the total iron in foods, but its absorption is low and is affected by the presence of soluble enhancers and inhibitors consumed before or with the meal [7, 8].

In plant-based diets, phytate is the main inhibitor of iron absorption [8]. Phytate occurs when phytic acid, a negatively charged molecule, binds to mono- and divalent dietary mineral cations to form highly stable phytate complexes at neutral pH. As a divalent cation, iron bioavailability in the gastrointestinal tract decreases, and the small intestine pH increases the dissociation and formation of phytate-divalent cation complexes that precipitate, thus lowering availability for absorption [9]. Polyphenols are widely present in coffee and tea can also strongly inhibit dietary non-heme iron absorption. Consumption of a cup of tea with a meal decreased iron absorption by 59% in people with IDA and 49% in healthy individuals [10]. In contrast, the presence of vitamin C (both synthetic and dietary) is the most significant enhancer of iron absorption, and can enhance iron absorption up to 6-fold in those who have low iron stores, thus overcoming the inhibitory effects of phytic acid. Vitamin C facilitates non-heme iron absorption by reducing ferric (Fe^{3+}) to ferrous (Fe^{2+}) iron, which is more easily absorbed [11].

The nutritional adequacy of iron in food is determined by the amount and quality of iron in the food consumed. The quality of iron is affected by its bioavailability, which is expressed as the proportion of iron consumed compared to iron that is absorbed and used for physiological functions. Iron storage is influenced by food and physical condition [12]. The bioavailability of iron can be estimated by calculating the molar ratio in the diet. A molar ratio of phytic acid to iron >1 indicates inhibition of iron absorption [13]. Meanwhile, consumption of vitamin C having a molar ratio of 2:1 can overcome the inhibition of iron absorption caused by phytic acid [14].

As an excellent source of iron, meat is an important factor that must be considered for prevention of IDA. However, meat consumption in developing countries remains low. In Indonesia, per capita beef consumption is only 2.7 kg per year, which is low compared to other countries in Southeast Asia, such as Malaysia (15 kg) and the Philippines (7 kg), and particularly low compared to other large countries such as Australia (90.2 kg), United States (90 kg), Argentina (86.5 kg), and Brazil (78 kg). Meanwhile, chicken and fish consumption in Indonesia is higher with per capita consumption around 15 kg and 32.4 kg per capita per year, respectively [15].

Indonesian diets are still dominated by plant-based foods [16]. Vegetable consumption in Indonesia in 2018 was approximately 54 kg per capita per year [17], which was higher than other Asian countries such as Malaysia (46.9 kg), Thailand (37.6 kg), Sri Lanka (31.6 kg), and Bangladesh (20.5 kg) [18].

As the largest archipelagic country in the world, the territory of Indonesia consists of 17 thousand islands inhabited by more than a thousand ethnicities with different food cultures. Up to 15.5% of the population in Indonesia is Sudanese, which is the second largest ethnic group in Indonesia. Many Sudanese originate from West Java [19]. Sundanese food includes many vegetables, such as karedok (raw vegetable salad in peanut sauce), lalapan (a variety of raw vegetables served with chili sauce), and sayur asem (vegetable tamarind soup). Hence, we examined whether the plant-based dietary habits of Sudanese in Indonesia significantly affect their health. We also assessed the correlation between iron consumption from a plant based-diet by considering the molar ratio of vitamin C and phytic acid to iron with the iron status in Sundanese adolescent girls studying at Islamic boarding schools in Indonesia.

Materials and Methods

Study Design and Subject

This was a quantitative study with an analytical design and a cross-sectional approach. The subjects were female students who were randomly selected from seven Islamic boarding schools in Tasikmalaya, West Java province, Indonesia. The sample size was calculated based on the formula for the minimum number of subjects for a cross-sectional study. Based on a previous local study that estimated a prevalence of anemia among junior high school students of 50%, the minimum sample size for this study was 171 adolescent girls. A total of 176 were enrolled to account for the possibility of nonresponse [20]. Female students who were both living and eating at the school who were willing to participate and provide informed consent were enrolled in the study.

Measurements

Data on subject characteristics were obtained through face-to-face interviews with subjects. Nutritional intake data were obtained using 24-hour dietary recall method for three non-consecutive days. Dietary patterns for sources of iron and enhancers and inhibitors of iron absorption were also assessed. Although students lived in the school dormitory and were provided three meals a day, some students bought food outside the school compound. Thus, we recorded the daily meal the students received at the school cafeteria and also food or snacks bought outside of school. Food intake was recorded in the form of household portions (e.g., tablespoons, teaspoons, cups, etc.). Pictures were used as a visual aid to determine portions consumed. Food intake was converted into grams and analyzed using Nutrisoft software to calculate nutritional intake. All nutritional intake (except phytic acid) was categorized into two groups, inadequate and adequate, according to the recommended dietary allowance (RDA) in Indonesia. Phytic acid intake was classified as inadequate if the amount consumed was ≤650 mg/day [21].

The moles of phytic acid, vitamin C, and iron were determined by dividing the weight per 100 grams of food by the atomic weight (phytic acid: 660 g/mol; vitamin C: 176.12 g/mol; iron: 56 g/mol). The molar ratio of phytic acid to iron was obtained after dividing the moles of phytic acid by the moles of iron. This method is also applied to calculate the ratio of vitamin C to iron [13, 22]. To increase iron absorption, the molar ratio of phytic acid to iron and molar ratio of vitamin C to iron should ideally be ≤ 1 and 2:1, respectively [13, 14].

Venous blood samples (3 mL total) for hematological analyses were drawn from each subject in the morning. The samples were analyzed in the laboratory using a Sysmex-XNL hematology analyzer. IDA status was measured using four parameters: hemoglobin, mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentration (MCHC). MCV was used to define red blood cell size, while MCH and MCHC were used to determine hemoglobin content [23]. Subjects were identified as anemic if their hemoglobin level was below 12 g/dL. IDA was determined by the following criteria: hemoglobin < 12 g/dL, MCV below normal value (< 82 fL for age group 11-14 years; < 85 fL for age group 15-75 years), MCH < 27 pg, and MCHC < 32 g/dL [24, 25]. Hematocrit was low if the value was ≤ 36% for children aged between 12-14 years and girls aged ≥15 years [26].

All participants obtained written informed consent after they were given a thorough explanation of the study aims, procedures, and associated risks. The study protocol was approved by the Health Research Ethics Committee Faculty of Public Health Diponegoro University (No. 29/EA/KEPK-FKM/2020).

Statistical Analysis

Data were analyzed using SPSS software version 23. The normality of the data was assessed using the Kolmogorov-Smirnov test and the Rank Spearman test was used to assess correlation of variables. The data were considered statistically significant with p-value < 0.05.

Results

A total of 176 female students were enrolled in this study. The mean age of the subjects was 15.2 years-old. Daily intake of iron was 6.59 mg and intake of non-heme iron was higher (5.05 mg) than heme iron (1.52 mg) and iron from meat, fish, and poultry (MFP) (1.16 mg). The molar ratio of phytic acid to iron was 8.72 while molar ratio of vitamin C to iron was 0.03 (Table 1).

Table 1. Average daily nutritional intake, molar ratios, and hematological characteristics of adolescent girls (*N* = 176)

Variables	Mean ± SD
Age (years)	15.21 ± 1.76
Nutritional intake	
Energy (kcal/day)	1365.36 ± 580.04
Protein (g/day)	30.22 ± 12.13
Iron (mg/day)	6.59 ± 2.87
Heme-iron (mg/day)	1.52 ± 1.65
Meat, fish, poultry (MFP) (mg/day)	1.16 ± 1.67
Non-heme iron (mg/day)	5.05 ± 2.45
Phytic acid (mg/day)	606.36 ± 274.94
Vitamin C (mg/day)	6.34 ±12.07
Molar ratio	
Phytic acid:iron	8.72 ± 4.32
Vitamin C:iron	0.03 ± 0.05
Hemoglobin (g/dL)	12.29 ± 1.39
Hematocrit (%)	37.72 ± 3.35
Erythrocyte (10^6/µL)	4.75 ± 0.41
MCV (fL)	79.83 ± 7.75
MCH (pg)	26.04 ± 3.18
MCHC (g/dL)	32.54 ± 1.36

Of the study subjects, 57 had anemia (32.4%) and 39 had iron-deficiency anemia (22.2%) (Table 2). The proportion of IDA was higher among students whose parents' education level was higher (50.0% and 27.8% when the mother and father had both attended college and had graduated senior high school, respectively). Those with inadequate nutritional intake had a higher frequency of IDA than those with adequate intake. For hematological parameters, the majority of students with IDA had below normal hematocrit (59.1%), MCH (43.8%), MCHC (78.0%), and MCV status (42.6%) (Table 3).

Table 2. Proportion of anemia and iron deficiency anemia (IDA) among adolescent girls (N = 176)

Variable	*N* (%)
Hemoglobin status	
Anemia	57 (32.4)
Normal	119 (67.6)
IDA status	
IDA	39 (22.2)
Non-IDA	137 (77.8)

Table 3. Sociodemographic characteristics, nutritional intake based on recommended dietary allowance and hematological parameter status of adolescent girls with and without iron deficiency anemia (N = 176)

Variable	Iron deficiency anemia		Total
	Yes N (%)	No N (%)	N (%)
Education level			
No Education	0 (0.0)	10 (100.0)	10 (5.7)
Elementary	1 (7.1)	13 (92.9)	14 (8.0)
Junior High	38 (25.0)	114 (75.0)	152 (86.4)
Father's education level			
No Education	0 (0.0)	2 (100.0)	2 (1.1)
Elementary	23 (21.3)	85 (78.7)	108 (61.4)
Junior High	10 (22.2)	35 (77.8)	45 (25.6)
Senior High	4 (23.5)	13 (76.5)	17 (9.7)
College	2 (50.0)	2 (50.0)	4 (2.3)
Mother's education level			
No Education	0 (0.0)	2 (100.0)	2 (1.1)
Elementary	23 (22.8)	78 (77.2)	101 (57.4)
Junior High	11 (20.8)	42 (79.2)	53 (30.1)
Senior High	5 (27.8)	13 (72.2)	18 (10.2)
College	0 (0.0)	2 (100.0)	2 (1.1)
Father's employment status			
Unemployed	0 (0.0)	1 (100.0)	1 (0.6)
Employed	39 (22.3)	136 (77.7)	175 (99.4)
Mother's employment status			
Unemployed	35 (23.3)	115 (76.7)	150 (85.2)
Employed	4 (15.4)	22 (84.6)	26 (14.8)
Energy intake (kcal)			
Inadequate	37 (23.7)	119 (76.3)	156 (88.6)
Adequate	2 (10.0)	18 (90.0)	20 (11.4)
Protein intake (g)			
Inadequate	39 (22.7)	133 (77.3)	172 (97.7)
Adequate	0 (0.0)	4 (100.0)	4 (2.3)
Iron intake (mg)			
Inadequate	39 (22.5)	134 (77.5)	173 (98.3)
Adequate	0 (0.0)	3 (100.0)	3 (1.7)
Phytic acid intake (mg)			
Inadequate	28 (25.0)	84 (75.0)	112 (63.6)
Adequate	11 (17.2)	53 (82.8)	64 (36.4)
Vitamin C intake (mg)			
Inadequate	39 (22.4)	135 (77.6)	174 (98.9)
Adequate	0 (0.0)	2 (100.0)	2 (1.1)
Hematocrit status			
≤ 36	26 (59.1)	18 (40.9)	44 (25.0)
> 36	13 (9.8)	119 (90.2)	132 (75.0)
MCH status			
< 27	39 (43.8)	50 (56.2)	89 (50.6)
≥ 27	0 (0.0)	87 (100.0)	87 (49.4)
MCHC status			
< 32	39 (78.0)	11 (22.0)	50 (28.4)
≥ 32	0 (0.0)	126 (100.0)	126 (71.6)

MCV status, n (%)			
Low	46 (42.6)	62 (57.4)	108 (61.4)
Normal	0 (0.0)	68 (100.0)	68 (38.6)

There was a significant correlation between hemoglobin and hematocrit with heme iron and MFP. Non-heme iron, vitamin C, and the molar ratio of phytic acid to iron and vitamin C to iron did not correlate with all hematological parameters (Table 4). Few students had adequate iron intake based on Indonesia RDA, but this was not significantly related to IDA. IDA was also not correlated with drinking tea or drinking tea and/or coffee (Table 5).

Table 4. Correlation between nutritional intake and iron status in Sudanese adolescent girls at Islamic boarding schools in Indonesia

Variable	(*r* ; *p*) value				
	Hemoglobin	Hematocrit	MCV	MCH	MCHC
Heme iron	0.195; 0.009	0.179; 0.018	-0.014; 0.850	0.038; 0.617	0.095; 0.210
MFP	0.195; 0.009	0.190; 0.011	0.003; 0.965;	0.040; 0.601	0.077; 0.308
Non-heme iron	0.075; 0.323	0.086; 0.254	0.103; 0.174	0.073; 0.334	0.032; 0.670
Phytic acid	0.074; 0.331	0.070; 0.358	0.073; 0.338	0.072; 0.344	0.078; 0.305
Vitamin C	0.020; 0.796	0.088; 0.245	-0.013; 0.869	-0.076; 0.315	-0.114; 0.132
Molar ratio of vitamin C:iron	-0.027; 0.724	0.039; 0.603	-0.060; 0.427	-0.118; 0.118	-0.127; 0.094
Molar ratio of phytic acid:iron	-0.116; 0.126	-0.097; 0.199	-0.031; 0.686	-0.057; 0.449	-0.049; 0.519

Table 5. Correlation of iron deficiency anemia with drinking tea and coffee

Variable	Iron deficiency anemia		*p*-value
	Yes (%)	No (%)	
Drinking tea			
Yes	9 (22.0)	32 (78.0)	0.971
No	30 (22.2)	105 (77.8)	
Drinking tea and/or coffee			
Yes	11 (22.4)	38 (77.6)	0.954
No	28 (22.0)	99 (78.0)	

Discussion

In this study the overall prevalence of anemia (32.4%) was slightly higher than that in the national report, which was 26.8% in children aged 5-14 years-old and 32.0% in young adults aged 15-24 years-old [27]. Based on WHO guidelines stating that a prevalence of anemia between 20.0-39.9% is of moderate public health significance [28], anemia is indeed a public health problem among the Sundanese adolescent girls in the area. Among all anemic subjects, the proportion of subjects with IDA was 22.2%, which was considerably higher than in other developing countries such as Iran (13.9%) [29], Ethiopia (11%) [6], and Thailand (5.7%) [30]. However, the prevalence of IDA was lower compared to other Asian countries such as Malaysia (34%) [31] and Bangladesh (32%) [32].

Low iron intake might be associated with the high prevalence of IDA observed in the present study. The mean iron intake of the subjects was only 6.59 mg/day, which is lower than the daily intake stated in the Indonesian RDA of 8 mg and 15 mg for females aged 10-12 years-old and 13-15 years-old, respectively [33]. Furthermore, the iron intake of the study subjects was mainly from non-heme iron that has lower bioavailability. However, this factor did not significantly affect iron status in terms of the plant-based Sundanese diet. On the other hand, heme iron and MFP showed a positive correlation with the hematological parameters hemoglobin and hematocrit value. A study in Korea supported this finding in which anemic adolescents girls, as indicated by low hemoglobin concentration, consumed less red meat than those without anemia [34]. Girls who consumed meat (beef, mutton, pork) < 4 times/week were more than twice as likely to have iron deficiency compared to those who consumed meat ≥ 4 times/week [35]. Low hemoglobin concentration was also associated with infrequent consumption of fish and poultry, as well as milk and dairy products [36]. Hematocrit value was also likely to be affected by socioeconomic status. People with low socioeconomic status tended to have low hematocrit value, which was related to poor intake and absorption of iron [37]. Moreover, the menu at boarding schools in developing countries generally offers a limited amount of animal-based food and lacks dietary diversity, thus increasing the risk of poor iron status [38].

Not all of the hematological parameters were related to intake of inhibitors and enhancers of iron absorption. The presence of phytic acid and vitamin C in the diet did not significantly affect iron status in the Sundanese girls in this study. This result could be because food sources for vitamin C and iron were not consumed together in the same meal so that vitamin C could not optimally function as an iron absorption enhancer. In addition, the amount of vitamin C consumed by the students (6.34 mg/day) was much lower than the recommended amount of 50 mg/day, 65 mg/day, and 75 mg/day for females aged 10-12 years, 13-15 years, and 16 years and older, respectively. A study in the Philippines showed that vitamin C could affect hemoglobin concentration if the intake exceeded 24 mg/day [39]. The molar ratio of phytate to iron and the molar ratio of vitamin C to iron were used as a determinant of iron absorption, wherein a higher ratio of phytate to iron indicated higher phytate intake and lower iron absorption. On the other hand, a higher molar ratio of vitamin C to iron could increase the absorption of iron and overcome the inhibition caused by phytate intake [40]. In this study, the molar ratio of phytate to iron was high, while the molar ratio of vitamin C to iron were low, indicating iron inhibition. However, the daily intake of phytate was inadequate and vitamin C intake was far lower than recommended. Thus, neither was likely to have had a considerable effect on iron status.

We also found no significant relationship between consuming tea and consuming tea and/or coffee with IDA. Several studies showed that coffee and tea were not associated with iron deficiency in healthy people with no risk of iron deficiency [41]. Hogenkamp, et al. found that iron deficiency and IDA were not significantly explained by black tea consumption in a black adult population in South Africa [42]. Sung et al. stated that green tea intake was not related to serum ferritin levels, but coffee consumption was associated with lower serum ferritin levels in Korean adults [43]. Another study found that the mean serum-ferritin concentration was not related to black, green and herbal tea consumption in men, pre- or postmenopausal women [41]. Evidence suggested that the type of food consumed had a greater influence on iron absorption than the effect of drinking coffee or tea. Coffee and tea were more likely to inhibit the absorption of non-heme iron from plant-based foods but have very little effect on heme iron from animal foods [8]. Similarly, tea consumption in 2,573 French men (n = 954) and women (n = 1,639) had no influence on iron status [44]. Another cross-sectional study with 157 Indian participants found no differences in anemia prevalence between men and women who consumed diets that contained high and low tannin amounts [41]. Hence, consuming tea and coffee could inhibit iron absorption to cause anemia, but IDA, on the other hand, was influenced by various factors such as the type of food consumed (heme or non-heme iron), when tea and coffee was consumed (preferably 1 hour before eating to not affect iron absorption), and the level consumption of substances that increase iron absorption from food. In the present study, most students only drank coffee and tea occasionally, for instance, after waking up in the morning and during late-night study to help them stay awake, and thus neither was likely to affect iron absorption that leads to IDA.

This study also found no association between red cell indices (MCV, MCH, and MCHC) and all variables analyzed. Nevertheless, MCV and MCH values were somewhat below normal, implying the presence of IDA. Considering that the Sundanese diet is similar to a vegetarian diet, we compared the MCV and MCH values in those two groups. This comparison showed that MCV values in our study were just above that for vegetarians (79.8 fL vs. 78.4 fL), while MCH values were slightly lower (26.0 pg vs. 27.2) [45]. Furthermore, MCV and MCH values in Sundanese girls were similar to those for high school girls who suffer iron deficiency without anemia in Nakhon Si Thammarat, Thailand (MCV = 80.0 fL; MCH = 26.9 pg) [30].

Conclusions

The prevalence of IDA was high in Sundanese adolescent girls studying at Islamic boarding schools in Indonesia. However, the Sundanese diet, which consists mostly of plant-based foods, was not a factor that caused IDA. Instead, IDA had a greater association with the consumption of less animal-based foods that have higher bioavailability of iron. Hence, to reduce incidence of IDA, animal-based foods should be offered more frequently at these schools.

Data Availability

The data used to support the findings of this study are available from the corresponding author upon request.

Conflicts of Interest

The authors declare that there is no conflict of interest regarding the publication of this paper.

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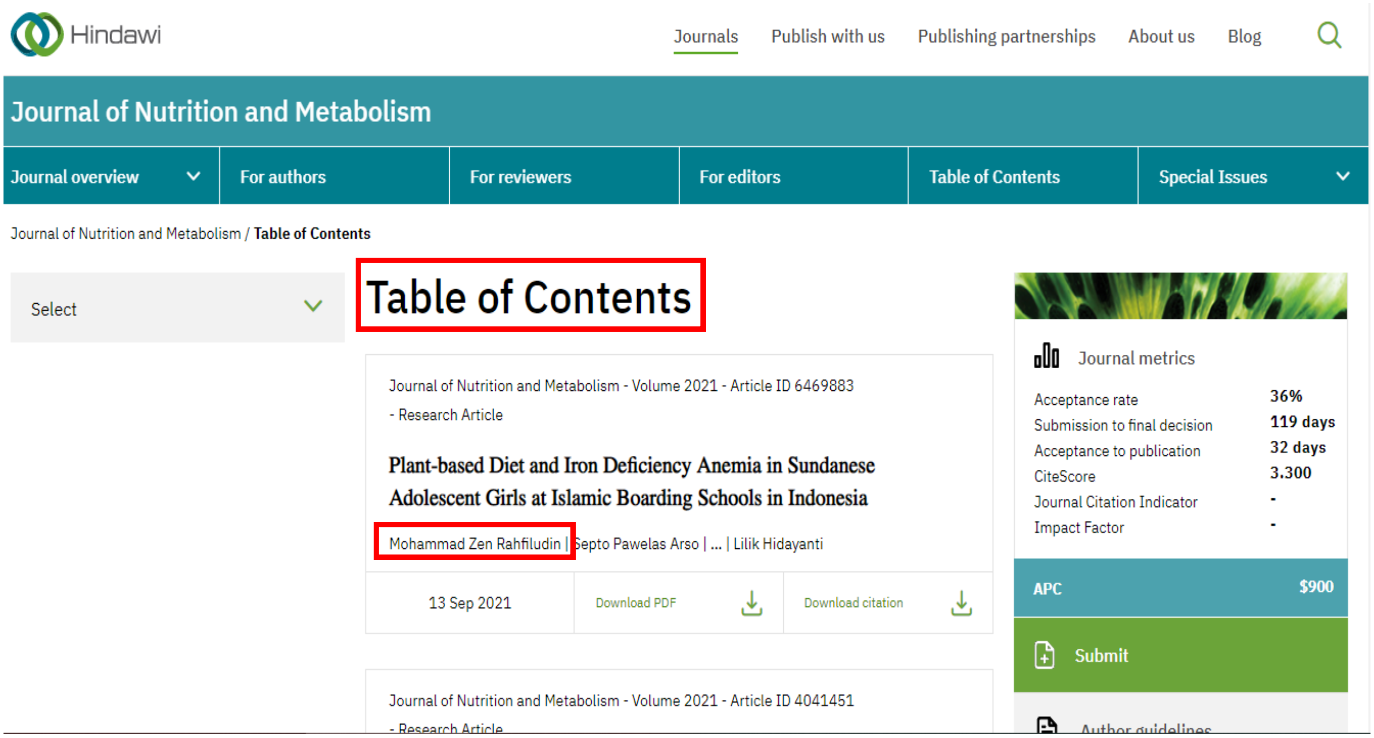

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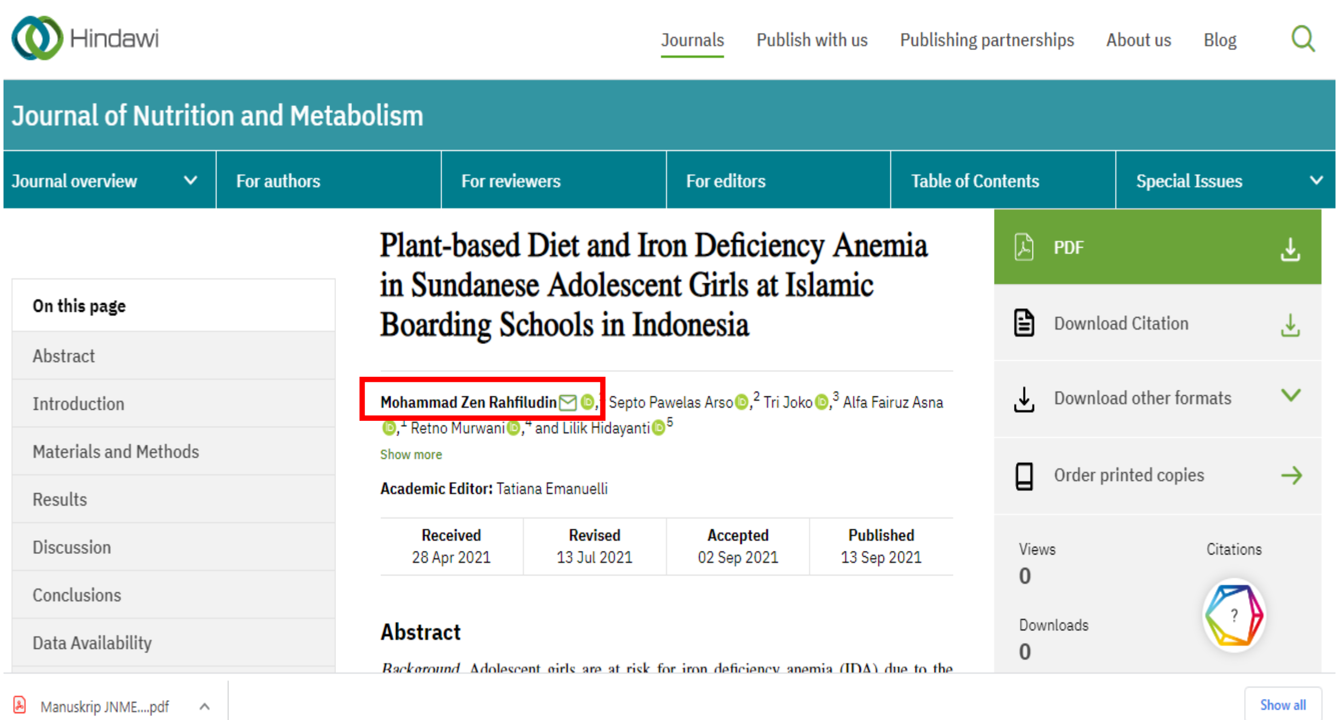

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Abstract

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