Effects of iron supplementation and nutrition education on haemoglobin, ferritin and oxidative stress in iron-deficient female adolescents in Palestine: randomized control trial

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Abstract

Background: Iron deficiency and iron-deficiency anaemia are associated with oxidative stress, but their role is largely unclear. Information is scarce on the effects of iron supplementation on biomarkers of oxidative stress in humans.

Aims: This study evaluated the effectiveness of iron supplementation and nutrition education on improving the levels of haemoglobin and ferritin, and decreasing oxidative stress among iron-deficient female adolescents in Gaza, Palestine.

Methods: A total 131 iron-deficient female adolescents were recruited and allocated randomly into 3 different groups. The iron supplementation group (A) received 200 mg of ferrous fumarate weekly during the 3-month intervention, the iron supplementation with nutrition education group (B) received iron supplements with nutrition education sessions, and the control group (C) did not receive any intervention. The levels of haemoglobin, ferritin and malonyl dialdehyde were measured at baseline, after 3 months (at which point the intervention was stopped), and then 3 months later. Trial registration number: ACTRN12618000960257.

Results: Haemoglobin levels increased significantly after supplementation in both groups A and B. At the follow-up stage (3 months after stopping the intervention), iron and haemoglobin levels in group B continued to increase and malonyl dialdehyde decreased. In Group A, haemoglobin, ferritin and malonyl dialdehyde levels decreased after 3 months of stopping the intervention. No changes were seen in Group C.

Conclusions: A nutrition programme should be adopted and integrated into comprehensive intervention programmes to target iron-deficiency anaemia among female adolescents in Palestine.

Keywords: Adolescent; female; anaemia, iron deficiency; oxidative stress; dietary supplements, Gaza

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Introduction

Iron deficiency is the most common form of nutritional disorder worldwide, affecting more than 2 billion people globally (1). Iron deficiency is not the only cause of anaemia but where anaemia is prevalent, iron deficiency is the most common cause (2). Most adolescents suffer from iron deficiency and its adverse health effects (2). In Gaza, Palestine, the prevalence of anaemia was reported as 33.3% (3), while the prevalence of iron deficiency was 23.6% among adolescents (4). Adolescents of today are the adult population of tomorrow and therefore their health and wellbeing are important (5).

Pregnancy increases the risk of iron deficiency and the approach to iron-deficiency anaemia in pregnancy has changed recently, from providing nutritional supplements during pregnancy to taking steps earlier to ensure that women, especially adolescents, have adequate iron stores before conception (6).

Previous studies have found that knowledge is one of the first steps to changing behaviour. Nutrition knowledge is therefore an essential basis for good dietary habits (7,8). Conversely, a lack of knowledge is a risk factor for malnutrition (9). Nutrition education programmes are needed to increase awareness in female adolescents about anaemia (10). Moreover, health education has proven to be very effective and has resulted in a substantial improvement in iron levels and nutrition knowledge (11,12).

Oxidative stress is defined as an imbalance between the oxidation and anti-oxidation systems, resulting in the excessive production of reactive oxygen species (13,14). Reactive oxygen species of erythrocytes is one of the principal causes of anaemia (15). Red blood cells are subjected to continuous oxidative stress during their lifetime. They are particularly susceptible to oxidative damage from high content of unsaturated fatty acid chains in the lipid bilayer combined with high oxygen...
levels (16).

As far as we know, no studies have been carried out to assess the effects of ferrous fumarate supplementation on the biomarkers of oxidative stress in female adolescents with iron deficiency and iron deficiency anaemia. Therefore, the aim of this study was to evaluate the effects of iron supplementation and nutritional education on haemoglobin (Hb) and ferritin levels and oxidative stress in female adolescents (aged 15–19 years) with iron deficiency and iron-deficiency anaemia in Gaza, Palestine.

Methods

Study design and setting

This randomized control trial was conducted in Gaza, Palestine on female adolescents aged 15–19 years with iron deficiency and iron-deficiency anaemia (mild and moderate). The study was conducted from 25 October 2015 to 25 April 2016.

Sample size and selection

The sample size was calculated based on the formula for 2 means:

\[ n = \left(\frac{Z_\alpha/2 + Z_\beta}{\sigma}\right)^2 \left(\frac{\mu_1 - \mu_2}{\sigma}\right)^2 \]

Where:
- \( \mu_1 \) = mean change in Hb after 3 months from iron supplements = 1.23 g/dL (17).
- \( \mu_2 \) = expected mean change in Hb after 3 months from iron supplements = 2 g/dL
- \( \sigma \) = standard deviation = 1.195
- \( Z_\alpha/2 \): at 5% level of significance = 1.96
- \( Z_\beta \): for a power of 80% = 0.84.

The calculation gave 38 respondents in each group. However, assuming a 15% drop-out from the trial, the number of participants was increased to 45 per group.

Eligibility

Participants were eligible for inclusion in the trial if they were unmarried and not pregnant. Participants were excluded if they: had severe anaemia (Hb < 8 g/dL); were suffering from acute or chronic infections that could affect their Hb and ferritin levels at the time of the blood sampling; had anaemia other than iron-deficiency anaemia; were underweight; were on medication; or were diagnosed with thalassemia trait.

Group allocation and intervention

This intervention programme was part of a 2-phase study in which anaemia and iron deficiency status were assessed among female adolescents attending secondary schools in Gaza (first phase).

In the first phase, a sample of female adolescents aged 15–19 years was selected from secondary schools in Gaza. As there are 5 governorates in Gaza, 1 school was selected from each governorate out of a total of 145 schools. Schools were listed by governorate and 1 was randomly selected from each governorate. Similarly classes were selected randomly from grades 10, 11 and 12 in the selected schools, 1 or 2 class(es) from each grade according to the population of the governorate. All the girls in the selected classes (330 girls) were assessed for iron deficiency and iron-deficiency anaemia. Preliminary screening for iron deficiency and iron-deficiency anaemia (Hb < 12 g/dL and ferritin < 15 µg/L) was done through vein blood samples. The girls were also interviewed in school by a female research assistant about their socioeconomic status and to determine if they met the eligibility criteria.

A total of 177 girls (54%) had iron deficiency or iron-deficiency anaemia. Of these, 43 did not meet the eligibility criteria and were excluded. Therefore 135 students with iron deficiency or iron-deficiency anaemia were invited to participate in the second phase of the study. None was taking taking any supplements. Those who agreed (n = 131) were randomized into 3 groups (Figure 1).

- Group A (iron supplementation) included 45 participants who received 200 mg of ferrous fumarate once weekly for 3 months; this was given by the researchers during school hours.
- Group B (iron supplementation with nutrition education) included 44 participants who received iron supplements (ferrous fumarate) once weekly and 9 nutritional education sessions (1.5 hours/session) for 3 months.
- Group C (controls) included 42 participants who did not receive any intervention throughout the study period.

The purpose of the nutrition education was to teach the participants about the importance of good nutrition, with an emphasis on iron deficiency and iron-deficiency anaemia. The nutrition education intervention consisted of lectures, posters, videos, booklets, and brochures. Nutrition lectures were delivered by the researchers using simple methods and vocabulary to present topics on nutrition in Arabic such as food groups, the food pyramid, a balanced diet, iron absorption enhancers and inhibitors, good sources of iron, and ways to improve the absorption of iron from foods.

After 3 months of the intervention, the supplementation and education sessions were stopped for Groups A and B, and the groups were followed up after a further 3 months.

After the study was completed, the intervention was offered to the girls in the control group; they were given a packet of iron supplement tablets (20 tablets) and 2 sessions of nutrition education.

Measurements

Three blood assessments were done: at baseline (25 October 2015), after 3 months (first post-intervention, 25 January 2016), and after 6 months of baseline (second post-intervention (25 April 2016).

Complete blood count, serum ferritin and malonyl dialdehyde (MDA) levels were measured. About 5 mL of
venous blood were drawn (0.5 mL in EDTA tubes and 3 mL in serum evacuated tubes) from each participant. All the samples were placed in tube racks and packed in an appropriate ice container (4 °C) and sent to a laboratory accredited by the Palestinian Ministry of Health. The samples were analysed on the same day. Blood samples were coded so assessors were unaware of the group a blood sample was from.

Anaemia was assessed by measuring Hb concentration in the complete blood count analysis (Horiba ABX Micros ES 60, France). Iron status was assessed by measuring the serum ferritin levels (Chemistry Autoanalyzer, Model: BS-120, Mindray Bio-Medical Electronic Co. Ltd, China) in duplicate, and recording the average results. An MDA adduct competitive ELISA kit (OxiSelect™, Cell Biolabs, Canada) was used to measure MDA levels.

Body weight of each participant was measured by using a calibrated scale (Seca model 750 1017009, Germany. Students were weighed barefooted to the nearest 0.5 kg. Standing height also was measured without shoes to the nearest 0.5 cm with a stadiometer (Seca body meter 206, Germany), with the shoulders in a relaxed position and the arms hanging freely. The body mass index (BMI) for age was be calculated using the Anthro Plus program of
the Centers for Disease Control and Prevention (version 7.1.5.2). Duplicate measurements of weight and height of the students were taken and the mean of value was determined.

**Statistical analysis**
All analyses were conducted using SPSS, version 22. Repeated measures analysis of variance (one-way repeated measures ANOVA) was used to evaluate changes in all the continuous variables (Hb, ferritin and MDA) over the study period. Repeated measures ANOVA measured the changes between the groups, within the group, and time and group interactions. A P-value < 0.05 was considered statistically significant.

**Ethical considerations**
Before data collection, written permission to carry out the study was obtained from the Helsinki Committee in Palestine and the Ministry of Education in Palestine (reference number PHRC/HC/3’/14). After reviewing the study protocol, the Human Ethics Committee of Universiti Kebangsaan Malaysia gave its ethical approval (reference number UKM.5.3.5/244/NN-025-2015).

In keeping with social and cultural norms, a female interviewer was hired. Written consent was obtained from all the respondents if they were aged ≥18 years or from their parents if they were under 18 years. It was explained that they were free to withdraw from the study at any time.

**Trial registration**
This trial is registered under ACTRN number: ACTRN12618000960257.

**Results**

**Descriptive results of the study sample**
Table 1 describes the baseline sociodemographic characteristics of the participants (age, region, educational grade), BMI, and blood analysis. Ferritin and MDA levels in group B were slightly higher than in the other 2 groups but no statistically significant differences were found. Thus, no adjustments were made in the outcome analysis on the basis of any of the variables.

**Effect of nutrition education and supplementation on Hb, ferritin and MDA status**

The main objective of the trial was to determine the effectiveness of iron supplementation and nutrition education on the levels of Hb, ferritin and MDA in female adolescents aged 15–19 years. The effect of the intervention was assessed using the 2 dimensions of repeated measures ANOVA; firstly, the within group effect to determine whether there was a difference in the blood parameters (Hb, ferritin and MDA), and secondly, to assess the interaction of treatment and time together.

Table 2 shows that the difference in the mean Hb concentration was statistically significant between within the same group at different intervals (P < 0.001). A post-hoc test using the Bonferroni correction showed that there was a statistically significant difference between all the timelines. The mean ferritin concentration also differed significantly between the timelines (P < 0.001). A post-hoc test using the Bonferroni correction showed that there was a statistically significant difference between the baseline and midline, and the baseline and end-line, but there was no statistically significant difference between the midline and end-line. There was a statistically significant difference between the 3 timelines in MDA concentrations also (P < 0.001). The Bonferroni test showed that the differences were statistically significant between the baseline and midline, the midline and end-line, but not between the baseline and end-line.

The results of the mean ferritin concentration were statistically significant between the groups at midline and end-line (post-intervention and post-intervention 2) (P < 0.001).

Figures 2, 3 and 4 explain the differences in the effect of nutrition education intervention between the 3 groups after 6 months. The trend in the plot showed the adjusted mean levels (estimated marginal mean) of Hb, ferritin and MDA for zero months (baseline), 3 months (intervention period) and 6 months (follow-up without intervention). The mean Hb, ferritin and MDA levels differed significantly between the timelines (Hb, ferritin and MDA), and secondly, to assess the interaction of treatment and time together.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Group A (n = 45)</th>
<th>Group B (n = 44)</th>
<th>Group C (n = 42)</th>
<th>Test value</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years) [mean (SD)]</td>
<td>16.11 (0.89)</td>
<td>16.37 (0.78)</td>
<td>16.51 (0.89)</td>
<td>2.422&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.09</td>
</tr>
<tr>
<td>Grade (10th, 11th, 12th) [No.]</td>
<td>18, 12, 15</td>
<td>16, 10, 18</td>
<td>9, 15, 18</td>
<td>4.452&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.34</td>
</tr>
<tr>
<td>Region (NG, GC, MG, KH, RF) [No.]</td>
<td>10, 10, 9, 6</td>
<td>4, 15, 14, 6, 5</td>
<td>12, 10, 10, 7, 3</td>
<td>7.915&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.44</td>
</tr>
<tr>
<td>Body mass index (kg/m²) [mean (SD)]</td>
<td>21.74 (3.88)</td>
<td>22.63 (3.57)</td>
<td>22.49 (4.13)</td>
<td>20.405&lt;sup&gt;d&lt;/sup&gt;</td>
<td>0.50</td>
</tr>
<tr>
<td>Haemoglobin (g/dL) [mean (SD)]</td>
<td>11.52 (0.96)</td>
<td>11.45 (1.18)</td>
<td>11.73 (0.59)</td>
<td>0.800&lt;sup&gt;e&lt;/sup&gt;</td>
<td>0.41</td>
</tr>
<tr>
<td>Ferritin (µg/dL) [median (IQR)]</td>
<td>8.60 (4.0)</td>
<td>10.50 (4.5)</td>
<td>9.50 (6.0)</td>
<td>1.471&lt;sup&gt;f&lt;/sup&gt;</td>
<td>0.47</td>
</tr>
<tr>
<td>Malonyl dialdehyde (Pmol/mL) [median (IQR)]</td>
<td>83.0 (43.0)</td>
<td>92.0 (31.0)</td>
<td>83.50 (65.0)</td>
<td>1.076&lt;sup&gt;g&lt;/sup&gt;</td>
<td>0.58</td>
</tr>
</tbody>
</table>

<sup>a</sup>One-way ANOVA F-test. <sup>b</sup>Kruskal–Wallis chi-squared test.

NG: North Gaza, GC: Gaza City, MG: Middle Governorate, KH: Khan Younis, RF: Rafah.

SD = standard deviation, IQR = interquartile range.
were almost equal for the 3 groups at the baseline. After 3 months of the intervention, the mean Hb, ferritin, and MDA levels increased in the 2 intervention groups but there were no changes in the control group. After 6 months of the baseline, the Hb and ferritin levels still increased in the nutrition education group, while the MDA level decreased in the same group. On the other hand, for the group that received iron supplementation alone, there was a decrease in the Hb and ferritin levels at the follow-up stage.

Discussion
This study evaluated the effects of an iron supplementation and nutrition education intervention on Hb, serum ferritin and MDA levels as indicators of oxidative stress among female adolescents with iron deficiency and iron-deficiency anaemia in Gaza, Palestine.

After 3 months of the intervention, mean Hb concentration had increased significantly in groups A and B. This finding is consistent with the results of studies in Malaysia and Ghana in 2012 (17,18).

The results of our study showed that weekly iron supplementation (200 mg of ferrous fumarate) led to an increase in Hb levels from a mean of 11.52 (SD 0.96) g/dL to 12.46 (SD 0.64) g/dL after 3 months. This result is consistent with that of previous studies. For example, a study in India found an increase in mean Hb levels from 10.51 (SD 0.35) g/dL to 12.49 (SD 0.65) g/dL after 3 months of weekly iron supplementation with folic acid among

Table 2 Changes in haemoglobin, ferritin and malonyl dialdehyde levels at different intervention times

<table>
<thead>
<tr>
<th>Blood analysis</th>
<th>Group A (n = 45)</th>
<th>Intervention groups</th>
<th>Group B (n = 44)</th>
<th>Group C (n = 42)</th>
<th>Mixed repeated-measure ANOVA</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean (SD)</td>
<td></td>
<td>Mean (SD)</td>
<td>Mean (SD)</td>
<td>Time effect</td>
</tr>
<tr>
<td></td>
<td>Baseline</td>
<td>Midline</td>
<td>End-line</td>
<td>Baseline</td>
<td>Interaction effect</td>
</tr>
<tr>
<td>Haemoglobin (g/dL)</td>
<td>11.52 (0.96)</td>
<td>11.45 (1.18)</td>
<td>11.73 (0.89)</td>
<td>0.373 (&lt; 0.001)</td>
<td>0.177 (&lt; 0.001)</td>
</tr>
<tr>
<td>Ferritin (µg/L)</td>
<td>9.92 (3.16)</td>
<td>9.19 (2.92)</td>
<td>9.70 (3.48)</td>
<td>0.511 (&lt; 0.001)</td>
<td>0.331 (&lt; 0.001)</td>
</tr>
<tr>
<td>Malonyl dialdehyde (Pmol/L)</td>
<td>11.92 (5.43)</td>
<td>14.75 (3.69)</td>
<td>10.13 (5.25)</td>
<td>0.437 (&lt; 0.001)</td>
<td>0.186 (&lt; 0.001)</td>
</tr>
</tbody>
</table>

*Significant at P < 0.05. ηp = partial eta, SD = standard deviation.

Figure 2 Mean ferritin levels of the groups pre- and post-intervention

Figure 3 Mean haemoglobin levels of the groups pre- and post-intervention
levels increased from 34.3 (SD 2.49) µg/L to 37.5 (SD 2.49) µg/L among female adolescents aged 12–17 years with anaemia but not iron deficiency following weekly iron supplementation for 3 months (23). This difference in the amount of increase may be because the Malaysian study targeted non-iron-deficient female adolescents. In addition, according to the results of our study, the change in the ferritin levels after the iron supplementation was lower than the study by Opoku, in which an increase in the ferritin levels was reported from a mean of 14.17 (SD 0.64) µg/L to 40.38 (SD 4.95) µg/L following 5-day a week iron supplementation for 10 weeks among students aged 6–11 years with iron-deficiency anaemia (18). This difference may have been due to the age differences between the studied samples.

Information is scarce on the effects of iron supplementation on the biomarkers of oxidative stress in humans (24). The lack of epidemiological data on oxidative damage in healthy human populations is a serious gap in the distribution, correlation and causative factors of oxidative damage (25). Iron deficiency and iron-deficiency anaemia are associated with oxidative stress, but their role in initiating stress is largely unclear. Also, oxidative stress induced by iron deficiency and iron-deficiency anaemia may also be caused by an inadequate supply of oxygen to tissues, resulting in increased concentrations of inflammatory mediators that activate leukocytes (26,27). There are inconsistencies in the results of these studies. Previous studies reported that MDA levels increased significantly in iron-deficiency anaemia (28–30).

In iron-deficiency anaemia, oxidative stress increases with the generation of free radicals, while therapeutic doses of iron supplements increase oxidative stress and antioxidant supplementation reduces oxidative stress. This increase and reduction in oxidative stress was studied among adults (31). Unlike in adults, oxidative stress in iron-deficiency anaemia is not aggravated by iron supplementation among children aged 10 months to 16 years (31). Tiwari et al. concluded that iron supplementation is effective in improving Hb levels but at the cost of increased oxidative stress among women with anaemia (29). An increase in MDA levels has been reported after 13 weeks of iron supplementation (32). An increase was also found in women after only 4 weeks of iron supplementation (33). The MDA level correlated with the serum ferritin level, suggesting that the iron status, having been modified by iron supplementation, increased the biomarker of lipid peroxidation (MDA).

In contrast, the findings of our study differ from other studies that reported iron supplementation for 6 weeks or 12 weeks caused a decrease in MDA levels (24,30), while another study reported no significant effects on lipid peroxidation and iron-deficiency anaemia after iron supplementation (33). These different findings might be explained by the different study designs, sample sizes, biomarkers, the consideration of certain covariates in some studies, and the health status of the participants. The use of various dosages of iron supplements and
Effets de la supplémentation en fer et de l’éducation nutritionnelle sur l’hémoglobine, la ferritine et le stress oxydatif chez des adolescentes carencées en fer (Palestine) : essai contrôlé randomisé

Résumé


Objectifs : La présente étude a évalué l’efficacité de la supplémentation en fer et de l’éducation nutritionnelle sur l’amélioration des taux d’hémoglobine et de ferritine, ainsi que sur la réduction du stress oxydatif chez les adolescentes carencées en fer à Gaza (Palestine).

Méthodes : Au total, 131 adolescentes carencées en fer ont été recrutées et réparties de façon aléatoire en trois groupes distincts. Le groupe de supplémentation en fer (A) a reçu 200 mg de fumarate de fer sur une base hebdomadaire au cours d’une intervention de trois mois. Le groupe de supplémentation en fer avec éducation nutritionnelle (B) a reçu des suppléments en fer et a assisté à des sessions d’éducation nutritionnelle. Le groupe témoin (C) n’a bénéficié d’aucune intervention. Les taux d’hémoglobine, de ferritine et de malonyl-dialdéhyde ont été mesurés à l’étape de suivi (trois mois après l’arrêt de l’intervention), les taux de fer et d’hémoglobine du groupe B continuaient d’augmenter et les taux de malonyl-dialdéhyde diminuaient. Dans le groupe A, les taux d’hémoglobine, de ferritine et de malonyl-dialdéhyde diminuaient trois mois après l’arrêt de l’intervention. Aucun changement n’a été observé dans le groupe C.

Conclusions : Un programme de nutrition devrait être adopté et intégré aux programmes d’intervention globaux de façon à cibler l’anémie ferriprive chez les adolescentes en Palestine.
أثر مكملات الحديد والتثقيف التغذوي على مستويات الهيموجلوبين والفيريتين والإجهاد التأكسدي لدى المراهقات اللائي تعانون من نقص الحديد في فلسطين: حِرْبة ضاِبِطَة عِشْوَائِة

مروان جلمبو1, نوريما كريم2, إيهاب نصر3، رازينة شاريف

الخلاصة
يرتبط نقص الحديد وفقر الدم الناجم عن نقص الحديد بالإجهاد التأكسدي، إلا أن دور كل منها يظل غير واضح بدرجة كبيرة. ولا توفر الخلفية: سوا معلومات شحيحة بشأن آثار مكملات الحديد على العلامات البيولوجية للإجهاد التأكسدي في البشر. عمدت هذه الدراسة إلى تقييم فعالية مكملات الحديد والتثقيف التغذوي على تحسن مستويات الهيموجلوبين والفيريتين، وتفاوت الأهداف: مستويات الإجهاد التأكسدي في صفوف المراهقات اللائي تعانون من نقص الحديد في قطاع غزة بفلسطين.

 metodologies: طرق البحث: ضمت هذه الدراسة 121 مراهقة من تعانون من نقص الحديد، تم توزيعهن بشكل عشوائي على 3 مجموعات مختلفة. تلقن مجموعة مكملات الحديد (المجموعة ألف) 200 مليجرام من فومارات الحديد أسبوعيا خلال فترة التدخل الممتدة لثلاثة أشهر، وتلقن مجموعة مكملات الحديد والتنقية الغذائية (المجموعة باء) مكملات الحديد مع عقد جلسات للتثقيف الغذائي، ولم تتلق المجموعة الضابطة (المجموعة جيم) أي تدخلات. وفي المجموعة ألف، انخفضت مستويات الهيموجلوبين والفيريتين وثنائي ألكدريد المالون قبل قبل ثلاثة أشهر، أي بعد ثلاثة أشهر من انتهاء التدخل.

النتائج: ارتفعت مستويات الهيموجلوبين، الفيريتين وثنائي ألكدريد المالون في المجموعتين ألف وباء. وفي المجموعة ألف، انخفضت مستويات الحديد وثنائي ألكدريد المالون بعد ثلاثة أشهر من أول سباعيات التدخل، ولم تشهد أي تغييرات في المجموعة جيم. الاستنتاجات: ينبغي اعتماد برنامج للتغذية وإدماجه في برامج التدخل الشاملة لاستهداف فقر الدم الناجم عن نقص الحديد في صفوف المراهقات في فلسطين.

References


