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Effects of prophylactic iron supplementation on outcome of nonanemic pregnant women: A non-randomized clinical trial

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Abstract

Background: The aim of the current study was to investigate the effects of prophylactic iron supplementation on the pregnancy outcome of nonanemic pregnant women in a sample of Iranian population.

Methods: This non-randomized clinical trial was conducted during a 2-year period in obstetrics clinics of Shiraz, southern Iran. We included a sample of singleton pregnancies registered in our clinics. Those with comorbidities were excluded. Serum ferritin was measured at baseline and participants were classified accordingly: those with normal serum ferritin levels (≥30 μg/dL) who received standard prophylactic iron supplementation during the pregnancy (Group 1); those who had minor thalassemia and elevated serum ferritin levels (≥30 μg/dL) who did not receive prophylactic iron supplementation or those with normal ferritin levels (≥30 μg/dL) who refused to receive iron supplementation due to gastrointestinal upset (Group 2); and those with iron deficiency anemia with low serum ferritin levels (<30 μg/dL) who received standard iron supplementation during pregnancy (Group 3). All the participants were followed to the delivery and maternal and neonatal outcomes were recorded and compared between three study groups.

Results: Overall we included 30 pregnant women in each group with mean age of the participants was 28.66 ± 6.02 years. There was no significant difference between three study groups regarding gestational age at delivery ($p = 0.250$), birthweight ($p = 0.893$), Apgar at 1 ($p = 0.532$) and 5 ($p = 0.590$) minutes, and route of delivery ($p = 0.590$). The overall rate of maternal complication of the pregnancy was comparable between the three study groups ($p = 0.188$). However, those in group 1, had significantly higher rate of gestational diabetes mellitus (GDM) when compared to other two groups ($p = 0.038$).

Conclusion: Prophylactic iron supplementation in pregnant women with normal ferritin levels is associated with increased risk of GDM. Other pregnancy and neonatal outcomes are not affected by the prophylactic iron supplementation.

Keywords: Anemia; Complications; Iron status; Iron supplementation; Pregnancy; Outcome

1. INTRODUCTION

Iron deficiency is one of the most common nutritional deficit in the world which is associated with significant morbidity and mortality worldwide, especially in Africa.[1] Epidemiologic studies have revealed that the prevalence of anemia in pregnant women is as high as 38.2% worldwide[2] and is associated with several adverse pregnancy outcomes including low birthweight, hypertensive disorder and premature birth.[3] Several meta-analysis of the observational studies have demonstrated that anemic mothers, experience adverse pregnancy outcomes in about half of the cases which lead to a significant burden of the disease.[4,5] The maternal iron requirement is considered to be approximately 1000 mg/d in women with normal weight.[6] Thus, WHO recommendation includes supplementation of 30 to 60 mg elemental iron and 400 mg folic acid per day for pregnant women regardless of the iron status for primary prevention.[7] There are several risk factors for anemia during pregnancy which include iron-deficient diet, malabsorption, short-pregnancy intervals, and preconception iron status.[8] However, in anemic women, the recommendation is 120 mg elemental iron per day for 3 months and vitamin A combined with iron that has greater effect in pregnant women who suffer from anemia in second trimester.[9]

In a Cochrane review, adverse effects of intermittent iron regimen and daily iron supplementation on pregnancy outcome was evaluated. There was similar maternal and neonatal outcome in both regimens. Also intermittent regimen had lower side effects compared with daily supplementation.[10] In another study, it was showed that prescription of iron supplementation in early pregnancy can improved the perinatal outcome.[11] Although several meta-analysis and systematic reviews have addressed the association between the maternal and neonatal outcome of pregnancy and the iron deficiency anemia, the results are inconclusive.[12,13] Several observational studies including cross-sectional, cohort, and case-control ones have demonstrated that the iron deficiency anemia during the pregnancy is associated with adverse

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pregnancy outcomes including the low birthweight,[14,15] premature birth,[16,17] and perinatal death.[18] However, the clinical data are inconclusive and does not support the results of these studies. The importance of the iron supplementation is also limited in asymptomatic nonanemic pregnant women. Recent meta-analysis demonstrated that there is insufficient evidence on the accuracy of screening in asymptomatic pregnant women but fair-quality evidence that treating asymptomatic iron deficiency anemia in pregnancy results in moderate health benefits.[14] Another meta-analysis demonstrated that there is inconclusive evidence that routine prenatal supplementation for anemia improves maternal or infant clinical health outcomes, but supplementation may improve maternal hematologic indices.[12] Maternal iron overload in pregnancy is associated with adverse pregnancy outcomes for both mother and fetus as well as its deficiency harmfulness.

To explain more, one meta-analysis showed the increased risk of gestational diabetic mellitus in the mothers who had elevated levels of Hb and Ferritin.[19]

And also, iron supplementation in nonanemic mothers has been proven to increase risk of GDM via oxidative stress pathway.[20–23]

Thus, we conducted this clinical trial to investigate the effects of prophylactic iron supplementation on the pregnancy outcome of nonanemic pregnant women in a sample of Iranian population.

2. METHODS

2.1. Study population

This non-randomized clinical trial was conducted during a 2-year period from March 2016 to March 2018 in obstetric clinics of Zeinabieh and Hafez hospitals, both tertiary healthcare centers affiliated with Shiraz University of Medical Sciences and both referral centers for obstetrics in southern Iran. We included all the singleton pregnancies within the first trimester who were between 18 and 40 years of age and were registered to our centers for prenatal care. We excluded those who comorbidities including diabetes, hypertension, liver and renal failure, cardiovascular disorders, and chronic obstructive pulmonary disease. We have also excluded those with previous history of pregnancy complications including pre-eclampsia, gestational diabetes, premature birth, abortion, stillbirth, low birthweight, and premature rupture of the membrane. Those who did not comply with complete and routine use of iron and folic acid supplementation and those who were lost to follow-up were also excluded from the study. The study protocol was approved by either institutional review board (IRB) and medical ethics committee of Shiraz University of Medical Sciences (Registration code: IR.SUMS.MED.REC.1396.21). The study protocol was also registered with the Iranian registry for clinical trials (www.irct.ir; IRCT2017051533976N1). All the patients provided their informed written consents before inclusion in the current study.

2.2. Study protocol and intervention

All the included women were registered in our clinics and the contact data were available. They were initially examined by an obstetrics and gynecology resident and the baseline characteristics and the clinical information were recorded into data gathering forms. The serum level of ferritin was measured at baseline to determine the iron status in pregnant women. The participants were then categorized based on the serum ferritin level to three groups: those with normal serum ferritin levels (≥30 μg/dL) who received standard prophylactic iron supplementation during the pregnancy (Group 1); those who had minor thalassemia and elevated serum ferritin levels (≥30 μg/dL) who did not receive prophylactic iron supplementation; those with normal ferritin levels (≥30 μg/dL) who refused to take iron supplementation due to gastrointestinal upset (Group 2); and those with iron deficiency anemia with low serum ferritin levels (<30 μg/dL) who received standard iron supplementation during pregnancy (Group 3). All the groups were fully matched regarding the baseline characteristics including the age, gestational age, parity, and gravidity.

2.3. Laboratory analysis

Serum ferritin and serum iron was measured during the first trimester and complete blood count (CBC) was measured in first, second, and third trimester in all the participants. The serum ferritin level was measured using the enzyme-linked immunosorbent assay (ELISA) in Hafez laboratory affiliated with Shiraz University of Medical Sciences. All the measurements were performed utilizing the Human Ferritin IRMA Kit (Isar Padtan Gostar, Arak, Iran) according to the manufacture instructions. The intra-assay and interassay coefficients of variation were <6% for all assays performed.

2.4. Follow-up and outcome assessment

The women were visited monthly till 28 weeks of gestation, twice weekly till 36 weeks, and then weekly till the end of pregnancy and the maternal and neonatal indices were measured and recorded. All the participants were followed till the end of the pregnancy and the clinical pregnancy outcome was recorded. The maternal adverse outcomes including the pre-eclampsia, gestational diabetes, premature birth, abortion, stillbirth, pregnancy duration, delivery type, and low birthweight were recorded. The neonatal outcomes included the Apgar score at 1 and 5 minutes, the aspiration pneumonia, and NICU admission. The results were compared between the study groups.

2.5. Statistical analysis

Based on the prevalence of anemia in pregnant women with an 80% power to detect 0.05 difference between main study outcomes with α equal to the 0.01, 27 subjects were required in each study group. In order to compensate for non-evaluable patients, we included 30 women in each study groups. All the data were entered into a computer database and were further analyzed using statistical package for social sciences (SPSS Inc., Chicago, Illinois, USA) version 16.0. All data are presented as mean ± SD as appropriate. The parametric variables with normal distributions were compared using one-way analysis of variance (ANOVA) with Tukey as the post hoc test. The parametric variables without normal distribution were compared using the Kruskal–Wallis test. Proportions were compared using chi square test. A two-sided p value of <0.05 was considered statistically significant.

3. RESULTS

Overall we included 90 pregnant women with singleton pregnancies who finished the study all. Thus, the final sample size which was included in the analysis was 90 (each group containing 30 women). The mean age of the participants was 28.66 ± 6.02 (ranging from 18 to 39 years). All the three study groups were found to be comparable regarding the baseline characteristics such as age, body mass index (BMI), gravidity, and parity. Those in group 3 had significantly lower ferritin levels compared to groups 1 ($p < 0.001$) and 2 ($p < 0.001$). The baseline characteristics of the participants is summarized in Table 1.

The indices of the CBC including the hemoglobin (Hb), RBC, mean corpuscular volume, and mean corpuscular hemoglobin were comparable between the three study groups at baseline. These indices were also comparable between the three study groups in second and third trimesters. Tables 3 compares the blood indices between the three study groups in first, second, and third trimesters.

Table 1

The baseline characteristics of 90 pregnant women with singleton pregnancies who participated in the current study and were categorized based on the serum ferritin levels

	Group 1 (n = 30)	Group 2 (n = 30)	Group 3 (n = 30)	*p*
Age (years)	29.6 ± 16.1	29.6 ± 12.3	27.5 ± 8.3	0.651
Weight (kg)	63.8 ± 4.26	64.7 ± 4.57	63.6 ± 6.19	0.862
Height (cm)	162.5 ± 6.3	159.4 ± 5.8	160.5 ± 3.8	0.151
BMI (kg/m^2)	24.3 ± 10.1	25.2 ± 12.9	24.2 ± 7.5	0.290
Serum Iron (µg/L)	105.3 ± 47.4	121.6 ± 0.5	95.3 ± 5.16	0.471
Ferritin (µg/dL)	52.2 ± 55.8	57.1 ± 56.2	16.7 ± 49.4	**<0.001**
Gravidity				
1 (%)	10 (33.3%)	11 (36.7%)	11 (36.7%)	0.281
2 (%)	8 (26.7%)	11 (36.7%)	12 (40.0%)	
3 (%)	7 (23.3%)	2 (6.7%)	2 (6.7%)	
4 (%)	5 (16.7%)	5 (16.7%)	3 (10.0%)	
5 (%)	0 (0.0%)	0 (0.0%)	2 (6.7%)	
6 (%)	0 (0.0%)	1 (3.3%)	0 (0.0%)	
Live				
1 (%)	11 (36.7%)	15 (50.0%)	11 (36.7%)	0.300
2 (%)	5 (16.7%)	0 (0.0%)	3 (10.0%)	
3 (%)	2 (6.7%)	2 (6.7%)	2 (6.7%)	
5 (%)	0 (0.0%)	1 (3.3%)	0 (0.0%)	

Table 2

Comparing the blood count indices between the three study groups within the three pregnancy trimesters.

	Group 1 (n = 30)	Group 2 (n = 30)	Group 3 (n = 30)	*p*
First trimester				
RBC (×10^6)	4.2 ± 0.42	4.4 ± 0.41	4.3 ± 0.47	0.151
Hb (g/dL)	14.75 ± 1.42	12.24 ± 1.50	12.54 ± 1.23	0.452
MCV (fl)	86.54 ± 6.50	82.50 ± 9.59	85.78 ± 6.93	0.101
MCH (pg)	28.73 ± 3.10	27.98 ± 4.47	28.73 ± 2.44	0.630
Second trimester				
RBC (×10^6)	4.34 ± 0.32	7.40 ± 1.65	4.31 ± 0.58	0.362
Hb (g/dL)	12.32 ± 1.27	11.88 ± 1.40	12.02 ± 1.06	0.387
MCV (fl)	81.95 ± 18.17	84.66 ± 9.49	84.57 ± 5.71	0.622
MCH (pg)	31.05 ± 9.82	27.31 ± 4.40	28.82 ± 3.56	0.091
Third trimester				
RBC (×10^6)	4.48 ± 0.35	4.41 ± 0.49	4.31 ± 0.58	0.458
Hb (g/dL)	12.79 ± 1.18	12.39 ± 2.29	12.46 ± 1.12	0.590
MCV (fl)	82.09 ± 14.19	80.71 ± 12.24	86.05 ± 6.36	0.171
MCH (pg)	28.65 ±2.96	27.87 ± 4.28	29.98 ± 5.43	0.228

Hb = hemoglobin; MCV = mean corpuscular volume; MCH = mean corpuscular hemoglobin; RBC = red blood cell.

Table 3

Maternal and neonatal outcome of 90 pregnant women with singleton pregnancies who participated in the current study and were categorized based on the serum ferritin levels.

	Group 1 (n = 30)	Group 2 (n = 30)	Group 3 (n = 30)	*p*
GA (weeks)	39.16 ± 2.11	38.56 ± 0.93	38.70 ± 0.98	0.250
Birthweight (gr)	3181.6 ± 333.5	3230.6 ± 459.3	3214.3 ± 417.5	0.893
Apgar				
1 min	8.73 ± 0.63	8.90 ± 0.30	8.76 ± 0.77	0.532
5 min	9.90 ± 0.40	9.93 ± 0.25	9.88 ± 0.38	0.590
Delivery				
NVD (%)	14 (46.7%)	12 (40.0%)	11 (36.7%)	0.725
CS (%)	16 (53.3%)	18 (60.0%)	19 (63.3%)	
Complications				
Pre-eclampsia (%)	2 (6.7%)	1 (3.3%)	3 (10.0%)	0.185
GDM (%)	5 (16.7%)	0 (0.0%)	0 (0.0%)	**0.038**
PB (%)	1 (3.3%)	0 (0.0%)	0 (0.0%)	0.723
IUGR (%)	1 (3.3%)	0 (0.0%)	1 (3.3%)	0.872

CS = Cesarean section; GA = gestational age; GDM = gestational diabetes mellitus; IUGR = intrauterine growth retardation; NVD = normal vaginal delivery; PB = preterm birth.

The maternal and neonatal outcomes are summarized in Table 3. As demonstrated the gestational age at delivery (p = 0.250) and birthweight (p = 0.893) were comparable between the three study groups. The frequency of cesarean section as a route of delivery was also comparable between the three study groups (p = 0.725). The neonatal Apgar at 1 (p = 0.532) and 5 (p = 0.590) minutes were also comparable between the three study groups. The overall rate of maternal complication of the pregnancy was comparable between the three study groups (p = 0.188). However, those in groups 1, had significantly higher rate of gestational diabetes mellitus (GDM) when compared to other 2 groups (p = 0.038). The rate of other complications was comparable between groups (Table 3).

4. DISCUSSION

The prophylactic iron supplementation in pregnant women is recommended by the WHO in order to prevent adverse pregnancy outcomes associated with iron deficiency anemia.[9] However, role of prophylactic iron supplementation in those with normal ferritin levels is still a matter of debate. Accordingly, we conducted this clinical trial in order to evaluate the effects of prophylactic iron supplementation on maternal and neonatal outcomes of singleton pregnancies in a sample of Iranian population. We observed that iron supplementation in those with normal ferritin levels is associated with increased risk of GDM. Other pregnancy outcomes were not affected. These findings are in line with previous reports in various populations.[4,11,12] To the best of our knowledge, this is the first study in Iranian population to study the role of iron supplementation in pregnant women with normal ferritin levels.

The importance of iron becomes ambiguous, when it acts like a two-edged sword. Although its deficiency has adverse effects on maternal and neonatal outcomes, elevated iron levels, especially in the third trimester, are also harmful and are associated with an increased risk of GDM.[19] Approximately, 18.1% of pregnancies are affected by GDM and the iron intake during the pregnancy increase the risk of GDM significantly.[20] In general, excess iron above body demands may result in increased levels of unabsorbed iron in the intestine, and also systemic oxidative stress, insulin resistance, and impairment of beta cells of the endocrine pancreas.[21] A recent meta-analysis by Iqbal and Ekmekcioglu[4] tried to perform an umbrella summary of meta-analyses to evaluate the effects and associations of iron supplementation or iron status on maternal and birth/neonatal outcomes. They showed that multi-micronutrient supplementation had beneficial effects on some neonatal outcomes. Furthermore, higher ferritin levels seem to increase the risk for GDM whereas maternal anemia was associated with adverse birth/neonatal outcomes.[4] In another meta-analysis, Cantor et al.[12] tried to investigate the effects of prophylactic iron supplementation on pregnancy outcome in asymptomatic nonanemic pregnancy women. They reported inconclusive evidence that routine prenatal supplementation for anemia improves maternal or infant clinical health outcomes, but supplementation may improve maternal hematologic indices.[12]

We also found that iron supplementation in nonanemic pregnant women is associated with increased risk of GDM. It has been previously demonstrated that high Hb or ferritin levels are associated with a higher risk of GDM.[4,21] Iron overload is thought to favor oxidative stress by increasing the production of reactive oxygen and nitrogen species and hydroxyl radicals via Fenton reactions. This could lead to severe biological damage to membranes, proteins, and DNA.[22] Therefore, although ferritin is a vital biomarker for iron status, increased levels of (free) iron/ferritin may be linked to impaired insulin sensitivity by increased oxidative stress.[23] It has been shown that high serum ferritin in early pregnancy is associated with GDM risk during gestation[24] and insulin resistance syndrome and risk of type 2 diabetes in different populations.[25] Furthermore, a well-established link exists between processed/red meat intake and type 2 diabetes risk described in recent reviews[26] and meta-analyses.[27] One mechanism may be the high bioavailability of heme iron from animal products. Therefore, the association of serum ferritin with GDM, might be due to iron induced oxidative stress, lipid oxidation, and DNA damage.[28] Furthermore, a strong relationship exists between ferritin and hepcidin since both are associated with pathogenesis of type 2 diabetes and serum hepcidin and ferritin were found to be higher in pregnant women with GDM.[29]

We note some limitation to the study. First, the sample size included in the current study was approximately small and this could lead to calculation bias. However, the power of the study was calculated to be 80% to detect 5% difference between the adverse pregnancy outcomes. Second, the design of the study was cohort. However, the best mythology for answering this research question could be a randomized clinical trial. This was perfumed because of resource limitation. We are now designing a clinical trial to address the issue more clearly. To the best of our knowledge, this is the first study to address the role of prophylactic iron supplementation in pregnant women with normal ferritin level.

In conclusion, prophylactic iron supplementation in pregnant women with normal ferritin levels is associated with increased risk of GDM. Other pregnancy and neonatal outcomes are not affected by the prophylactic iron supplementation. Further studies are required to shed light on the issues.

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REFERENCES

1. Stoltzfus RJ. Iron deficiency: global prevalence and consequences. *Food Nutr Bull* 2003;**24**(4 Suppl):S99–103.
2. Organization WHO. *The global prevalence of anaemia in 2011. The global prevalence of anaemia in 2011*. 2015;**43**.
3. Tunkyi K, Moodley J. Anemia and pregnancy outcomes: a longitudinal study. *J Matern Fetal Neonatal Med* 2018;**31**:2594–8.
4. Iqbal S, Ekmekcioglu C. Maternal and neonatal outcomes related to iron supplementation or iron status: a summary of meta-analyses. *J Matern Fetal Neonatal Med* 2019;**32**:1528–40.
5. Rahman MM, Abe SK, Rahman MS, Kanda M, Narita S, Bilano V, et al. Maternal anemia and risk of adverse birth and health outcomes in low- and middle-income countries: systematic review and meta-analysis. *Am J Clin Nutr* 2016;**103**:495–504.
6. Bothwell TH. Iron requirements in pregnancy and strategies to meet them. *Am J Clin Nutr* 2000;**72**:257s–64s.
7. Organization WHO. Guideline: daily iron and folic acid supplementation in pregnant women: *World Health Organization*; 2012.
8. ACOG Practice Bulletin No. 95: anemia in pregnancy. *Obstet Gynecol* 2008;**112**:201–7.
9. Organization WHO. *Standards for maternal and neonatal care*; 2007.
10. Pena-Rosas JP, De-Regil LM, Gomez Malave H, Flores-Urrutia MC, Dowswell T. Intermittent oral iron supplementation during pregnancy. *Cochrane Database Syst Rev* 2015:Cd009997.
11. Siega-Riz AM, Hartzema AG, Turnbull C, Thorp J, McDonald T, Cogswell ME. The effects of prophylactic iron given in prenatal supplements on iron status and birth outcomes: a randomized controlled trial. *Am J Obstet Gynecol* 2006;**194**:512–9.

12. Cantor AG, Bougatsos C, Dana T, Blazina I, McDonagh M. Routine iron supplementation and screening for iron deficiency anemia in pregnancy: a systematic review for the U.S. Preventive Services Task Force. *Ann Intern Med* 2015;**162**:566–76.
13. Pena-Rosas JP, De-Regil LM, Garcia-Casal MN, Dowswell T. Daily oral iron supplementation during pregnancy. *Cochrane Database Syst Rev* 2015:Cd004736.
14. Rahmati S, Delpishe A, Azami M, Hafezi Ahmadi MR, Sayehmiri K. Maternal anemia during pregnancy and infant low birth weight: a systematic review and meta-analysis. *Int J Reprod Biomed (Yazd)* 2017;**15**:125–34.
15. Zerfu TA, Umeta M, Baye K. Dietary diversity during pregnancy is associated with reduced risk of maternal anemia, preterm delivery, and low birth weight in a prospective cohort study in rural Ethiopia. *Am J Clin Nutr* 2016;**103**:1482–8.
16. Dotters-Katz SK, Grotegut CA, Heine RP. The effects of anemia on pregnancy outcome in patients with pyelonephritis. *Infect Dis Obstet Gynecol* 2013;**2013**:780960.
17. Yi SW, Han YJ, Ohrr H. Anemia before pregnancy and risk of preterm birth, low birth weight and small-for-gestational-age birth in Korean women. *Eur J Clin Nutr* 2013;**67**:337–42.
18. Patel A, Prakash AA, Das PK, Gupta S, Pusdekar YV, Hibberd PL. Maternal anemia and underweight as determinants of pregnancy outcomes: cohort study in eastern rural Maharashtra, India. *BMJ Open* 2018;**8**:e021623.
19. Fernandez-Cao JC, Aranda N, Ribot B, Tous M, Arija V. Elevated iron status and risk of gestational diabetes mellitus: a systematic review and meta-analysis. *Matern Child Nutr* 2017;**13**:e12400.
20. Helin A, Kinnunen TI, Raitanen J, Ahonen S,Virtanen SM, Luoto R. Iron intake, haemoglobin and risk of gestational diabetes: a prospective cohort study. *BMJ Open* 2012;**2**:pii: e001730.
21. Khambalia AZ, Aimone A, Nagubandi P, Roberts CL, McElduff A, Morris JM, et al. High maternal iron status, dietary iron intake and iron supplement use in pregnancy and risk of gestational diabetes mellitus: a prospective study and systematic review. *Diabet Med* 2016;**33**:1211–21.
22. Puntarulo S. Iron, oxidative stress and human health. *Mol Aspects Med* 2005;**26**:299–312.
23. Syrovatka P, Kraml P, Potockova J, Fialova L, Vejrazka M, Crkovska J, et al. Relationship between increased body iron stores, oxidative stress and insulin resistance in healthy men. *Ann Nutr Metab* 2009;**54**:268–74.
24. Chen X, Scholl TO, Stein TP. Association of elevated serum ferritin levels and the risk of gestational diabetes mellitus in pregnant women: the Camden study. *Diabetes Care* 2006;**29**:1077–82.
25. Rajpathak SN, Crandall JP, Wylie-Rosett J, Kabat GC, Rohan TE, Hu FB. The role of iron in type 2 diabetes in humans. *Biochim Biophys Acta* 2009;**1790**:671–81.
26. Ekmekcioglu C, Wallner P, Kundi M, Weisz U, Haas W, Hutter HP. Red meat, diseases, and healthy alternatives: a critical review. *Crit Rev Food Sci Nutr* 2018;**58**:247–61.
27. Pan A, Sun Q, Bernstein AM, Schulze MB, Manson JE, Willett WC, et al. Red meat consumption and risk of type 2 diabetes: 3 cohorts of US adults and an updated meta-analysis. *Am J Clin Nutr* 2011;**94**:1088–96.
28. Zhuang T, Han H, Yang Z. Iron, oxidative stress and gestational diabetes. *Nutrients* 2014;**6**:3968–80.
29. Derbent AU, Simavli SA, Kaygusuz I, Gumus II, Yılmaz S, Yildirim M, et al. Serum hepcidin is associated with parameters of glucose metabolism in women with gestational diabetes mellitus. *J Matern Fetal Neonatal Med* 2013;**26**:1112–5.