Original Article

Serum Zinc Levels in Iron Deficiency Anemia, Non-iron Deficiency Anemia, and Normal Pregnant Women and Its Correlation with Iron Status and Hematological Parameters

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Abstract

Background: There is limited information on zinc deficiency in pregnant women. The present study aimed to assess the serum zinc (S. zinc) levels and its relation to iron deficiency anemia (IDA) in pregnant women. **Patients and Methods:** In this case–control study, S. zinc concentrations of 34 individuals diagnosed with IDA, 20 non-iron deficiency anemic pregnant women, and 32 non-anemic apparently healthy individuals were measured. **Results:** S. zinc was significantly lower in the IDA group (49.59 ng/dL) compared to the healthy controls (55.78; $P = 0.018$). The individuals in three groups were comparable in the number of persons with zinc deficiency. The study showed that S. zinc has a positive correlation with Hb ($r = 0.281$, $P = 0.011$). In addition, S. zinc had a positive correlation with hematocrit (HCT, $r = 0.305$, $P = 0.005$) and a negative correlation with serum iron (S. iron, $r = 0.242$, $P = 0.029$). **Conclusions:** This investigation showed that the patients with IDA have a significantly lower concentration of S. zinc and it was substantially positively correlated with Hb, red blood cell, and HCT and negatively with S. iron. Further studies are still needed to evaluate the benefits of zinc and iron supplementation in IDA patients.

Keywords: Iron deficiency anemia, pregnant women, serum zinc

Introduction

Zinc is an essential mineral for human health. It is relatively abundant in nature, yet at the same time, overwhelming data suggest that zinc deficiency is one of the most prevalent micronutrient deficiencies worldwide.[1]

It is recognized that zinc is important for many basic metabolic processes and hence essential for optimal growth and immunocompetence.[2] It plays an important role in many biological functions, including protein synthesis, cellular division, and nucleic acid metabolism.[3] It also plays a critical role in hemoglobin synthesis and erythropoiesis and may, therefore, play a vital role in the etiology of anemia.[4]

Scientific attention to the role of specific nutrients, especially micronutrients, in healthy pregnancy has grown steadily in recent years, beginning with numerous fields of studies of pregnancy in developing countries. Zinc and iron are micronutrients whose requirements increase with pregnancy.[5] Pregnant women worldwide are frequently iron and zinc deficient.[6] Low birth weight is associated with maternal anemia and, in some circumstances, with low iron and zinc status.[7] Therefore, cosupplementation of iron and zinc during pregnancy is common. Although iron supplementation programs are successful, studies suggest that zinc supplementation negatively affects maternal iron metabolism.[6]

Although severe zinc deficiency is relatively rare in human populations, mild-to-moderate depletion appears to be quite prevalent.[3] Information on zinc deficiency is limited; 1.2 billion people worldwide are at risk of inadequate zinc

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intake, and presumably, many are zinc deficient.[8] However, very little is known regarding the impact of zinc deficiency on iron status indicators in pregnant women.[6] The present study aimed to assess the serum zinc (S. zinc) levels and its relation to iron deficiency anemia (IDA) in pregnant women.

Patients and Methods

Study design and sampling

The present study was a case–control study that included 86 pregnant women who attended the Bahdinan, Duban, and private clinics and living in different areas of Duhok governorate. The study included 140 pregnant women, from whom 86 individuals from different gestational ages: 19 from the first trimester, 34 from the second trimester, and 33 from the third trimester, were selected based on the eligibility criteria.

The patients were assigned to the following three groups based on the eligibility criteria: the first group, case/patient group: including 34 individuals diagnosed with IDA; the second group, positive control group: comprised 20 non-iron deficiency anemic pregnant women; and finally, the third group, negative control group, included 32 non-anemic, apparently healthy individuals. The data collection was carried out from August 2018 to March 2019.

Inclusion and exclusion criteria

The patients met eligibility criteria if they were aged 16 years and older and regardless of the sociodemographic perspectives. Patients with jaundice, liver diseases, hemoglobinopathies, and hemolytic diseases were excluded owing to interferences with zinc analysis.

Diagnostic and measurement criteria

A pretested questionnaire was designed to obtain information on age, gestational age, parity, and type of delivery. The diagnoses of the study groups were established according to the following criteria: case/patient group: Pregnant women diagnosed with IDA if Hb <11.5 g/dL, serum ferritin <10 ng/mL, and transferrin saturation percentage (TS%) <15%; positive control group: Pregnant women with non-IDA if Hb <11.5 g/dL, serum ferritin >10 ng/mL, and TS% >15%; and negative control group, included nonanemic, apparently healthy pregnant women if Hb >11.5 g/dL, serum ferritin >10 ng/mL, and TS% >15%.

Laboratory measurement

The blood samples were obtained from a suitable forearm vein; 7 mL of venous blood was drawn from each participant; 2 mL was placed into an EDTA containing tube and sent for the assessment of complete blood count using hematology auto-analyzer (Medonic Coulter Counter – Sweden). The remaining 5 mL was placed into a plain gel-tube and let stand for 30 min at room temperature to clot and then centrifuged at 3000 rpm for 10 min at 4°C. The obtained serum was stored in capped Eppendorf tubes and stored frozen at −28°C for future analysis. For this purpose, we divided the obtained serum from each participant into two separate capped tubes, one for iron studies (serum ferritin, serum iron [S. iron], total iron binding capacity [TIBC], and TS%) and the other for the analysis of the S. zinc.

Both S. iron and unsaturated iron binding capacity (UIBC) were identified by an enzymatic colorimetric method, called FerroZine method, using the Cobas Integra Iron Gen. 2 (Iron2) Kit and UIBC Kit, respectively, for the quantitative detection of human S. iron and UIBC, respectively, on COBAS INTEGRA 400/800 system (Roche Diagnostics GmbH, Germany).

TIBC was calculated as S. iron + UIBC = TIBC. In addition, TS% was calculated by TS% =100 × S. iron/TIBC.[9]

Serum ferritin was identified by the sandwich principle of electrochemiluminescence immunoassay "ECLIA" using the Cobas Integra Ferritin Kit for the quantitative detection of human serum ferritin on COBAS INTEGRA 400/800 system (Roche Diagnostics GmbH, Germany).

S. zinc was identified by colorimetric determination by a specific kit (presents by LTA s.r.l. via Milano, Italy) composed of two reagents (Reagent A: Borate buffer 0.37 M and pH 8.2, salicylaldoxime 12.5 mM, dimethylglyoxime 1.25 mM; Reagent B: NITRO-PAPS 0.4 mM) and a standard (zinc ion 200 μg/dl).

S. zinc reacted with the chromogen present in the reagent forming a colored compound which the color intensity is proportional to the zinc concentration present in the sample.

S. zinc reference values, according to the WHO guidelines, are severe deficiency: <50 μg/dL, mild-to-moderate deficiency: 50–70 μg/dL, and normal level: more than 70 μg/dL.

Statistical analysis

The descriptive purposes of the study were presented in mean and standard deviation or frequency and percentage. The comparison of general information between IDA group and positive and negative groups was examined in independent *t*-test or Pearson's Chi-square tests. The comparison of hematological parameters between IDA and other groups was examined in independent *t*-test. Correlation of S. zinc with hematological factors was examined by Pearson's correlation. In this analysis, the adjustment was made for age, gestational age, and delivery mode, and study groups. ANOVA one-way was performed to assess the S. zinc level in patients with different gestational age and delivery mode. A $P < 0.05$ was considered a statistically significant difference. The statistical calculations were performed by Statistical Package for the Social Sciences 24:00 (SPSS 24:00; BM, USA).

Ethical considerations

The local health ethics committee of Duhok Directorate General of Health approved the study. The study was registered as the reference number of 26062018-5 on June 26, 2018. The

confidentiality of the personal information of the subjects was protected throughout the study steps.

Results

Thirty-four pregnant women with IDA were compared to 20 pregnant women with non-IDA as positive control and 32 healthy pregnant women as a negative control, in this case–control study. The mean age was 29.76 years in the IDA group, 27.60 years in the positive control group, and 27.25 years in the negative control group. There was no statistical difference between the age groups (P = 0.217 and 0.122). The study groups were comparable in most of the general characteristics ($P \geq 0.05$) except gravida between IDA and negative control (P = 0.041) and abortion between IDA and positive control (P = 0.034), as shown in Table 1.

The patients in the IDA groups had significantly lower levels of Hb, MCV, MCHC, red blood cell (RBC), hematocrit (HCT), ferritin, S. iron, and TS compared to those in positive and negative controls. S. zinc was significantly lower in the IDA group (49.59 ng/dL) compared to the negative control (55.78 ng/dL; P = 0.018), while its concentration was comparable with the positive control (45.90 ng/dL; P = 0.303). The individuals in three groups were comparable in the number of persons with zinc deficiency (severe zinc deficiency: 58.8%, 55.0%, and 31.3% in IDA, positive, and negative control groups, respectively) [Table 2].

The correlation of S. zinc with hematological parameters with adjustment for age, gestational age, delivery, and study groups showed that S. zinc has a positive correlation with Hb (r = 0.281, P = 0.011). The analysis did not show any correlation with ferritin (P = 0.682) and TS (P = 0.051) [Table 3].

The correlation of S. zinc with hematological and biochemical parameters with adjustment for age, gestational age, delivery mode, and study groups showed that S. zinc has a positive correlation with Hb (r = 0.281; P = 0.011), RBC (r = 0.233; P = 0.035), and HCT (r = 0.305; P = 0.005) and a significant negative with S. iron (r = −0.242; P = 0.029), as shown in Table 3.

The concentration of the S. zinc in patients with different gestational age (P = 0.372) and delivery modes (P = 0.591) was not different substantially [Table 4].

The correlation of S. zinc with gravida, para, and abortion and stillbirth was not significant. In this correlation, the analysis was adjusted for age, gestational age, delivery, and study groups [Table 5].

Discussion

Although trace elements are found in minimal quantities, they have essential roles in homeostasis. Two of the most important trace elements are iron and zinc.[10] Iron and zinc are essential micronutrients for human health. Deficiencies in these two nutrients remain a global problem, especially among women and children in developing countries.[11]

Impaired iron absorption is caused by a decrease in zinc levels, which is found in the structure of enzymes that coordinate or catalyst iron metabolism.[12] Coexistence of zinc and iron deficiency has attracted the interest of many researchers, and there have been many studies about this subject.[10]

Micronutrient interactions are particularly important during pregnancy because the developing fetus is very vulnerable to inappropriate micronutrient status.[13] Micronutrient deficiency, whether clinical or subclinical, may affect growth, cognition, and reproductive performance. In pregnant women, moderate-to-severe deficiencies of iron and zinc has been shown to increase

Table 1: Comparison of general information between the patients in the iron deficiency anemia group with positive and negative controls

Patients' characteristics	IDA (n=34)	Positive control (n=20)	P (two-sided)	Negative control (n=32)	P (two-sided)
Age (year)	29.76 (1.13)	27.60 (1.31)	0.217	27.25 (1.40)	0.122
Gravida	3.76 (0.375)	2.85 (0.418)	0.111	2.69 (0.355)	**0.041**
Para	2.32 (0.326)	1.65 (0.350)	0.166	1.47 (0.330)	0.070
Abortion	0.35 (0.093)	0.10 (0.069)	**0.034**	0.16 (0.065)	0.089
Stillbirth	0.09 (0.049)	0.10 (0.069)	0.890	0.06 (0.043)	0.697
Gestational age					
First trimester	4 (11.8)	4 (20.0)	0.059*	11 (34.4)	0.079*
Second trimester	12 (35.3)	12 (60.0)		10 (31.3)	
Third trimester	18 (52.9)	4 (20.0)		11 (34.4)	
Delivery mode					
NVD	4 (11.8)	4 (20.0)	0.824*	11 (34.4)	0.087*
C/S	24 (70.6)	12 (60.0)		14 (43.8)	
Primi	4 (11.8)	3 (15.0)		6 (18.8)	
Multimethod	2 (5.9)	1 (5.0)		1 (3.1)	

*Fishers' exact test was performed for statistical analysis. The bold numbers show significant differences. Independent t-test was performed for statistical analyses. The first P value is for between IDA and positive control, and the second P value is for between IDA and negative control. First trimester: 1-12 weeks; Second trimester: 12-28 weeks, and Third trimester: 28 and above. NVD: Natural vaginal delivery, C/S: Cesarean section, IDA: Iron deficiency anemia

Table 2: Comparison of hematological parameters between the subjects in the iron deficiency anemia group with the positive and negative controls

Patients' characteristics	IDA ($n=34$)	Positive control ($n=20$)	P (two-sided)	Negative control ($n=32$)	P (two-sided)
Hb (g/dl)	9.92 (0.16)	10.51 (0.18)	**0.020**	12.59 (0.13)	**<0.001**
MCV	77.24 (1.40)	83.06 (2.84)	0.076	92.51 (0.74)	**<0.001**
MCH	24.65 (0.48)	27.55 (0.94)	**0.004**	29.62 (0.23)	**<0.001**
MCHC	31.92 (0.28)	32.93 (0.24)	**0.010**	32.08 (0.20)	0.666
RBC	4.08 (0.07)	3.89 (0.11)	0.150	4.26 (0.05)	**0.046**
Hct	31.05 (0.51)	31.96 (0.57)	0.238	39.36 (0.49)	**<0.001**
WBC	9.63 (0.35)	8.93 (0.45)	0.220	9.62 (0.42)	0.976
PLT	210.79 (10.82)	208.75 (12.26)	0.901	197.38 (8.66)	0.337
Ferritin	5.42 (0.30)	41.99 (11.19)	**0.004**	29.62 (3.35)	**<0.001**
Serum iron	27.41 (1.78)	93.05 (9.56)	**<0.001**	96.63 (5.94)	**<0.001**
TIBC	452.09 (11.42)	330.25 (15.08)	**<0.001**	365.72 (14.26)	**<0.001**
TS%	6.15 (0.41)	28.50 (2.69)	**<0.001**	26.84 (1.44)	**<0.001**
Serum zinc	49.59 (1.53)	45.90 (3.17)	0.303	55.78 (2.04)	**0.018**
Severe zinc deficiency (%)	20 (58.8)	11 (55.0)	0.860*	10 (31.3)	0.063*
Mild zinc deficiency (%)	13 (38.2)	9 (45.0)		18 (56.3)	
Normal (%)	1 (2.9)	0 (0.0)		4 (12.5)	

*Pearson Chi-squared test was performed for statistical analysis. Independent *t*-test was performed for statistical analyses. The first *P* value is for between IDA and positive control, and the second *P* value is for between IDA and negative control. The bold numbers show significant differences. Serum zinc range: Severe zinc deficiency (<50 ng/dL); Mild zinc deficiency (50-70 ng/dL); and Normal zinc level (>70 ng/dL). Hb: Hemoglobin, MCV: Mean corpuscular volume, MCH: Mean corpuscular hemoglobin, MCHC: Mean corpuscular hemoglobin concentration, RBC: Red blood cell, HCT: Hematocrit, WBC: White blood cell, PLT: Platelet, TIBC: Total iron-binding capacity, TS: Transferrin saturation, IDA: Iron deficiency anemia

Table 3: Correlation of serum zinc with hematological parameters with adjustment of age, gestational age, and delivery mode, and study groups

Hematological parameters	Serum zinc	
	Correlation	Significance (two-tailed)
Hb (g/dl)	0.281	0.011
Ferritin	−0.046	0.682
TS%	−0.217	0.051
Serum iron	−0.242	**0.029**
TIBC	0.164	0.141
RBC	0.233	**0.035**
HCT	0.305	**0.005**
MCV	0.020	0.860
MCH	−0.058	0.603
MCHC	−0.097	0.387

The analysis was adjusted for age and gestational age and delivery and study groups. The bold numbers show the significant correlations. Hb: Hemoglobin, MCV: Mean corpuscular volume, MCH: Mean corpuscular hemoglobin, MCHC: Mean corpuscular hemoglobin concentration, RBC: Red blood cell, HCT: Hematocrit, TIBC: Total iron-binding capacity, TS: Transferrin saturation

the risk of low birth weight, pregnancy complications, and birth defects.[14]

Several reports demonstrate that maternal zinc deficiency during pregnancy is linked with adverse pregnancy outcomes, including abortion, preterm delivery, stillbirth, and fetal neural tube defects.[15]

In this study, S. zinc was significantly lower in IDA group (49.59 ng/dL) compared to the negative control (55.78; $P = 0.018$), while its concentration was comparable with the positive control (45.90; $P = 0.303$). In a study conducted by Ece *et al*., the S. zinc levels were lower in the IDA group than the control group ($P = 0.017$). There was a statistically significant difference between the two groups, as in our study. As a result, it has been suggested that S. zinc levels should be checked in pregnant women with iron deficiency.[16]

Another study conducted by Kelkitli *et al*. also recorded lower S. zinc levels in anemic patients than in the control groups ($P < 0.001$).[17] These results were all compatible with our results.

Saaka[4] included pregnant women who attended antenatal care in a region in Ghana. The patients were randomly and double-blind assigned into an interventional group who received a combined supplement of 40 mg zinc as zinc gluconate and 40 mg iron as ferrous sulfate. However, the control group received 40 mg elemental iron only. They found that women who had low plasma zinc levels were three-fold increased odds of developing deficiency (odds ratio 3.41, 95% confidence interval: 1.19–9.76). They concluded that iron–zinc supplementation is effective in increasing Hb levels and serum ferritin values in women with IDA in early pregnancy but not in women with sufficient iron level.

One of the reasons for iron deficiency occurring with zinc deficiency, other than diet, is the increase in production of zinc protoporphyrin and usage of zinc, instead of iron in the protoporphyrin structure.[18]

In the present study, there was a positive correlation between zinc with Hb and HCT; however, a weak negative correlation was found between zinc with S. iron and ferritin. This finding is in consistence to the finding of Arijanty *et al*.;[19] they also

Table 4: Comparison of serum zinc concentration in iron deficiency anemia patients with different gestational age and delivery modes

Patients characteristics ($n=34$)	Serum zinc			
	Mean (SD)	SE	Range	*P* (two-sided)
Gestational age				
First trimester	55.50 (8.02)	4.01	46-65	0.372
Second trimester	49.17 (10.76)	3.11	36-77	
Third trimester	48.56 (7.59)	1.79	39-63	
Delivery mode				
Primi	50.50 (5.20)	2.60	46-58	0.591
Normal delivery	50.46 (9.02)	1.84	37-77	
Cesarean section	47.25 (11.90)	5.95	40-65	
Multimethod	42.00 (8.46)	6.00	36-48	

ANOVA one-way was performed for statistical analyses. SD: Standard deviation, SE: Standard error

Table 5: Correlation of serum zinc with GPA with adjustment of age, gestational age, and delivery mode, and study groups

Control variables Serum zinc	Gravida	Para	Abortions	Stillbirth
Correlation	0.099	0.121	−0.018	−0.021
Significance (two-tailed)	0.375	0.277	0.873	0.849

The correlation was controlled for the following variables: Gestational age, delivery, age, and study groups. GPA: Gravida, Para, Abortion

found a weak negative correlation between plasma zinc level and ferritin level, which could be due to competitive inhibition of zinc by ferritin.

Nonsignificant statistical difference was found regarding the concentration of S. zinc between IDA group with the positive and negative control groups, with $P = 0.059$ and 0.79, respectively. The concentration of S. zinc between different gestational age groups ($P = 0.372$) and delivery mode ($P = 0.591$) did not show substantial difference too. The correlation of S. zinc with gravida, para, and abortion and stillbirth was also not significant.

Due to lack of studies regarding zinc status in pregnant women and the high prevalence of IDA in pregnancy, this study was conducted in pregnant women with IDA. We tried to find out whether zinc deficiency coexists with iron deficiency or not.

With the help of this study, iron and zinc supplementation instead of only iron replacement may be considered in cases of iron deficiency. Further studies are still needed to evaluate the benefit of zinc and iron supplementation in IDA patients.

Strengths and limitations

To our knowledge, this the first study in this field using two control (+ve and −ve) groups to demonstrate the relation of zinc to non-IDA. The most obvious limitation of the present study was the small sample size. We recommend a larger sample size and an interventional type of study by supplying zinc to the patients with IDA and follow-up changes in hematological parameters after correction of zinc status.

Conclusions

This investigation showed that the patients with IDA have a significantly lower concentration of S. zinc and it was substantially positively correlated with Hb, RBC, and HCT and negatively with S. iron. Further studies are still needed to evaluate the benefit of zinc and iron supplementation in IDA patients.

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Conflicts of interest

There are no conflicts of interest.

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