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Concentrations of Serum Zinc, Hemoglobin and Ferritin among Pregnant Women and their Effects on Birth Outcomes in Kashan, Iran

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Abstract

Objectives: Zinc and Iron are essential micronutrients in fetus growth and development. The aim of this study was to assess the relationship of maternal serum Zinc, Hemoglobin and Ferritin levels with their newborns weight, height and head circumference.
Methods: This cross sectional-analytical study carried out among pregnant women referred to Naghavi Polyclinic, Kashan, Iran from November 2009 to October 2010. One hundred and twenty-nine pregnant women in the third trimester were selected and anthropometric factors of their newborns (weight, height and head circumference) and their relation to serum Zn, Hemoglobin and Ferritin concentrations were assessed.
Results: Serum Hemoglobin deficiency (<11g/dl), serum Ferritin deficiency (<12 μg/L) and serum Zn deficiency (<66 μg/dl) were present in 11 (8.5%), 9 (7%) and 9 (7%) women, respectively. The proportion of newborns with birth weight of 2500-2999 g was greater among mothers with lower Hemoglobin (<11 g/dl) compared to mothers with normal Hemoglobin (≥11 g/dl; $p=0.04$). Multiple regression analysis showed that among biochemical characteristics of pregnant women, serum Hemoglobin levels were positively correlated with low birth weight ($\beta=0.26$; $p=0.04$). But serum Zn and Ferritin levels were not significantly associated with weight, height and head circumference.
Conclusion: Low maternal serum hemoglobin levels are associated with low birth weight and does not have any association with birth height or head circumference; but there was no significant association between maternal serum zinc and Ferritin levels with weight, height and head circumference.

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Keywords: Zinc; Hemoglobin; Birth weight; Birth height; Birth head circumference.

Introduction

The relation of maternal Zn,[1-6] Hemoglobin status and pregnancy outcome in humans has been evaluated in past decades by many investigators.[5,7,8] It is known that serum status of Hemoglobin and zinc during pregnancy is crucial for health of mothers, growth of fetus and optimal outcomes of the mother and fetus.[9,10] Although some studies have shown a great deal of self-medications worldwide and throughout the Middle-East,[11,12] micronutrient deficiency is a major problem in women of reproductive age in many developing countries.[13-16]

One of the most common micronutrient deficiencies in developing countries is zinc deficiency. It is estimated that 82% of all pregnant women in the world suffer from zinc deficiency.[17,18] Low Zn levels during pregnancy are associated with growth retardation; congenital abnormalities; low birth weight; abnormalities in gene replication, activation and repression, as well as transcription and translation of DNA and protein synthesis in rapid growth periods, thus emphasizing its important role in gestation and fetal life.[9,10,19,20] WHO has estimated that the prevalence of anemia in developed and developing countries among pregnant women is 14% and 51%, respectively.[21]

Low status of hemoglobin in pregnancy is associated with increased morbidity, infections, a substantial proportion of maternal deaths (due to ante-partum and post-partum hemorrhage), premature births, lower birth weight and higher perinatal mortality in the babies.[9,17,21,22] The aim of this cross-sectional study was the determination of the relationship between maternal serum Zinc, Hemoglobin and Ferritin status and birth weight, height and head circumference.

Methods

The target population of this study was 129 mother-infant pairs which was calculated according to an estimate of proportion of low Zn serum retinol derived from a pilot study performed on the same population. On the basis of the suggested formula for the sample size, $n=(Z^2(_{1-\alpha/2})\times p(1-p)/d$,[23] we determined that 129 patients were needed for adequate power. Participants were recruited from women attending prenatal care after 28 weeks of pregnancy (P 28), at the maternity clinic (Naghavi Specialitis and Subspecialitis Polyclinic, affiliated with Kashan University of Medical Sciences), Kashan, Iran. Mother-infant pairs were excluded if any of the following items were diagnosed: multiple pregnancies, meconium-stained amniotic fluid, febrile illness prior to delivery, maternal hypertension, liver or renal disease, gestational diabetes, drug intake (other than vitamins) in the week prior to delivery, major congenital malformations, Apgar score <7 at 5 min or perinatal death. These conditions may affect Zn and Iron status in mothers and infants or the birth weight, and may therefore confound the results. Among the subjects, due to the diagnosis of maternal preeclampsia; three newborns with birth weight <2500 g were excluded from the study (n=3).

Health, intake of supplements and lifestyle information were obtained by trained interviewers via a standardized face-to-face interview and questionnaire at enrollment from all participants. Gestational age was assessed by the mother's estimated date of last menstrual period. The present study was conducted according to the guidelines in the Declaration of Helsinki and all procedures involving human subjects were approved by the Ethics Committee at KUMS. Written informed consent was obtained from all subjects.

For biochemical assessment, fasting early morning blood (10 ml) was collected at P 28 and anthropometry was performed at P 28 and term of pregnancy (P 37-41). Immediately after blood samples were collected, they were fractionated using the standard procedures, stored at $^{-}80^{\circ C}$ until analysis at the Reference Laboratory in Tehran.

Atomic absorption spectrophotometer (Chem Tech Analytical CTA 2000, Germany) was used for serum zinc measurement. Serum Hemoglobin was measured by colorimetery (Sysmex, Model XS 8001, Japan) using the Electronic enzyme kit (Solpholizer SLS, Japan); and serum Ferritin was measured by radioimmunoassay using the System Ortho kit (System vitros, Ortho company, U.S).Other variables such as maternal weight and BMI, at pre-pregnancy, third trimester and delivery were measured (digital floor scale; Seca, Hamburg, Germany) with 0.1 kg accuracy without shoes and with minimum clothing. Height was measured with 0.1 cm accuracy with a non-stretchable tape (Seca, Hamburg, Germany). While BMI was determined through dividing body weight by height squared (kg/m^2).

During the first 24 hrs after birth in the obstetrics clinics, the height (Rollametre, London, United Kingdom) and weight (Seca, Birmingham, United Kingdom) of newborns were measured to the nearest 1 mm and 10 g, respectively. The head circumference of newborns was measured to the largest diameter of the anterior and posterior fontanels to the nearest 1 mm with a Seca girth measuring tape.

In this study, the pregnant mother-neonate pairs were classified into 2 groups: normal and deficiency group, according to serum zinc, hemoglobin and ferritin status. Serum zinc levels ≥66 µg/dl were considered a safe range, while levels <66 µg/dl were considered as as deficiency.[17] For hemoglobin, serum levels ≥11 g/dl were defined as the safe range, while levels <11 g/dl were considered as deficiency.[17,24] For ferritin, serum concentrations ≥12 µg/L were considered the safe range and levels <12 µg/L were classified as deficiency.[17] Also, the weight of newborns was classified into groups: 2500-2999g and ≥3000g. All pregnant women were taking 400 µg of folic acid daily from the beginning of pregnancy, 50 mg of ferrous sulfate from the second trimester and multivitamin supplement from the 16th week of pregnancy to delivery.

To ensure the normal distribution of variables, statistical analysis was done using the Histogram and Kolmogrov-Smirnov test. The Student *t test* was used to detect differences between groups in terms of serum zinc, hemoglobin and ferritin concentrations. Multivariate regression models were used to estimate the effects of independent variables such as maternal age, gestational age, zinc, hemoglobin and ferritin on the birth weight, height and head circumference of newborns. Also, R^2 and adjusted R^2 values were presented according to multivariate regression models. In all instances, a value $p<0.05$ was considered statistically significant. The SPSS 17 statistical package (SPSS Inc., Chicago, Illinois, USA) was used for data analysis.

Results

A sample size of 129 mother-infant pairs was studied during the years 2009 and 2010; and complete epidemiological and clinical data were obtained from all of the studied participants. Independent t-test showed that there was a statistically significant difference between the group with normal maternal serum Zn, Hb and Ferritin concentrations and the group with deficient serum Zn, Hb and Ferritin concentrations ($p<0.0001$). (Table 1)

The proportion of infants with birth weight ranging from 2500-2999 g was greater among mothers with lower serum hemoglobin (<11 g/dl; n=19), compared to mothers with normal serum hemoglobin (≥11 g/dl; n=110). The difference was statistically significant, ($p=0.04$). (Table 2)

In the current study, possible factors related to birth weight were investigated by multivariate analysis (Table 3). Independent variables in the equation were: maternal age; gestational age; maternal serum zinc, and hemoglobin, and ferritin concentrations. All independent variables together (R^2) accounted for 32.9% of the variability in birth weight (adjusted R^2= 7%). The results showed that maternal zinc and ferritin did not significantly contribute to the variability of birth weight, while maternal hemoglobin significantly contributed to this variability. (Table 3)

Table 1: General and clinical characteristics of the study mother-infant pairs.

Variable	N (%)	Mean (minimum-maximum)	p^1
Mother			
Age (years)	129 (100)	25.1 (18 - 38)	-
Parity			
1	80 (62)	-	-
2	36 (28)	-	-
3+	13 (10)	-	-
Height (cm)	65 (50.4)	160 (145-173)	-
Pre-pregnancy weight (kg)	129(100) 65 (50.4)	66.7 (48-95)	-
Weight in the third trimester (kg)	129 (100)	73.6 (54.5-103)	-
Weight at delivery (kg)	129 (100)	77 (56.5-107)	-
Pre-pregnancy BMI (kg/m^2)	129 (100)	26 (18.9-37.5)	-
BMI in the third trimester (kg/m^2)	129 (100)	28.7 (21.2-40.7)	-
BMI at delivery (kg/m^2)	129 (100)	30.1 (22-42.3)	-
Zn Status (μg/dl)[2]			
Normal (≥66)	120 (93)	91.5 (66-135)	
Zn Deficiency (< 66)	9 (7)	60.2 (54.5-65)	
Total	129 (100)	89.3 (54.5-135)	<0.0001
Gestation age (weeks)	129 (100)	39.1 (37-41)	-
Hb Status (g/dl)[2]			
Normal (≥11)	118 (91.5)	12.2 (11-14.5)	
Hb Deficiency (<11)	11 (8.5)	10.4 (9.3- 10.9)	
Total	129 (100)	12.1 (9.3-14.5)	<0.0001
Fer Status (μg/L)[2]			
Normal (≥12)	120 (93)	91.5 (12-174)	
Fer Deficiency (< 12)	9 (7)	60.2 (11.9-65)	
Total	129 (100)	26 (7.1-174)	<0.0001
Infants			
Birth weight (g)	129 (100)	3336.9 (2550-4750)	-
Birth Height (cm)	129 (100)	50.7 (43-59)	-
Head circumference (cm)	129 (100)	34.8 (31.5-39)	-

[1]*obtained from independent -samples t test*
[2] *Measured during third trimester*

Table 2: Maternal serum Zn, Hb and Ferritin concentrations in relation to Birth weight (2500-2999 and ≥3000) among Iranian pregnant women during third trimester.

Parameter	Birth weight		p^1
	≥3000 g	**2500-2999 g**	
Mothers serum zinc			
N (%)	110 (85.9)	19 (14.1)	
(mean± SD)	89±18.1	91.5±18.9	0.57
Mothers serum hemoglobin			
N (%)	110 (85.9)	19 (14.1)	
(mean± SD)	12.1±0.8	11.7±0.7	0.04
Mothers serum ferritin			
N (%)	110 (85.9)	19 (14.1)	
(mean± SD)	24.1±15.8	27.9±13.3	0.30

obtained from independent -Samples t test [1]

Table 3: Results of multiple regression analysis between maternal age, gestational age, maternal Zn, Hemoglobin and Ferritin concentrations during third semester of pregnancy with birth size.

	Standardized regression, β	95% CI, β	*p*[4]
Determinants of birth weight[1]			
Maternal age (year)	0.17	-0.07; 32.21	0.05
Gestational age (weak)	0.22	20.78;151.46	0.01
Zn (0:<66 µg /dl; 1:≥66 µg /dl)	0.08	-128.19;410.45	0.3
Hemoglobin (0:<11 g/dl)	0.17	11.45;500.52	0.04
Ferritin (0:<12 µg/L; 1:≥12 µg/L)	-0.06	-282.27;131.56	0.47
Determinants of birth hieght[2]			
Maternal age (year)	-0.1	-0.14; 0.04	0.26
Gestational age (weak)	-0.01	-0.4;0.33	0.86
Zn (0:<66 µg /dl; 1:≥66 µg /dl)	0.07	-0.87; 2.16	0.4
Hemoglobin (0:<11 g/dl; 1:≥11 g/dl)	0.03	-1.11; 1.64	0.7
Ferritin (0:<12 µg/L; 1:≥12 µg/L)	-0.15	-2.19; 0.14	0.08
Determinants of birth head circumference[3]			
Maternal age (year)	0.17	-0.003; 0.11	0.06
Gestational age (weak)	0.07	-0.13;0.32	0.42
Zn (0:<66 µg /dl; 1:≥66 µg /dl)	0.009	-0.91; 1	0.92
Hemoglobin (0:<11 g/dl)	0.04	-0.67; 1	0.65
Ferritin (0:<12 µg/L)	0.02	-0.64; 0.82	0.81

[1] R^2= 0.32 (Adjusted R^2= 0.07); [2] R^2= 0.21 (Adjusted R^2= 0.006)

[3] R^2= 0.17 (Adjusted R^2=- 0.008); [4] Obtained from multiple regression analysis

For the determination of height at birth; the possible factors associated with birth height were investigated by multivariate analysis in another part of the study. Independent variables in the equation included: maternal age; gestational age; maternal serum zinc, hemoglobin, and ferritin concentrations. All independent variables together (R^2) accounted for 21.2% of the variability in birth height (adjusted R^2= 0.6%). As depicted in Table 3; maternal Zn, hemoglobin and Ferritin did not significantly contribute to the variability of birth height. (Table 3)

The last part of the study investigated the possible factors associated with birth head circumference using multivariate analysis. The independent variables included: maternal age; gestational age; maternal serum zinc, hemoglobin and ferritin concentrations. Altogether, the independent variables (R^2) accounted for 17.7% of the variability in birth head circumference (adjusted R^2=-0.8%). Moreover, maternal serum zinc, hemoglobin and ferritin did not significantly contribute to the variability in head circumference at birth. (Table 3)

Discussion

The present study revealed that the prevalence of zinc and hemoglobin deficiencies to be 8.5% (n=7) among pregnant Iranian women in Kashan city. Previous studies have shown a greater degree of hemoglobin deficiency in other countries. In 2004, a study conducted in China reported 50.5% of hemoglobin deficiency among pregnant women,[17] and the value had increased to approximately 58.6% by 2009.[22] Another study performed on pregnant women in South East Asia revealed 50% or more for hemoglobin deficiency.[15] Also, a study in Turkey revealed 43% of hemoglobin deficiency among pregnant women in Istanbul.[25]

On the other hand, in 2004; a study in India reported 73.5% Zn deficiency among pregnant women in a rural block of Haryana state.[21] In 2008, another study in Haryana state, India, reported 64.6% Zn deficiency among pregnant women.[26]

Pregnant women are very susceptible to iron and zinc deficiency, particularly in the third trimester due to expanded blood volume, increased iron demands and poor bio-absorption or intake of this important micronutrient.[17] Also, the rate of prenatal care could have an impact on serum iron and zinc levels. Emerging evidence now indicates that the use of micronutrient-containing prenatal supplements pre-pregnancy is associated with reduced risk of congenital defects, preterm delivery, low infant birth weight, preeclampsia and improvement of serum micronutrients.[27] Iron deficiency during pregnancy could also result in hemoglobin deficiency.[17] Iron and hemoglobin deficiency during pregnancy are associated with serious short and long term consequences, including fetal growth decrease and cardiovascular problems in the adult offspring.[17] Furthermore, it has also been reported that zinc deficiency during pregnancy, particularly in the third trimester, may be associated with decreased intrauterine growth, congenital malformations and spontaneous abortion, as well as preterm births and feto-maternal complications.[28]

The current study also showed that through longitudinal regression modeling that low prenatal hemoglobin status was associated with lower birth weight in neonates. These findings are in agreement with other studies; Akhter et al. (2010) revealed that there is a significant association between anemia in pregnancy and low birth weight.[29] This relationship has also been reported among pregnant women in Korea and Turkey,[30,31] and has also been emphasized in Chinese birth weight.[22] On the other hand, some larger studies in developed countries have shown a U-shaped relationship between maternal hemoglobin concentration and birth weight; these studies found greater birth weights at both ends of the distribution; and the highest birth weight was found at hemoglobin concentrations below the cutoff for anemia.[32] This may reflect the expansion of plasma volume as an important maternal adaptation to increase uteroplacental perfusion.[32] However, a study in Oman found no significant association between anemia in pregnancy and low birth weight.[33]

Some studies have indicated that factors responsible for adverse maternal and perinatal outcomes are associated with anemia. Moreover, a higher prevalence of low birth weight, perinatal and maternal morbidity and mortality have also been shown among anemic women.[21] Depression of the immune system as a result of anemia and the consequent increase in morbidity due to infection (especially urinary tract infection), could be a responsible factor for low birth weight in babies from anemic mothers.[21] In the other part of this study documenting the longitudinal regression modeling; low prenatal zinc was not associated with greater birth size in neonates.

The findings from the current study are in agreement with two large trials conducted in Bangladesh,[3] and Peru,[34] which revealed that low zinc status does not have any effect on birth weight. However, another study based on 580 US mothers,[35] and a study conducted in Peru (2008),[36] as well as data from 20 observational studies (many from developing countries) have shown an association between low zinc status and low birth weight.[32,36]

The mechanisms whereby postnatal growth patterns are affected by prenatal zinc status are unknown. Zinc deficiency during pregnancy could result in growth retardation in infants through effects on the development of the body's immune system.[36] In contrast, animal studies have revealed that this micronutrient regulates IGF-I activity in the formation of osteoblasts and regulates bone growth, in particular.[37] Overall, there are specific enzymes and growth hormones requiring zinc during pregnancy, which may play an important role in growth pathways at later stages.[38] For example, Placental alkaline phosphatase which stimulates DNA synthesis and cell proliferation in pregnancy requires zinc.[39]

One of the limitations in this study is the fact that it was not possible to control the participants' food intake, and we were unable to obtain another blood sample from most of the participants.

Conclusion

The current study found that low maternal serum hemoglobin is associated with low birth weight in newborns in Kashan, Iran. This situation warrants appropriate public health interventions, including nutritional education and appropriate supplementation for pregnant women with low hemoglobin status or those who are anemic. Furthermore, larger studies with larger sample size are recommended.

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