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ORIGINAL ARTICLE

Serum Level of Some Minerals during Three Trimesters of Pregnancy in Iranian Women and Their Newborns: A Longitudinal Study

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Abstract Concentrations of various trace elements are altered during pregnancy with changes in the mother's physiology and the requirements of growing fetus. The aim of the present longitudinal study was to learn the changes of micronutrients Iron (Fe), Calcium (Ca), zinc (Zn) Magnesium (Mg) and copper (Cu) of pregnant woman and their relations with newborns levels. Serum levels of iron, calcium, zinc, magnesium and copper of 162 pregnant women and their newborns were determined by an inductively couple plasma mass spectrometer (ICP/MS). The results showed that majority (41 %) of pregnant women were in age group 26–36 years 55 % had high school and diploma levels of education and the total income ranged between 3 and 5 Rials million per month There was significant difference in iron levels during first, second and third trimesters, 76.0 ± 17.8, 63.5 ± 15.2 and 70.1 ± 14.4 μg/dl respectively. Significant difference was shown in zinc levels 79.5 ± 15, 74.5 ± 16.1, and 65.3 ± 14.9 μg/dl during three trimesters. Copper levels during pregnancy were significantly different (130.9 ± 43.5, 172.0 ± 38.94, 193.2 ± 28.5 μg/dl. The serum levels of calcium and magnesium during pregnancy were constant (Ca: 8.96 ± 0.48, 8.86 ± 0.47, 8.91 ± 0.42 mg/dl and Mg: 2.10 ± 0.21, 2.08 ± 0.28, 2.09 ± 0.29 mg/dl). Results showed that 13 % of pregnant women had hypocalcaemia and hypomagnesaemia. Thirty eight percent and 42 % of pregnant women had iron and zinc deficiency respectively. In this study, unlike zinc, no pregnant women were found deficient in serum copper levels. Calcium, iron, zinc, copper and magnesium levels in the newborn's cord blood were 8.93 ± 0.43, 106.0 ± 26.1, 85.35 ± 16.6, 57.04 ± 13.8 and 1.99 ± 0.27 mg/dl respectively. In the present study the levels of iron and zinc in cord blood were higher than the levels of iron and zinc in maternal serum. The mean level of copper in cord blood serum in the current study was lower than maternal values. The mean serum calcium and magnesium in the serum cord blood and in the serum of the pregnant women were similar.

Keywords Pregnancy period · Newborn · Micronutrients · Deficiency · Longitudinal study

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Introduction

Pregnancy is a natural phenomenon during which women encounter a wide range of internal physical physiological changes [1]. It is a period of rapid growth and cell differentiation, both for the mother and the fetus. Consequently, it is a period when both are very susceptible to alterations in dietary supply, especially of nutrients which are marginal under normal circumstances.

The period of intrauterine nourishment, growth and development is one of the most vulnerable periods which affect nutrition status of fetus [2]. During this time, inadequate stores or intake of micronutrients can have adverse effects on the mother, such as anemia, hypertension, complications of labor, or even death [3]. Furthermore, the fetus can be affected, resulting in a stillbirth, preterm delivery, intrauterine growth retardation, congenital malformations, reduced immunocompetence, and abnormal organ developments [4].

It has been shown that concentrations of various trace elements are altered during pregnancy by many researches. But most of those researches were about single micronutrient, cross-sectional researches [5], of different women in different duration of pregnancy [6]. Longitudinal studies with larger sample size on a wide range of micronutrients of normal pregnant women and their cord blood in Iran have not been reported. In this longitudinal study, we investigated the changes on serum blood concentrations of selected trace elements during three trimesters of pregnancy changes of statistical significance and cord blood after delivery in healthy women.

Materials and Methods

The selected urban area, the city of Khoy is located in the Western Azerbaijan, situated in North West of Iran. A total number of 162 healthy women with confirmed pregnancy (17–45 years) who started their prenatal care were selected based on their willingness. All subjects were requested by the head midwife of the center to take part in this longitudinal study. Written consent letters from all the subjects were obtained and they agreed to be the subjects until the birth of the babies. The pregnant women with diabetes mellitus and cardio vascular disease (CVD), multiple pregnancies, mothers with placenta previa and placenta abruptia were excluded from this study. Venous blood specimens were collected from the participating pregnant women at the end of first, second and third trimester of pregnancy. New-born cord blood sample was collected before delivery of placenta. The collected blood was poured into metal-free plain tubes and was allowed to clot at room temperature. Plain tubes were centrifuged for 15 min at 3,500 rpm and the serum was separated and kept in trace elements-free tubes and stored at -40 °C until analysis. Finally samples were kept in dried ice and were sent for biochemical analysis. Inductively couple plasma mass spectrometer (ICP/MS) [7] was selected for serum calcium, iron, zinc, copper and magnesium analysis. The analysed items consisted of calcium (mg/dl), iron (μg/dl), zinc (μg/dl), copper (μg/dl) and magnesium (mg/dl). The data that was elicited from different schedules were fed to spread sheet of SPSS version 16 (SPSS Inc., Chicago, Illinois). All other data obtained were subjected to suitable statistical analysis. Mean, standard deviation (Mean ± SD) and percentages were calculated wherever necessary and suitable tables were prepared. The statistical differences among the groups were analysed by *t* test and one-way ANOVA followed by post hoc test.

Results

The present longitudinal method investigation was planned to study the changes of some micronutrients like; Iron (Fe), Calcium (Ca), Zinc (Zn) Magnesium (Mg) and Copper (Cu) of normal pregnant woman at the end of each trimester of pregnancy. Blood specimens were collected from 162 healthy pregnant women aged 16–40 years and from cord blood of their neonates.

Information on Pregnant Women, Their Family Background and Type of Diet Consumed

The mean age of pregnant women was 26.1 ± 5.8 years and the age range was 18-40 years. Majority (41 %) of pregnant women were in age group 26–36 years, followed by the age group 20–26 years (36 %). Majority of subjects (55 %) had high school and diploma levels of education. Eighty-seven percent of subjects were home maker. Based on what the subjects and their family declared, the total income of a majority of them was Rials 3–5 million per month. The percentage of subjects with income <Rials 3 (million/month) and above Rials 5 million/month were 27 and 25 respectively. Mean height, weight and BMI were 159.4 ± 4.7, 58.1 ± 8.2 and 22.1 ± 1.9 respectively. All the Iranian pregnant women were non-vegetarians. All subjects generally followed the traditional Iranian food pattern. Analysis of the frequency of consumption of various food items by the Iranian pregnant women showed that wheat in the form of bread was the most common cereal that was used daily by the subjects. The main meal of the day was lunch or dinner. Breakfast consisted of tea, with bread, yogurt and cheese and, in some cases, milk. Consumption of butter, jam and honey for breakfast was rare. Bread and potato were the staple foods. Rice was consumed three to four times per week. In general, if one meal included rice, the other meal was without rice. Snacks were usually bread, cheese and tea and occasionally fruits.

Profile of Serum Calcium, Iron, Zinc, Copper and Magnesium Levels of Mothers During Three Trimesters of Pregnancy

The profile of serum calcium, iron, zinc, copper and magnesium levels of the pregnant women during three trimesters of pregnancy is given in Table 1. The mean serum calcium and magnesium during the first, second and third trimesters were the same (8.96 ± 0.48, 8.86 ± 0.47, 8.91 ± 0.42 and 2.10 ± 0.21, 2.08 ± 0.28 and 2.09 ± 0.29 mg/dl). Our findings showed that there were significant difference in iron, zinc and copper levels during three trimesters of pregnancy. In comparison with the values in the first trimester, serum iron concentration kept decrease in the third trimester (<0.05) Table 1.

Micronutrients Levels in Maternal and Umbilical Cord Blood

Serum levels of calcium, iron, zinc, copper and magnesium in maternal serum blood and umbilical cord blood in the third trimester are presented in Table 2.

Calcium, iron, zinc, copper and magnesium levels in the newborn's cord blood were 8.93 ± 0.43, 106.0 ± 26.1, 85.35 ± 16.6, 57.04 ± 13.8 and 1.99 ± 0.27 mg/dl respectively. As shown in the table iron and zinc levels in maternal serum were significantly lower than that in cord blood. The mean level of copper in cord blood serum in the current study was lower than maternal values. The mean serum magnesium and calcium in the serum cord blood and in the serum of pregnant women was similar Table 2.

Prevalence of Serum Calcium, Iron, Zinc, Copper and Magnesium Deficiency in Third Trimester of Pregnancy

As it is clear from the Table 3, this study found that ~13 % of pregnant women were hypocalcemic. In the present study, 38 % of pregnant women had iron deficiency. We observed zinc deficiency in 42 % of the pregnant women. In this study, unlike zinc, no pregnant women were found deficient in serum copper levels. Prevalence of deficiency in magnesium was 13 % in the present study Table 3.

Discussion

As reported, maternal calcium levels of all the three gestation stages in the present study were found no difference among themselves, although some studies found that the serum calcium level did not change during the pregnancy [8, 9]. As mentioned above, the level of calcium during all the trimesters was constant whereas due to the plasma dilation, a decline in the level of the calcium was expected but we could not get the predicted result. So the level of calcium was similar in all the three trimesters of pregnancy. Optimum level and any sort of changes in the level of serum calcium play a vital role in the health and well-being of the mother as well as of the neonate. The very high circulatory concentrations of estrogen and progesterone alter the concentration of many substances including calcium in the maternal blood during pregnancy [10]. Studies of calcium homeostasis responses during pregnancy have shown increase in both intestinal calcium absorption and urinary calcium excretion during pregnancy and increased rate of bone turnover during pregnancy [11]. Moreover, the increase production of maternal 1,25-dihydroxyvitamin D3 is an independent phenomenon leads to a marked increase in intestinal calcium absorption and may allow the maternal skeleton to store calcium in advance of peak fetal demands later in pregnancy [12]. All of them can explain the insignificant changes during the different gestational weeks in the present study.

Iron is an important micronutrient and is necessary for hemoglobin (Hb) synthesis and several other important functions in the body. Iron deficiency can result not only in reduced oxygen carrying capacity due to lowered hemoglobin levels, but can also affect immunity and growth and development [13]. During pregnancy, iron needs are usually very high to meet the requirements for the fetus, placenta, and maternal red cell expansion. Worldwide, poor pregnancy outcome has been most commonly associated with anemia caused by low plasma levels of iron. Our results showed that in comparison with the values in the first trimester, serum iron concentration kept decrease in the third trimester. This reflects the fact that the iron stores in pregnant body, gradually fell during pregnancy and also it seems pregnant women did not take their iron

Table 1 Serum levels of calcium, iron, zinc, copper and magnesium in maternal serum during pregnancy (*n* = 162)

Serum levels (mg/dl)	Reference values[e]	Trimesters			F values
		First	Second	Third	
Calcium	8.9–10.4	8.96 ± 0.48	8.86 ± 0.47	8.91 ± 0.42	NS
Iron	60–150	76.0 ± 17.8[a]	63.5 ± 15.2[b]	70.1 ± 14.4[c]	25.0*
Zinc	70–102	79.5 ± 15[a]	74.5 ± 16.1[b]	65.3 ± 14.9[c]	33.8*
Copper	70–150	130.9 ± 43.5[a]	172.0 ± 38.94[b]	193.2 ± 28.5[c]	115.7*
Magnesium	1.8–3	2.10 ± 0.21	2.08 ± 0.28	2.09 ± 0.29	NS

Different superscripts indicate significant difference at 5 % level as shown by Post hoc Bonferroni test

NS not significant

* Significant at the 0.05 level

[e] Reference values for non pregnant women [10]

Table 2 Serum levels of calcium, iron, zinc, copper and magnesium in maternal serum blood and umbilical cord blood in third trimester ($n = 162$)

Elements in serum levels (mg/dl)	Maternal blood	Cord blood	*t* test
Calcium	8.9 ± 0.41	8.93 ± 0.43	NS
Iron	70.1 ± 14.4	106.0 ± 26.1	15.7*
Zinc	65.3 ± 14.9	85.35 ± 16.6	10.7*
Copper	193.2 ± 28.5	57.04 ± 13.8	54.4*
Magnesium	1.98 ± 0.26	1.99 ± 0.27	NS

NS not significant

* Significant at the 0.05 level

supplements during pregnancy regularly. Similar variation in serum iron during pregnancy was shown in a study in south Korea [14], and India [15].

Zinc was considered one of the necessary compositions of more than 200 enzymes in the human body and plays an important role in nucleic acid metabolism, cell replication, wound healing, and growth through its functions in nucleic acid polymerases. Zinc is required for cellular division and differentiation, and is an essential nutrient for normal embryogenesis. Zinc is a cofactor for the synthesis of a number of enzymes, DNA, and RNA [16]. Zinc deficiency has been associated with complications of pregnancy and delivery, as well as with growth retardation and congenital abnormalities in the fetus [4]. A comparison between the mean serum zinc during the three trimesters of pregnancy in the current study with a study in China [9], findings showed that the mean serum zinc levels was higher than in the current findings. Zinc levels of subjects kept decreasing during pregnancy from first trimester to third trimester. Similar results were shown in other studies in Turkey [17] and in China [9]. The decline may be explained by a disproportionate increase in plasma volume, as well as the maternal–fetal transfer. The other reasons possibly be decrease in zinc binding [18], or low dietary bioavailability [19], or very high amounts of copper or iron in the diet that compete with zinc at absorption sites [20].

Table 3 maternal serum micronutrients deficiency in third trimester of pregnancy ($n = 162$)

Parameters (mg/dl)	Reference values[a]	Levels	*N*	Percent
Calcium	9.2–11	≤8.7 (Hypocalcaemia)	21	13
		8.8–9	87	54
		>9.1	54	33
Iron	60–150	<60 (Deficient)	61	38
		60–70	48	29
		>70	53	33
Zinc	70–110	<70 (Deficient)	68	42
		70–80	39	24
		>80	55	34
Cooper	70–150	<150	14	9
		150–200	85	52
		>200	63	39
Magnesium	1.8–3.0	<1.8 (Deficient)	21	13
		1.8-2	87	54
		>2	54	33

[a] Refrence values in non pregnant women [10]

Copper is an essential micronutrient and has many important functional roles in immune system and its response [21]. Trace element copper is involved in the function of several cuproenzymes that are essential for life. Copper deficiency affects many cuproenzymes, leading to defects in ATP production; lipid peroxidation; hormone activation; angiogenesis; and abnormalities of vasculature, skeleton, and lung [22]. Similar mean serum levels of copper were shown in other studies carried out in Ethiopia [23] and in China [9]. Our results show that copper levels rise significantly with increasing gestational periods. The increase of copper with the progression of pregnancy could be partly related to synthesis of ceruloplasmin, a major copper binding protein, as a result of elevated levels of maternal estrogen. Another reason may be the decreased biliary copper excretion induced by hormonal changes, typical during pregnancy [24].

Magnesium, an essential trace element for the human body, is needed for proper bone formation and in various intracellular enzymatic processes [25]. Magnesium has established its role in obstetrics, it being an essential element to fetal wellbeing. Deficiency of magnesium may be possibly associated with pre-eclampsia and pre-term delivery and possibly with low birth weight [26]. It has been documented that magnesium deficiency during gestation significantly increases neonatal mortality and morbidity [27]. As shown in the present study, the measurements of serum magnesium during the three trimesters of pregnancy showed a slightly decrease in serum magnesium during second trimester of pregnancy but it was not significant. The similar result was reported in Argentina [11.]. Generally, the lower serum magnesium during second trimester may be associated with hemodilution, renal clearance during pregnancy, and consumption of minerals by the growing fetus [4, 28].

The results of the current study showed that, 13 % of pregnant women were hypocalcemic. A study in Iran [29], showed that 19 % of pregnant women had hypocalcemia (i.e. serum calcium level below 8.5 mg/dl). Factors affect the low blood calcium levels is inadequate vitamin D, low calcium intake or things that interfere with the absorption of calcium. The reason for calcium disabsorption may be due to the nutrients such as wheat in the form of bread, the staple cereal that is used daily by Iranian, which can impair intestinal calcium absorption, because of the presence of high amounts of phytic acid in wheat flour [30].

Vitamin D is essential for the maintenance of calcium homeostasis. In general, vitamin D deficiency was prominent in people living in high latitudes, during the winter season, the use of sunscreens and people who wear cultural clothing such as veiling and concealing clothing. Concealed clothing blocks the absorbance of UV light therefore fails to produce vitamin D. Furthermore, Iranian women spend less time outdoor over men, which make them and their children more prone to the deficiency [31].

In the present study, 38 % of pregnant women had iron deficiency. The prevalence of iron deficiency in pregnant women in the studies conducted in UK [32]; in China [33]; and in India [16] was 34, 42.6 and 73.4 % respectively. The high prevalent of iron deficiency amongst pregnant women found in the present study was possibly due to the inadequate iron consumption or the bread eaten by Iranian is made from flour of high extraction and without added leaven. As a result, it contains high concentrations of phytic acid. Phytate has been shown by several investigators to interfere with absorption of iron [34].

We observed zinc deficiency in 42 % of the pregnant women. This finding is comparable to studies on pregnant women from elsewhere, where prevalence ranging from 22 % to as high as 73.5 % were reported in India and China [35–37]. The higher prevalence of zinc deficiency found in the present study could be due to an inadequate zinc intake and poor bio-absorption. It is worthy to note that several dietary factors are known to affect zinc absorption as a result of physico-chemical interactions in the intestine. Phytate, a component in plants with the highest concentration found in seeds (cereal grains/legumes/nuts), inhibits zinc absorption [38]. This might hold true in the subjects of the present study, as the staple foods in Iran are cereal based.

Copper deficiency caused inadequate maternal dietary intake is very rare, whereas moderate copper deficiency attributed to secondary causes, such as diseases status, during interactions and nutritional genetic factors, are more common and may result pregnancy complication. Deficient of copper in pregnancy can result in early embryonic death and gross structural abnormalities including skeletal, pulmonary central nervous system and cardiovascular defects [39]. In this study, unlike zinc, no pregnant women were found deficient in serum copper levels. In line with our observation, a very low prevalence (2.7 %) of copper deficiency was reported from pregnant women in India [16] and in China [35].

Prevalence of deficiency in magnesium was 13 % (serum levels <1.80 mg/dl).in the present study. However, this prevalence of magnesium deficiency is <43.6 % showed in a study in India [16], and 51.5 % in China [35]. Low serum magnesium status during pregnancy could be due to an active transport of the element across the placenta into the fetus estrogen domination to which these women are exposed [40].

The mean calcium in cord blood in the studies conducted in the USA [41]; Greek and Albania [42] was similar to the mean calcium in cord blood in the current study.

The current study showed iron levels in maternal serum were higher than that in cord blood. Similar results were shown in the other studies carried out in Amman [43] and India [15] which indicate selective uptake of iron by fetus [44].

In the present study the levels of zinc in cord blood were higher than the levels of zinc in maternal serum. Similar findings have been reported in the other studies in India [45] and Amman [43]. Our study exhibited that zinc level of neonates were more than their mothers which could be caused by active transport of zinc from mother to fetus through placenta as a result of high zinc requirement in the fetus, sufficient transport from mother to the fetus and increased absorption in the fetus. On the other hand, it could be caused by band valency reduction of maternal zinc during pregnancy and physiologic dilution because of enhanced maternal blood volumes. This could be due to rising neonatal zinc level as compared to their mothers.

The mean level of copper in cord blood serum in the current study was lower than maternal values; these values are in agreement with the data obtained from India [15] and Amman [43]. Copper transport is done actively from mother to fetus through the placenta as a result of fetal need.

The mean serum magnesium in the serum cord blood and in the serum of pregnant women was similar. Comparable results were shown in other countries namely the USA [41]; Greek and Albania [42].

In conclusion the present study has several advantages over previous studies that examined micronutrient status during pregnancy, since a wide range of micronutrients was examined simultaneously in a longitudinal study. These values will be helpful in assessing the health status of pregnant women with a socioeconomic and racial background similar to those of our study participants and give treatments to them promptly. There was no published data concerning micronutrient changes and their deficiencies among pregnant Iranian women. Therefore, the aim of this study was to determine the changes of micronutrients and the prevalence of micronutrients deficiencies among pregnant women of Iranian. Identifying the magnitude of micronutrient deficiencies among pregnant women is essential for evidence-based intervention modalities. Thus, studies investigating these parameters are vital and may be of great interest, so as to provide health planners and caregivers with fundamental guidelines for the implementation of preventive measures. The actual consumption of supplementary nutrients was not monitored and this is one of the limitation of the study.

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