European Review for Medical and Pharmacological Sciences 2015; 19: 4690-4697

Four trace elements in pregnant women and their relationships with adverse pregnancy outcomes

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Abstract. – OBJECTIVE: Lack of trace elements during pregnancy is detrimental to maternal and fetal health. Our aim is to study the changes in trace element levels in Chinese pregnant women and their association with adverse pregnancy outcomes.

PATIENTS AND METHODS: 1568 cases of Chinese pregnant women in remote areas were collected for a prospective cohort study. Serum copper, zinc, calcium and iron levels were measured at pre-pregnancy, 1st trimester (7w-12w), 2nd trimester (24w-28w) and 3rd trimester (35w-40w).

RESULTS: (1) Serum copper levels was significantly higher after pregnancy than before, calcium and iron levels decreased, but zinc levels did not change significantly. (2) Copper and zinc deficiency in pregnant women was not a common finding, but lack of iron and calcium was frequently encountered; iron deficiency was especially common in the 3rd trimester (42.27%). (3) Serum zinc and iron levels in patients who either had a miscarriage or a preterm delivery were significantly lower than in the control group ($p < 0.05$). In patients with premature rupture of membranes, serum zinc levels were significantly lower ($p < 0.05$). In patients with intrauterine growth restriction (IUGR), serum copper, zinc, calcium and iron were significantly lower ($p < 0.05$).

CONCLUSIONS: Trace elements is closely associated with fetal growth and development during pregnancy. Deficiency can lead to adverse pregnancy outcomes. Therefore, we should have a reasonable diet, replenish trace elements, therefore reducing the occurrence of adverse pregnancy outcomes.

Key Words:
Pregnancy, Trace element levels, Adverse pregnancy outcomes.

Introduction

Maternal nutritional status directly affects fetal growth and development. Levels of serum trace elements is an important indicator of maternal nutritional status during pregnancy[1]. Trace elements play an important role during pregnancy and any reduction may lead to physical abnormalities and diseases, which may increase the incidence of adverse pregnancy outcomes[2]. Elements associated with adverse pregnancy outcomes include copper, zinc, calcium, magnesium, iron and iodine among others[3]. The vast majority of copper exists as ceruloplasmin and their main function is to protect cells from the toxic superoxide anion and ensure normal fetal growth and immune function. Copper also participates in the maintenance of normal hematopoietic function and maintenance of the central nervous system, which promotes bone, blood vessels and skin health[4]. Studies have shown that pregnant women presenting with copper deficiency are prone to preterm delivery, premature rupture of membranes and fetal nervous system damage[5]. In humans, zinc is a component of a variety of enzymes and nucleic acid. It is directly involved in the body's synthesis of DNA and RNA and transcription and replication, which plays an important role in the human immune system and the development of the fetal nervous system[6]. Studies have shown that lack of zinc intake in late pregnancy can cause fetal growth retardation, congenital malformations, and even fetal death[7]. Calcium is the main component of the teeth and skeleton and it plays an important role in the activation of muscle-keeping, nervous excitement and enzyme activation. Studies have shown that calcium deficiency in pregnant women can cause gestational hypertension[8] and aggravate postpartum hemorrhage. Calcium deficiency can cause ossification disorder, when maternal calcium mobilization compensate for the lack of fetal calcium, which may lead to an increased maternal deficiency[9]. Iron is the main

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component of hemoglobin and myoglobin, it is not only involved in oxygen transport, storage and use, but also involved in the synthesis of cytochrome enzymes, peroxidase enzymes and hormones[10]. Past studies have shown that anemia occurs in pregnant women who have a lack of iron, leading to fetal chronic hypoxia, premature birth and perinatal fetus mortality[11,12].

The imbalance of the four trace elements mentioned above may be closely related to complications during pregnancy, including miscarriage, preterm delivery, stillbirth, intrauterine growth restriction, fetal malformations, premature rupture of membranes and other adverse pregnancy outcomes. However, owing to differences between races, difference in diet and living environments, changes of these trace elements during pregnancy in Chinese women is not well understood. Lack of trace elements in pregnant women and the way they affect pregnancy outcome is also not clear. Our research will prospectively observe changes of maternal serum trace elements in pregnant Chinese women and analyze their relationship with adverse pregnancy outcomes, so as to be able to guide women during their pregnancy when it comes to intake of trace elements, and to minimize the occurrence of adverse pregnancy outcome.

Clinical Subjects

A total of 1568 cases were collected in antenatal care clinics in our hospital from February 2013 to January 2014. They were all from downtown areas. Their age ranged from 18 year old to 39 year old, and mean age was 26.4 year old. There were 1435 cases of primiparas and 133 cases were multiparas. We conducted a prospective cohort study for our research. In pre-pregnancy, first trimester (9-12w), second trimester (24-28w) and third trimester (35-40w), measurement of trace elements level were undertaken. Pregnancy outcomes of clinical subjects were collected, including gestational age at delivery, miscarriage, preterm delivery, gestational hypertension, intrauterine growth restriction (IUGR), fetal malformations and premature rupture of fetal membranes, etc. Almost all the clinical subjects were not on any treatment plan of trace elements during pregnancy. Five women were excluded for trace elements supplements before pregnancy, and 15 pregnant women who planned to add trace elements after pregnancy were also excluded. Finally, 1065 cases of pregnant women had given birth in our hospital, but 58 cases had a miscarriage. Stillbirth and fetal malformation were not recorded. Maternal data and detection in each gestational age was shown in Figure 1.

Laboratory Tests

In the different pregnancy period, 5 ml fasting blood was extracted for analysis. After mixing with anticoagulant EDTA-K2, trace elements were detected. Serum was separated at a rotational speed of 1000-2000 r/min in the centrifuge for 10 min, and measured for copper, zinc, calcium and iron content. BH-5100 five-channel atomic absorption spectrometer was used for trace elements detection (Beijing Bo Hui Innovative Photovoltaic Technology Co., Ltd). Dilution of trace elements, control solution and calibration solution were provided by that company. Normal reference range of trace elements provided by the kit reference range showed 11.8-39.3 μmol /L for copper, 76.5-170 μmol/L for zinc, 1.55-2.10 mmol/L for calcium and 7.52-11.80 mmol/L for iron. Detection value that was less than the lower limit of the normal reference value or greater than upper limit of normal reference values were determined to be abnormal.

Statistical Analysis

SAS 9.2 (USA) was used for statistical analysis in this study. Test results such as trace elements were shown as Mean ± SD. Comparison between multiple groups was done with ANOVA analysis and the authors also used the post-hoc test. The Authors should use also post-hoc test. Values of trace elements detection between pregnant women with adverse pregnancy outcomes and normal pregnancy women were compared with the *t*-test. $p < 0.05$ was considered statistically significant.

Results

Changes of 4 Trace Elements During Pregnancy

In our study, trace elements in different gestational age of pregnant women were measured. As pregnancy advances, serum copper levels were significantly increased. In the 1st, 2nd and 3rd trimester of pregnancy, it was significantly higher than in pregnancy before. Serum zinc level was not significantly changed before and after pregnancy. Serum calcium levels was reduced during pregnancy, but had no significant difference compared to previous pregnancies. Serum iron levels decreased after pregnancy, and in the

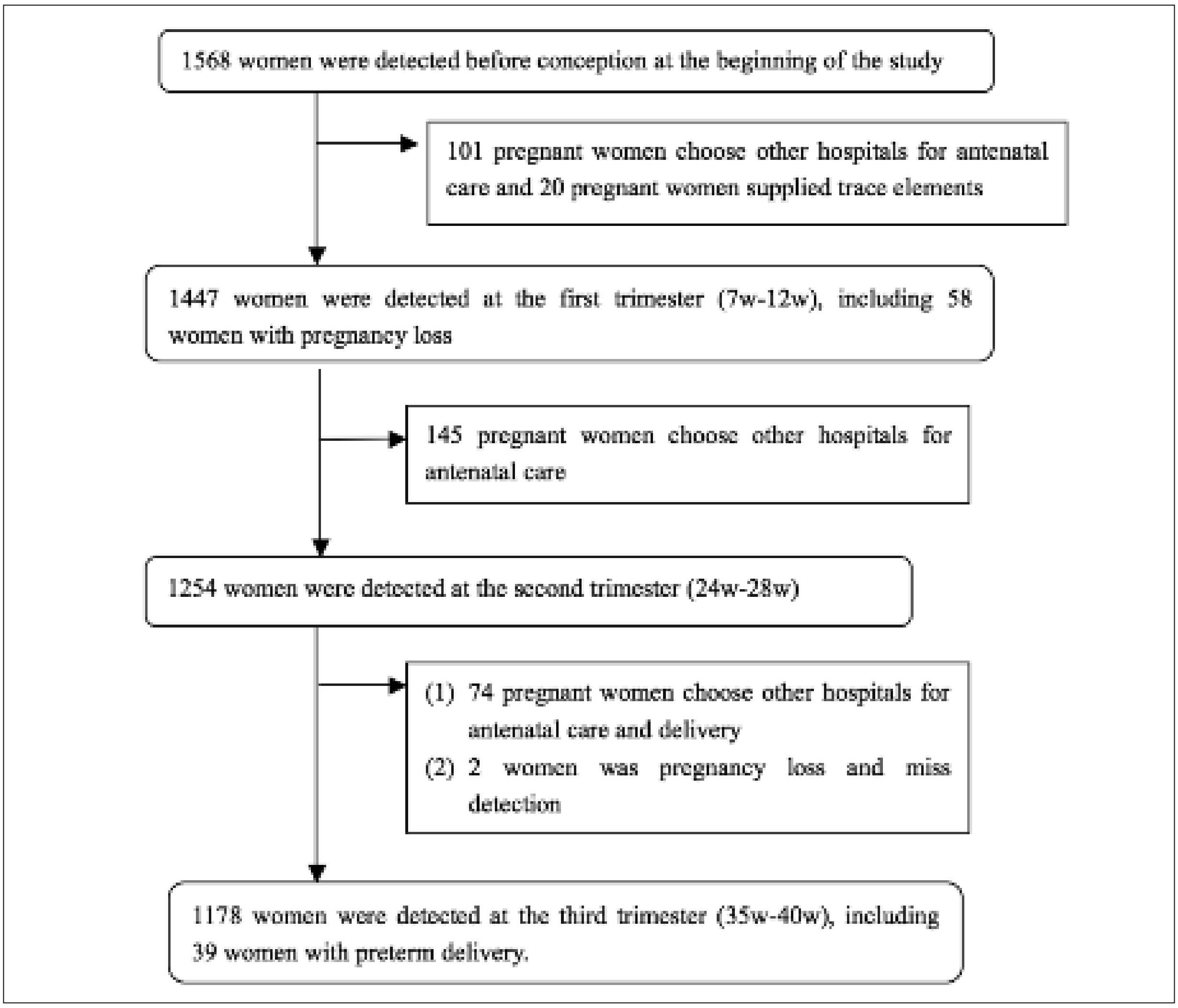

Figure 1. Number of pregnant women included in the detection at different periods.

third trimester, it was significantly lower than before pregnancy. Specific data and the trends of 4 trace elements during pregnancy were shown in Table I and Figures 2 to 5.

Trace elements Deficiency in Pregnant Women

Specific data of trace elements deficiency are shown in Table II. We found that the lack of cop-

Table I. Trace elements levels in different detection period in pregnant women.

	Copper (μmol/L)	Zinc (μmol/L)	Calcium (mmol/L)	Iron (mmol/L)
Pre-pregnancy (n = 1568)	23.43 ± 5.74	87.32 ± 12.76	1.72 ± 0.18	8.10 ± 1.01
1st trimester (n = 1447)	30.45 ± 5.21[a]	79.32 ± 12.87	1.55 ± 0.21	7.43 ± 1.04
2nd trimester (n = 1254)	29.77 ± 5.02[a]	84.13 ± 12.96	1.59 ± 0.19	7.35 ± 1.18
3rd trmester (n = 1178)	31.02 ± 6.03[a]	84.55 ± 14.38	1.57 ± 0.20	6.82 ± 0.92[a]
F	9.362	1.232	1.432	6.543
P	< 0.001	0.364	0.201	0.013

[a]Compared with pre-pregnancy, $p < 0.05$.

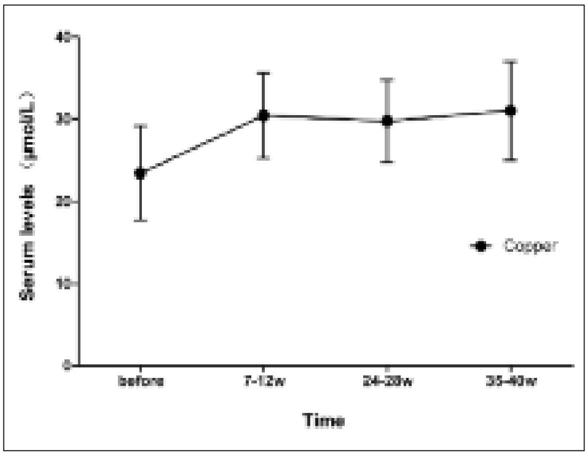

Figure 2. Serum copper levels at different detection periods.

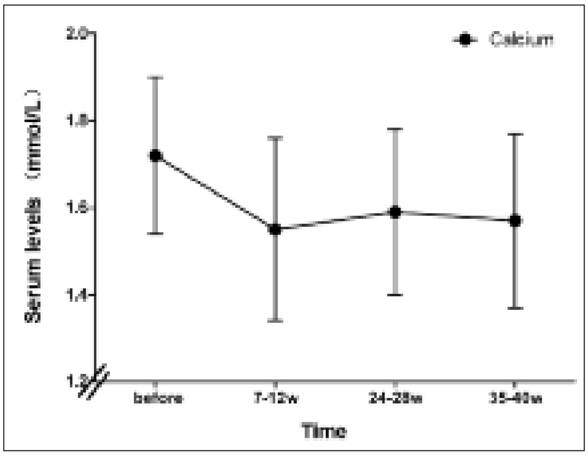

Figure 4. Serum calcium levels at different detection periods.

per and zinc in pregnant women was less frequently encountered, but a relatively larger proportion of pregnant women had an iron and calcium deficiency and iron deficiency was a common finding in the 3rd trimester of pregnancy (42.27%).

Maternal Trace Elements Deficiency and its Relationship with Pregnancy Outcome

Pregnancy loss (n = 58, Figure 1) and major adverse pregnancy outcomes, including premature birth (n = 39, Figure 1), gestational hypertension (including pre-eclampsia, eclampsia), premature rupture of membranes, IUGR were followed up. Of 1178 cases between the 37th and 40th week of gestation, gestational hypertension was diagnosed in 79 cases, and there was 101 cases of premature rupture of membranes and 25 cases of IUGR were also found. The remaining 973 cases which were in the 3rd trimester were classified as the control group (n = 1389 in 1st trimester were used as the control group, see Figure 1). Comparison of four trace elements is shown in Table III. It was found that serum zinc and iron levels in patients with miscarriage and preterm delivery were significantly lower than in the control group. In patients with premature rupture of membranes, serum zinc levels were significantly lower than in the control group. In IUGR patients, serum copper, zinc, calcium and iron were significantly lower than in the control group.

Discussion

Trace elements play a crucial role in normal metabolism in pregnant women, fetal growth and

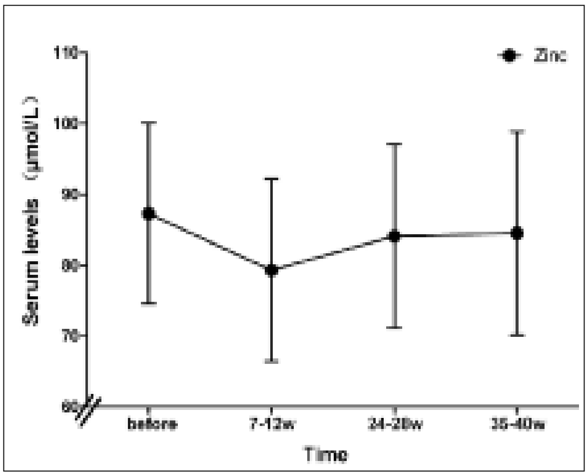

Figure 3. Serum zinc levels at different detection periods.

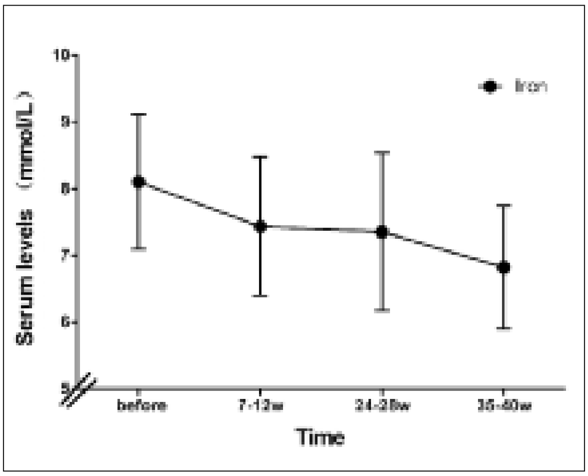

Figure 5. Serum iron levels at different detection periods.

Table II. Accounting of cases in trace elements deficiency.

	Copper	Zinc	Calcium	Iron
Pre-pregnancy (n=1568)	11 (0.70%)	65 (4.15%)	220 (14.03%)	165 (10.52%)
1st trimester (n=1447)	12 (0.83%)	121 (8.36%)	454 (31.37%)	482 (33.31%)
2nd trimester (n=1254)	9 (0.72%)	89 (7.10%)	319 (25.44%)	447 (35.65%)
3rd trmester (n=1178)	8 (0.68%)	78 (6.62%)	348 (29.54%)	498 (42.27%)

immune function[13]. Copper, iron, zinc and calcium are essential trace elements, which exist in a variety of enzyme systems and play an important role in maintaining cell proliferation[14,15]. Deficiency of trace elements is also closely related to many complications of pregnancy, including miscarriage, preterm delivery, stillbirth, poor intrauterine growth, deformities, premature rupture of membranes and other adverse pregnancy outcomes.

The physiological role of copper is to primarily protect the body cells from the toxicity of superoxide ions, thereby promoting the growth and development. Progesterone stimulates the liver to increase synthesis of ceruloplasmin which can promote fetal growth and development, supply nutrition and strengthen the immune function[16]. In patients presenting with copper deficiency, cytochrome synthesis and bone collagen synthesis are impaired, therefore, bone development is also inhibited. Zinc is also known as an "intelligence" element. It plays an active role in a variety of enzymes, such as carbonic anhydrase, DNA polymerase, RNA polymerase, etc., participating in important biochemical nucleic acids, proteins and other metabolic processes, in order to promote growth and development[17]. Therefore, zinc deficiency in pregnant women can directly or indirectly affect the growth and development of the fetus. If there is zinc deficiency in pregnant women, they may have loss of appetite, which invariably affects nutrients intake and leads to poor fetal development[18]. Our results showed that serum zinc in IUGR cases was significantly lower than in healthy pregnant women, which was due to a discrepancy in supply and demand, that is, inadequate intake by the pregnant mother which was insufficient for proper fetus development. Therefore, monitoring of serum zinc in pregnant women during pregnancy is important so as to ensure proper fetal development.

Iron is an important component in the synthesis of hemoglobin. During pregnancy, blood volume augments fast in pregnant women and their fetuses. The body requires a large amount of iron for the synthesis of hemoglobin. Iron deficiency can cause anemia, decreasing the capacity of carrying the oxygen by hemoglobin, resulting in chronic lack of oxygen in mother and fetus[19]. As a result of inadequate intake of iron, iron deficiency or anemia will directly affect fetal growth and development. Therefore, during pregnancy, pregnant women should increase the intake of iron, so as to constantly meet the physical needs of pregnant women and fetuses. Our study found that serum iron levels gradually decreased as the pregnancy progressed. Also, pregnant women had evident iron deficiency, and especially in late pregnancy,

Table III. Maternal trace elements deficiency and its relationship with pregnancy outcome

	Copper (μmol/L)	Zinc (μmol/L)	Calcium (mmol/L)	Iron (mmol/L)
Miscarriage (n = 58)	29.96 ± 5.27	72.67 ± 11.98[a]	1.50 ± 0.19	6.96 ± 0.98[a]
Control group (n = 1389)	31.24 ± 5.07	83.25 ± 12.79	1.58 ± 0.22	7.59 ± 1.01
Preterm delivery (n = 39)	29.30 ± 5.80	73.55 ± 14.09[a]	1.53 ± 0.20	6.51 ± 1.19[a]
Gestational hypertension (n = 79)	30.32 ± 5.98	75.32 ± 14.8[a]	1.49 ± 0.19[a]	6.89 ± 1.08
Premature rupture of membranes (n = 101)	30.21 ± 5.78	73.21 ± 13.9[a]	1.52 ± 0.21	6.73 ± 1.12
IUGR (n = 25)	23.28 ± 5.27[a]	71.28 ± 12.99[a]	1.45 ± 0.15[a]	6.39 ± 1.01[a]
Control group (n = 973)	32.74 ± 6.23	87.29 ± 14.87	1.64 ± 0.21	7.09 ± 1.21

[a]Compared with the control group, $p < 0.05$.

iron deficiency was very frequently encountered (42.27%). Pregnant women should have iron supplementation in late pregnancy, in order to maintain normal physiological needs. Calcium plays an important role in the development process of fetal bones, nerves and muscles. Maternal serum calcium levels directly affect fetal serum calcium concentration. Calcium is very important in the growth and development of the fetus[20]]. During pregnancy, as a result of an increasing need of calcium in mother and fetus, calcium deficiency is a commonly encountered issue[21]. Severe calcium deficiency can cause lower limbs cramps in mothers, fetal growth restriction and so on[22]. Our study also found that there was a severe lack of calcium in IUGR pregnancies.

Gestational hypertension is the most common complication in pregnant women and is a common cause of maternal and perinatal morbidity and mortality. It has been found that lack of calcium, magnesium, zinc, selenium and other elements is closely related to the development of pre-eclampsia[23]. Calcium and zinc imbalance, especially play a very important role in the pathogenesis of pre-eclampsia[24]. Levels of serum zinc in cord blood is dependent on placental transport function. Gestational hypertension or prolonged pregnancy patients, who are more liable to have placental dysfunction, had a reduced ability of transporting zinc through the placenta, resulting in a decrease in serum zinc in the cord blood, such that the fetal serum zinc level was relatively lower[25]. The results of our study showed that serum zinc levels in patients with gestational hypertension was significantly lower than in the control group. In recent years, studies have found that serum calcium levels in PIH (pregnancy induced hypertension) is significantly decreased as compared to relatively normal pregnancies[26]. A Study by Ephraim et al[27] also confirmed this finding. This study comprised of 120 women with PIH, 100 women with PE (pre-eclampsia) and 160 healthy, age-matched pregnant women (controls). Women with hypertensive disorders (PE and PIH) had significantly lower serum calcium levels than those in the control group ($p < 0.0001$). Our study found that serum calcium levels in PIH were significantly lower than in the control group. In addition to this, trace elements are also closely related to threatened abortion, miscarriage, preterm delivery and premature rupture of membranes[28]. Our research found that in patients with preterm delivery and abortion, serum zinc and iron levels were significantly lower than those of the control group. Zinc in patients with premature rupture of membranes was lower than in the control group. This points to the fact that these two elements are potentially important factors affecting fetal growth and development, but the underlying mechanism is still not fully understood. Zinc can promote growth and development, accelerate the speed of cell division and maintain a high level of cell metabolism[29]. In addition, iron can also enhance the resistance of white blood cells, and strengthen fetal immune system[30]. Therefore, lack of these two elements are closely related to preterm delivery and miscarriage.

Lack of trace elements can lead to fetal growth restriction in pregnant women[31]. Usually, there is a lack of vitamins and trace elements in IUGR patients. A study by Brough et al[32] confirmed this finding. They conducted a randomized, double-blind, placebo-controlled trial of multiple-micronutrient supplementation including 20 mg Iron and 400 microg folic acid from the first trimester of pregnancy. In the compliant subset (n = 149), placebo mothers had more small-for-gestational age (SGA) infants (eight SGA vs. thirteen; $p = 0.042$) than the treatment mothers. Therefore, inadequate intake of trace elements will directly affect fetal growth and development, potentially leading to IUGR. The results in this study also support this view.

Conclusions

In this study we have showed that trace elements deficiency is a real problem in pregnant Chinese women. They should be promptly supplied trace elements according to individual circumstances, especially in late pregnancy. When pregnant women are supplied adequate levels of trace elements, they can maintain healthy and well developed fetuses. Therefore, pregnant women should maintain nutritional balance; have regular medical examinations, serum trace elements should also be investigated, thereby reducing adverse pregnancy outcomes.

Acknowledgements

This study was supported by: The Fund of Shanghai Health and Family Planning (ZK2012B06).

Conflict of Interest

The Authors declare that there are no conflicts of interests.

References

1) Reddy YS, Y A, Ramalaksmi BA, Kumar BD. Lead and trace element levels in placenta, maternal and cord blood: a cross-sectional pilot study. J Obstet Gynaecol Res 2014; 40: 2184-2190.
2) Sakamoto M, Yasutake A, Domingo JL, Chan HM, Kubota M, Murata K. Relationships between trace element concentrations in chorionic tissue of placenta and umbilical cord tissue: potential use as indicators for prenatal exposure. Environ Int 2013; 60: 106-111.
3) Frías-Espericueta MG, Cardenas-Nava NG, Márquez-Farías JF, Osuna-López JI, Muy-Rangel MD, Rubio-Carrasco W, Voltolina D. Cadmium, copper, lead and zinc concentrations in female and embryonic Pacific sharpnose shark (Rhizoprionodon longurio) tissues. Bull Environ Contam Toxicol 2014; 93: 532-535.
4) Ergaz Z, Guillemin C, Neeman-Azulay M,Weinstein-Fudim L, Stodgell CJ, Miller RK, Szyf M, Ornoy A. Placental oxidative stress and decreased global DNA methylation are corrected by copper in the Cohen diabetic rat. Toxicol Appl Pharmacol 2014; 276: 220-230.
5) Ergaz Z, Shoshani-Dror D, Guillemin C, Neeman-azulay M, Fudim L, Weksler-Zangen S, Stodgell CJ, Miller RK, Ornoy A. The effect of copper deficiency on fetal growth and liver anti-oxidant capacity in the Cohen diabetic rat model. Toxicol Appl Pharmacol 2012; 265: 209-220.
6) Dickinson N, Rankin J, Pollard M, Maleta K, Robertson C, Hursthouse A. Evaluating environmental and social influences on iron and zinc status of pregnant subsistence farmers in two geographically contrasting regions of Southern Malawi. Sci Total Environ 2014; 500-501: 199-210.
7) Dickinson N, Gulliver J, MacPherson G, Atkinson J, Rankin J, Cummings M, Nisbet Z, Hursthouse A, Taylor A, Robertson C, Burghardt W. A framework to explore micronutrient deficiency in maternal and child health in Malawi, Southern Africa. Environ Health 2009: 8 Suppl 1: S13.
8) Esteban-Vasallo MD, Aragonés N, Pollan M, López-Abente G, Perez-Gomez B. Mercury, cadmium, and lead levels in human placenta: a systematic review. Environ Health Perspect 2012; 120: 1369-1377.
9) Taylor CM, Golding J, Emond AM. Lead, cadmium and mercury levels in pregnancy: the need for international consensus on levels of concern. J Dev Orig Health Dis 2014; 5: 16-30.
10) Kapil U, Bhadoria AS. National Iron-plus initiative guidelines for control of iron deficiency anaemia in India, 2013. Natl Med J India 2014; 27: 27-29.
11) Narasinga Rao BS. Anaemia and micronutrient deficiency. Natl Med J India 2003; 16 Suppl 2: 46-50.
12) Pathak P, Kapil U, Kapoor SK,Saxena R, Kumar A, Gupta N, Dwivedi SN, Singh R, Singh P. Prevalence of multiple micronutrient deficiencies amongst pregnant women in a rural area of Haryana. Indian J Pediatr 2004; 71: 1007-1014.
13) Freeland-Graves JH, Sanjeevi N, Lee JJ. Global perspectives on trace element requirements. J Trace Elem Med Biol 2015; 31: 135-141.
14) Zheng G, Zhong H, Guo Z,Wu Z, Zhang H, Wang C, Zhou Y, Zuo Z. Levels of heavy metals and trace elements in umbilical cord blood and the risk of adverse pregnancy outcomes: a population-based study. Biol Trace Elem Res 2014; 160: 437-444.
15) Al-Jameil N, Tabassum H, Al-Mayouf H, Aljohar HI, Alenzi ND, Hijazy SM, Khan FA. Analysis of serum trace elements-copper, manganese and zinc in preeclamptic pregnant women by inductively coupled plasma optical emission spectrometry: a prospective case controlled study in Riyadh, Saudi Arabia. Int J Clin Exp Pathol 2014; 7: 1900-1910.
16) Vukeli J, Kapamadzija A, Petrovi D, Gruji Z, Novakov-Miki A, Kopitovi V, Bjelica A. Variations of serum copper values in pregnancy. Srp Arh Celok Lek 2012; 140: 42-46.
17) Pathak P, Kapil U, Dwivedi SN, Singh R. Serum zinc levels amongst pregnant women in a rural block of Haryana state, India. Asia Pac J Clin Nutr 2008; 17: 276-279.
18) Hanachi P, Norrozi M, Moosavi RM. The correlation of prenatal zinc concentration and deficiency with anthropometric factors. J Family Reprod Health. 2014; 8: 21-26.
19) Nwaru BI, Hayes H, Gambling L, Craig LC, Allan K, Prabhu N, Turner SW, McNeill G, Erkkola M, Seaton A, McArdle HJ, Devereux G. An exploratory study of the associations between maternal iron status in pregnancy and childhood wheeze and atopy. Br J Nutr 2014; 112: 2018-2027.
20) Tang R, Tang IC, Henry A, Welsh A. Limited evidence for calcium supplementation in preeclampsia prevention: a meta-analysis and systematic review. Hypertens Pregnancy 2014; 30: 1-23.
21) Hofmeyr GJ, Lawrie TA, Atallah AN, Duley L, Torloni MR. Calcium supplementation during pregnancy for preventing hypertensive disorders and related problems. Cochrane Database Syst Rev 2014; 6: CD001059.
22) Flenady V, Wojcieszek AM, Papatsonis DN, Stock OM, Murray L, Jardine LA, Carbonne B. Calcium channel blockers for inhibiting preterm labour and birth. Cochrane Database Syst Rev 2014; 6: CD002255.
23) Adam B, Malatyalio lu E, Alvur M, Talu C. Magnesium, zinc and iron levels in pre-eclampsia. J Matern Fetal Med 2001; 10: 246-250.
24) Jain S, Sharma P, Kulshreshtha S, Mohan G, Singh S. The role of calcium, magnesium, and zinc in preeclampsia. Biol Trace Elem Res 2010; 133: 162-170.

25) Gupta S, Jain NP, Avasthi K, Wander GS. Plasma and erythrocyte zinc in pre-eclampsia and its correlation with foetal outcome. J Assoc Physicians India 2014; 62: 306-310.

26) Hofmeyr GJ, Lawrie TA, Atallah AN, Duley L, Torloni MR. Calcium supplementation during pregnancy for preventing hypertensive disorders and related problems. Cochrane Database Syst Rev 2014; 6: CD001059.

27) Ephraim RK, Osakunor DN, Denkyira SW, Eshun H, Amoah S, Anto EO. Serum calcium and magnesium levels in women presenting with pre-eclampsia and pregnancy-induced hypertension: a case control study in the Cape Coast metropolis, Ghana. BMC Pregnancy Childbirth 2014; 14: 390.

28) Rahmanian M, Jahed FS, Yousefi B, Ghorbani R. Maternal serum copper and zinc levels and premature rupture of the foetal membranes. J Pak Med Assoc 2014; 64: 770-774.

29) Gómez T, Bequer L, Mollineda A, González O, Diaz M, Fernández D. Serum zinc levels of cord blood: Relation to birth weight and gestational period. J Trace Elem Med Biol 2015; 30: 180-183.

30) Pathak P, Kapil U, Yajnik CS, Kapoor SK, Dwivedi SN, Singh R. Iron, folate, and vitamin B12 stores among pregnant women in a rural area of Haryana State, India. Food Nutr Bull 2007; 28: 435-438.

31) Hovdenak N, Haram K. Influence of mineral and vitamin supplements on pregnancy outcome. Eur J Obstet Gynecol Reprod Biol 2012; 164: 127-132.

32) Brough L, Rees GA, Crawford MA, Morton RH, Dorman EK. Effect of multiple-micronutrient supplementation on maternal nutrient status, infant birth weight and gestational age at birth in a low-income, multi-ethnic population. Br J Nutr 2010; 104: 437-445.