Comparative assessment of serum level of selenium and iron in 1st trimester of pregnancy with non-pregnant women

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A R T I C L E   I N F O

Article history:
Received 07-08-2019
Accepted 10-09-2019
Available online 14-12-2019

Keywords:
Selenium
Iron
Atomic absorption spectrophotometry

A B S T R A C T

Introduction: Pregnancy is dynamic anabolic state in women’s life. During pregnancy women undergo profound hormonal, metabolic and physical changes. Aim of the present study was to compare the serum level of selenium and iron between pregnant and non-pregnant women.

Materials and Methods: The present study was carried out in 50 first trimester pregnant women and 50 non pregnant women at tertiary care hospital in Surat, in age group of <25 years, 25 to 30 years and ≥30 years and in BMI groups of <23 kg/m², 23-24.99 kg/m², 25-29.9 kg/m² and 30 or more kg/m² groups. Blood was collected and serum was analyzed for selenium and iron concentration in atomic absorption spectrophotometry (AAS). We have also measured anthropological parameter like height, weight and based on this BMI calculated. SPSS software used for statistical analysis.

Result: There was a significant lower level of serum selenium (p<0.01) and serum iron (p<0.05) was found in 1st trimester pregnant women compared to non-pregnant women. There was a significant decreased in selenium level in all BMI group in pregnant women except in ≥30 kg/m² group and there was a decreased in serum iron level in all BMI group in pregnant women compared to non-pregnant women.

Conclusion: There was a significant decrease in serum selenium and iron level in 1st trimester of pregnant women compared to non-pregnant women, which indicate that during pregnancy requirement of trace elements increase to meet demand for fetus growth. A reduction in maternal selenium concentration during pregnancy can lead to various disorders such as miscarriage, premature birth, preeclampsia, pregnancy include hypertension, low birth weight, retinopathy of prematurity etc.

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1. Introduction

During pregnancy women undergo profound hormonal, metabolic and physical changes. Pregnancy is dynamic anabolic state with increase nutritional requirement for growth of mother and fetus, and preparation of maternal tissue for lactation.¹ Additional nutrient requirement to be met by increased maternal intake through diet and supplement.²

Pregnancy is associated with the increase demand of all the nutrient. The minerals are critical for development the fetus.³ Many minerals are the transferred to the fetus for fetal storage in the latter part of the pregnancy, which also play important role throughout the pregnancy. Trace element is normally found in the body as a component of metalloproteinase and performs a variety of enzymatic and non-enzymatic function.⁴

There is decrease in ionized and total trace element level with increase gestational age during normal pregnancy. Various trace elements play a vital role in the health of human and particularly in pregnant population. Iron is involved in oxygen and carbon dioxide transport in associated with hemoglobin and selenium act as antioxidant in associated with glutathione peroxidase.⁵
Among all the nutrients, we had mainly focused in trace elements like iron and selenium in the study. These trace elements have been experimentally showed to be essential, as their deficiency lead to abnormal pathological changes.

The total content of iron in an adult body is 3-5 g. About 70% of this occur in the erythrocytes of blood as a constituent of hemoglobin, bone marrow and muscles. Iron is present in most of the cells. Heme is most predominant iron containing substance. It is a constituent of several protein/enzyme hem protein.

The body iron requirement for an average pregnancy is approximately 1,000 mg from which calculated that 350mg of iron is lost to the fetus. Thus the iron requirement of a pregnancy average about 1,040mg. During pregnancy the total maternal need for extra iron average close to 800mg, of which about 300mg is for the fetus and the placenta and the remaining is the maternal hemoglobin mass expansion.

Among healthy human being, pregnant women and rapidly growing infant are most vulnerable to iron deficiency. Both group have to absorb substantially more iron that is lost from the body, and both considerable risk of developing iron deficiency under ordinary dietary circumstance. During the pregnancy, more iron is needed primarily to supply the growing fetus and placenta. Higher level of serum iron lead to increased hemoglobin concentration which lead to increased oxygen carrying capacity and also provide buffer against blood loss that will happen during delivery.

Iron deficiency and overload are the major disorder of iron metabolism. However, altered iron metabolism has been observed to a number of other diseases including anaemia, cardiovascular diseases, and infection.

During pregnancy, iron is required in high levels to support fetal development. With fetal demand increasing significantly during late gestation, maternal requirements also increased and as such the risk of anaemia increases with advancing gestation, which is called physiological anaemia of pregnancy. Despite the up-regulation of placental transporters, low maternal iron levels can compromise fetal iron needs and may lead to altered metabolism and development in offspring, including reduced cognitive function. In current era iron supplementation is advisable in pregnancy because most of women have low level of iron store after pregnancy. Women in developing countries are always in a state of low iron balance during their reproductive year. Their iron store is not well developed because of poor nutritional intake, recurrent infections, menstrual blood loss, and repeated pregnancies.

Reactive oxygen species are product of oxygen, when brought into contact with a n iron, form a very reactive free radical, the hydroxyl radical have a potential to damage cells, organs, tissue in the body. Oxidative stress over time is now through to be component of the processes of aging, and the development of cardiovascular disease. Iron overload and the associated oxidative stress contribute to the pathogenesis and increase risk of type 2 diabetes and other disorder.

Most of the selenium in tissue is present in two forms: selenocystein and selenomethionine. Selenomethionine cannot be synthesis in the body and which must be supplied by the diet. It can replace methionine in a variety of protein. Selenomethionine is regarded as an unregulated storage compartment for selenium; when the dietary selenium supply is interrupted, this pool turn over and supplies selenium to the organism. A daily intake of 50-200 mg of selenium has been recommended for adults. The good source of selenium are organ meat and sea food. It is absorbed from duodenum. Normal level of selenium is 13μg/dl. Selenocystein is an essential component of the enzyme glutathione peroxidase. These enzymes protect the cell against the damage caused by H2O2. It appears from recent studies that selenocystein is directly incorporated during protein biosynthesis. Therefore, selenocystein is considered as a separate amino acid. Selenium deficiency lead to muscular degeneration which is seen in combined selenium vitamin E deficiency. The daily dietary intake of selenium depend on many factors, including: the amount of selenium in the soil of a given country, type of food consume, place a residence. Pregnant women consume selenium in the form of selenocystein or Selenomethionine. Selenoprotein P is involved in the antioxidant defense of the body. Their level depends on the concentration of the selenium.

Maternal selenium concentrations and glutathione peroxidase activity fall during pregnancy. Worldwide differences exist in assessment of selenium requirements, adequacy, and intakes. Anti-oxidant activity of selenium via glutathione peroxidase are same as like vitamin C and E. It has been observed that babies on average have lower selenium concentrations compared to the, which is expected as selenium is transported via the placenta. Although speculative, and requiring larger placebo-controlled randomised trials, women with recurrent early pregnancy loss may benefit from selenium supplement.

Recently, our group and others have demonstrated, through retrospective studies, the association between low serum selenium concentrations and reduced antioxidant function of the associated antioxidant glutathione peroxidase enzymes in women with preeclampsia. It has been suggested that adequate selenium status is important for antioxidant defense and may be a potential factor in women at risk of preeclampsia; this hypothesis has been further justified by the reduced expression and activities of glutathione peroxidase found in maternal, fetal, and placental samples taken from 25 preeclampsia pregnancies, when compared to 27 normal controls in our recent cross-
The growing fetus requires selenium. Selenium is transported across the placenta by passive diffusion down a concentration gradient. It has been reported about the association of selenium with infertility, abortion, and retention of the placenta. New born from selenium-deficient mothers suffer from muscular weakness. The selenium requirements of a pregnant and lactation mother are increased. Because of selenium content in dietary intake are low, maternal selenium concentration fall during pregnancy, being the lowest at delivery compared with non-pregnant controls. In contrast, in areas of very high soil selenium content, it would appear that there is no gestational trend in serum selenium concentration. The first trimester of pregnancy ended with a miscarriage, selenium concentration was significantly lower compare with first trimester of healthy pregnant women. A person with low content of selenium in the body has lower concentration of this element in the plasma. In the women with high concentration of selenium in plasma changes in its concentration during pregnancy differ significantly in comparison to pregnant women having low concentration of selenium, which determine selenium concentration in human pregnancy at various gestation.

Preeclampsia affected about 5% of pregnancy and is more common among women who are expediency their first pregnancy. This condition is characteristic by hypertension, proteinuria and oedema, usually late in second trimester or in the third trimester. When untreated preeclampsia can be lead convulsions and is then called as eclampsia, which are related abnormal endothelial reactivity that leads to intravascular deposition fibrin sequence organ damage. In present study we had compared serum level of iron and selenium in pregnant women with non-pregnant women and its role in pregnancy and in foetus development. We had also make an effort to compare the finding of iron and selenium between various age groups and BMI groups of both pregnant and non-pregnant women.

2. Materials and Methods

The present study was carried out in the Biochemistry departments of Surat Municipal Institute of medical Education & Research (SMIMER), Surat. A total of 100 (50 first trimester pregnant and 50 non-pregnant women of reproductive age) female subject were taken up for this study. Subject were selected from outdoor patient departments. Estimation of trace element like serum iron and selenium was done by atomic absorption spectrophotometry (AAS) fully auto analyzer. We had excluded diabetic, thyroid or any other gynecological disorder. We have also measure anthropogenic parameter like height (m), weight (kg) and BMI in kg/m². Also measure blood pressure in mmHg (systolic and diastolic blood pressure). We had divided pregnant and non-pregnant women in different age wise like <25 years, 25-30 years and more or 30 years and different BMI group wise like <23 kg/m², 23-24.9 kg/ m², 25-29.9 kg/ m² and ≥30 kg/m².

Blood sample collected in fasting condition. Serum iron and selenium were measured by atomic absorption spectrophotometry. Data compiled by using Microsoft excel software and statistical analysis was done by statistical software SPSS (version 16). We had calculated p value by using student t test and p value <0.01 are considered to be highly statistical significant and p<0.05 are considered to be statistical significant.

3. Result and Discussion

In present study we had measured serum level of iron and selenium in pregnant and non-pregnant women, we had divided subjects by various age group and BMI wise. Following results were obtained from current study.

Table 1 shows that, <25 years of age group 22 were pregnant and 20 were non-pregnant, between 25 to 30 years, 23 were pregnant and 23 were non-pregnant and ≥30 year of age group 5 are pregnant and 7 are non-pregnant.

Table 2 shows that, <23 kg/m² of BMI group, 5 were pregnant and 28 were non-pregnant, between 23-24.99 kg/m² of BMI, 22 are pregnant and 8 were non-pregnant and 25-29.99 kg/m² of BMI group 23 were pregnant and 11 were non-pregnant and ≥30 kg/m² of BMI group 5 were pregnant and 3 were non-pregnant.

Table 3 there was a statistical significant (p<0.01) decreased in serum selenium and significant (p<0.05) decreased in iron level in pregnant women compared to non-pregnant.

Table 4 there was significant (p<0.01) decrease in serum selenium level in pregnant women compared to non-pregnant women in age group 25 to 30 years and ≥ 30 years but non-significant reduction seen in <25 years of age group. There was a significant (p<0.05) decrease in serum iron level in pregnant women compared to non-pregnant in age group of 25-30 years age group and non-significant decreased level in remaining age group.

Table 5 there was a significant (p<0.01 for <23 and 25-29.9 kg/m² groups and p<0.05 for 23-24.9 kg/m² group) decreased in serum selenium level in pregnant women compared to non-pregnant women in all BMI group except ≥30 kg/m². There was a decrease in serum iron level in all BMI group in pregnant women compared to non-pregnant but statistical not significant.

Table 6 mentioned that, there was significant (p<0.05) decreased in systolic blood pressure and non-significant decreased in diastolic blood pressure in pregnant women compared to non-pregnant. Average age and BMI of both group were almost similar.
Table 1: Age wise distribution of pregnant and non-pregnant women

| Age (Years) | Pregnant women | Non pregnant women |
|-------------|----------------|-------------------|
| <25         | 22             | 20                |
| 25-30       | 23             | 23                |
| ≥30         | 5              | 7                 |

Table 2: BMI wise distribution of pregnant and non-pregnant women

| BMI (kg/m²) | Pregnant women | Non pregnant women |
|-------------|----------------|-------------------|
| <23         | 5              | 28                |
| 23-24.99    | 22             | 8                 |
| 25-29.99    | 23             | 11                |
| ≥30         | 5              | 3                 |

Table 3: Comparison of serum selenium and iron level in pregnant women with non-pregnant women

| Parameter         | Pregnant women mean ± SD (n= 50) | Non-pregnant women mean ± SD (n= 50) | P value |
|-------------------|-----------------------------------|--------------------------------------|---------|
| Selenium (µg/dl)  | 101.99 ± 39                       | 124.18 ± 45.72                      | <0.01** |
| Iron (µg/dl)      | 77.68 ± 43.7                      | 104.33 ± 41                         | <0.05*  |

Table 4: Comparison of serum selenium and iron level in pregnant and non-pregnant women in age group of < 25 years, 25 – 29 years and ≥ 30 years

| Parameter         | <25 years | 25-30 years | ≥30 years | Non-pregnant women | P value |
|-------------------|-----------|-------------|-----------|--------------------|---------|
| Selenium (µg/dl)  | 107.66 ± 52.86 | 117.89 ± 47.2 | 101 ± 24.31 | 128.83 ± 50.72 | <0.01** |
| Iron (µg/dl)      | 83.62 ± 52.50 | 105.04 ± 52.34 | 66.7 ± 21.25 | 107.72 ± 34.66 | <0.01** |

Table 5: Comparison of serum selenium and iron and level in pregnant and non-pregnant women in BMI (body mass index) of < 23 kg/m², 23-24.9 kg/m², 25 – 29.9 kg/m² and ≥ 30 kg/m²

| BMI Parameter | <23 kg/m² | 23-24.9 kg/m² | 25-29.9 kg/m² | ≥30 kg/m² | P value |
|---------------|-----------|---------------|---------------|-----------|---------|
| Selenium (µg/dl) | 98.80 ± 43.06 | 101.94 ± 135.19 | 94.09 ± 114.31 | 138.58 ± 157.42 | <0.01** |
| Iron (µg/dl)   | 86.70 ± 56.82 | 70.89 ± 129.62 | 67.59 ± 89.3 | 68.29 ± 100.11 | <0.01** |

Table 6: Comparison of anthropological parameter between pregnant and non-pregnant women

| Parameter          | Pregnant women mean ± SD (n= 50) | Non-pregnant women mean ± SD (n= 50) | P value |
|--------------------|-----------------------------------|--------------------------------------|---------|
| Age (years)        | 25 ± 3.6                          | 26 ± 3.6                             | >0.99   |
| BMI (kg/m²)        | 23.52 ± 4.6                       | 22.6 ± 3.98                          | 0.314   |
| Systolic blood pressure (mmhg) | 114 ± 9.3                   | 121 ± 7                             | <0.05*  |
| Diastolic blood pressure (mmhg) | 75.8 ± 5.9                   | 82 ± 4.7                            | 0.114   |
4. Discussion

Trace elements have important influence on the health of pregnant women and the growing fetus.28 Pregnancy is associated with increased demand of all the nutrient like iron, copper, zinc, selenium and vitamins like folic acid and ascorbic acid29 and deficiency of any of these could affect pregnancy, delivery and outcome of pregnancy. Iron deficiency result in anemia, which may increase the risk of death from hemorrhage during delivery however, its effects on fetal development and birth outcome still needs further elucidation.

It is a common experience that anemia of pregnancy is sometime not corrected despite iron supplementation, which may be due to underling deficiency of other micronutrients, which affected pregnancy, childbirth or fetal development. Tissue iron stores depletion can lead to cellular hypoxia, which could lead to the abnormal proliferation of blood vessels in order to compensate for the oxygen deficiency in the brain. In experimental studies, iron deficiency result in increased angiogenesis in the brain of rats, which have a negative impact on further development of the fetus.30 The iron level in non- pregnant women 106.9 ± 20.5 μg/dl and pregnant women 72.7 ±7.3 μg/dl was found in study conducted by Chitra Upadhyay et al 2004 (31). There was a significant decreased irons level in pregnant women. In our study, serum iron level was pregnant women 77.67 ± 43.71 μg/dl and non- pregnant women was 104.3 ± 41.02 μg/dl, which shows that in our study, there was a significant decreased iron level in pregnant women and compared to non- pregnant women, which were similar to above study. It is suggested that the maternal selenium concentration has an influence on neonatal anthropometric measurement showed that triceps skin fold ratio was negatively associated with first trimester selenium intake. There was a no any statistically significant difference in cord blood selenium level of women who received daily selenium supplementation and control group as per study conducted by Chitra upadhya et al 2004.31

Study conducted by Chitra Upadhyay et al 2004,31 had shown that an increase d copper to zinc ratio and decreased selenium serum level may be marker for preeclampsia risk pregnant women show that, supplementation of minerals with antioxidant function, had a positive effect on the reduction of preeclampsia incidence during the initial stage of pregnancy in women not diagnosed with diabetes type 1.

The concentration of selenium in the blood of women during pregnancy varies from country to country. According to study done by Suliburska J et al,32 as pregnancy progresses, the level of the selenium progressively decreased, while others have not observed any differences between the beginning and the end of pregnancy. The results may depend on the content of selenium in the body of pregnant women.

Study conducted by Jariwala M. et a33 believe that the selenium requirement of pregnant women were increased as a result of selenium transport to the fetus. The fetus accumulates selenium especially at the end of pregnancy by storing it in the liver. In women with low selenium content in the body, its concentration in plasma decreases with the progress of pregnancy.

A reduction in maternal selenium concentration during pregnancy can lead to various disorders such as miscarriage, premature birth, preeclampsia, pregnancy induced hypertension, low birth weight, retinopathy of prematurity and some others,29 which showed that in the first trimester in women whose pregnancy ended with a miscarriage, selenium serum concentration was significantly lower compared with the first trimester of healthy pregnant women (54.7 μg/L vs. 65.3 μg/L, respectively; p<0.01).

In study of Iwona Lewicka et al 2017,34 there was a selenium level in pregnant women 70 ± 15μg/dl, which was significantly lower than non- pregnant women. In our study selenium level in non-pregnant women 124.18 ± 45.72μg/dl, shown in our study there was decreased d level of selenium in first trimester of pregnant women compared to non- pregnant women, which show finding of our study correlate with finding of this study.

5. Conclusion

There was a significant lower level of selenium found in pregnant women compared to non-pregnant women. There was a significant lower level of iron found in pregnant women compared to non-pregnant women. There was a decrease d in serum selenium and iron level, which indicate that during pregnancy requirement of trace elements increase to meet demand for fetus growth. A reduction in maternal selenium concentration during pregnancy can lead to various disorders such as miscarriage, premature birth, preeclampsia, pregnancy include hypertension, low birth weight, retinopathy of prematurity etc. So we need to focused more on serum selenium and iron level apart from other routine investigation, which is required for screening before pregnancy and during pregnancy for better management of maternal and fetal health.

6. Source of funding

None.

7. Conflict of interest

None.

References

1. Bowman BA, Russell RM, Allen LH. Pregnancy and lactation. Present knowledge in nutrition; 2006-. p. 529–543. 9th ed.
2. Brien O, K. Pregnancy and iron homeostasis: an update. Nutr Rev. 2013;71(1):35–51.
3. Otten JJ, Hellwig JP, Meyers LD. Dietary Reference Intakes: the essential guide to nutrient requirements In: (2006). The National
Academies Press.

King JC. Physiology of pregnancy and nutrient metabolism. *Am J Clin Nutr.* 2000;71(12):1218–1225. Suppl.

Olaussson H, Goldberg GR, Laskey MA. Calcium economy in human pregnancy and lactation. *Nutr Res Rev.* 2012;25:40–67.

Lenton EA, Weston GA, Cooke ID. Problems in using basal body temperature recordings in an infertility clinic. *Br Med J.* 1977;1:803–805.

Hallberg L, Hogdahl A, Nilsson L, Rybo G. Menstrual blood loss—a population study. *Acta Obstet Gynecol Scand.* 1966;45:320–351.

Bonnar J, Goldberg A, Smith JA. Do pregnant women take their iron? *Lancet.* 1969;1:457–458.

Milman N, Ibsen KK, Christensen JM. Serum ferritin and iron status in mothers and newborn infants. *Acta Obstet Gynecol Scand.* 1987;66:205–211.

Pierson RN, Holt PR, Watson RM. Aspirin and gastrointestinal bleeding: chromate blood loss studies. *Am J Med.* 1961;31:259–265.

Taylor DJ, Mallen C, McDougall N. Effect of iron supplementation on serum ferritin levels during and after pregnancy. *Br J Obstet Gynaecol.* 1982;89:1011–1017.

LSRO (Life Sciences Research Office). “Assessment of the Iron Nutritional Status of the U.S. Population Based on Data Collected in the Second National Health and Nutrition Examination Survey, 1976–1980”. Federation of American Societies for Experimental Biology, Bethesda, Md ; 1984., p. 120.

Milman N, Ibsen KK, Christensen JM. Serum ferritin and iron status in mothers and newborn infants. *Acta Obstet Gynecol Scand.* 1987;66:205–211.

Baltussen R, Knai C, Sharan M. Iron fortification and iron supplementation are cost effective interventions to reduce iron deficiency in four sub regions of the world. *J Nutr.* 2004;134:2678–2684.

Horton S, Ross J. The economics of iron deficiency. *Food Policy.* 2003;28:51–75.

Bothwell TH, Macphail AP. The potential role of NaFe, EDTA as an iron fortificant. *Int J Vitam Nutr Res.* 2004;74:421–434.

Wegmuller R, Camara F, Zimmermann MB. Salt dual fortified with iodine and micronized ground ferric pyrophosphate affects iron status but not hemoglobin in children in Cote d'Ivoire. *J Nutr.* 2006;136:1814–1820.

National Nutritional Anemia Control Programme in India. *Indian J Public Health.* 1999;43:3–5.

Robberecht H, Deelstra H. Factors influencing blood selenium concentration values a literature review. *J Trace Elem Electrolyte Health Dis.* 1994;8:129–143.

Nandakumar M, Dashi HM, Al-Saleh E. Transport kinetics of zinc, copper, selenium, and iron in perfused human placental lobule in vitro. *Mol Cell Biochem.* 2003;252:91–96.

Zachara BA, Wardak C, Didkowski W. Changes in blood selenium and glutathione concentrations and glutathione peroxidase activity in human pregnancy. *Gynecol Obstet Invest.* 1993;35:12–17.

Navarro M, Lopez H, Perez V. Serum selenium levels during normal pregnancy in healthy Spanish women. *Sci Total Environ.* 1996;186:237–242.

Benemariya H, Robberecht H, Deelstra H. Daily dietary intake of copper, zinc and selenium by different population groups in Burundi. *Africa Sci Total Environ.* 1993;136:49–76.

Zachara BA, Pawluk H, Korenkiewicz J, J. Selenium levels in kidney, liver and heart of newborns and infants. *Early Hum Dev.* 2001;63:103–111.

Schroeder HA, Frost DV, Balassa JJ. Essential trace metals in man: selenium. *J Chronic Dis.* 1970;23:227–243.

Neve J. Selenium and pregnancy. *Rev Fr Gynecol Obstet.* 1990;85:29–33.

Oldfield JE. Selenium – World Atlas. STDA. 1999.

Marseglia L, Angelo GD, Manti S. Oxidative stress-mediated aging during the fetal and perinatal periods. *Oxid Med Cell Longev.* 2014;p. 358–375.

Wibowo N, Purwosunu Y, Sekizawa Y. Antioxidant supplementation in pregnant women with low antioxidant status. *J Obstet Gynaecol Res.* 2012;38(9):1152–1161.

Thomas WB, T SS, Thu AN. Fetal and neonatal iron deficiency but coper deficiency increases vascular complexity in developing rat brai. *Nutr Neurosci.* 2015;18(8):365–375.

Chitra U, Sandhya M, Peeyush A. Serum iron, coper and zinc status in maternal and cord blood. *HCB.* 2004;p. 48–52.

Suliburska J, Kocyowski R, Komorowicz I. Concentrations of mineral in amniotic fluid and their relations to selected maternal and fetal parameters. *Biol Trace Elem Res.* 2016;172(1):37–45.

Jariwala M, Suvarna S, Kumar GK. Study of the concentration of trace elements fe, zn, cu, se and their correlation in maternal serum, cord serum and colostrums. *Indian J Clin Biochem.* 2014;29(2):181–188.

Iwona L, Rafal K, Mariusz G. Selected trace elements concentrations in serum ferritin levels during and after pregnancy. *Br J Obstet Gynaecol.* 2000;107:1961–1965.

Horton S, Ross J. The economics of iron deficiency. *Food Policy.* 2003;28:51–75.

Bothwell TH, Macphail AP. The potential role of NaFe, EDTA as an iron fortificant. *Int J Vitam Nutr Res.* 2004;74:421–434.

Wegmuller R, Camara F, Zimmermann MB. Salt dual fortified with iodine and micronized ground ferric pyrophosphate affects iron status but not hemoglobin in children in Cote d'Ivoire. *J Nutr.* 2006;136:1814–1820.

National Nutritional Anemia Control Programme in India. *Indian J Public Health.* 1999;43:3–5.

Robberecht H, Deelstra H. Factors influencing blood selenium concentration values a literature review. *J Trace Elem Electrolyte Health Dis.* 1994;8:129–143.

Nandakumar M, Dashi HM, Al-Saleh E. Transport kinetics of zinc, copper, selenium, and iron in perfused human placental lobule in vitro. *Mol Cell Biochem.* 2003;252:91–96.

Zachara BA, Wardak C, Didkowski W. Changes in blood selenium and glutathione concentrations and glutathione peroxidase activity in human pregnancy. *Gynecol Obstet Invest.* 1993;35:12–17.

Navarro M, Lopez H, Perez V. Serum selenium levels during normal pregnancy in healthy Spanish women. *Sci Total Environ.* 1996;186:237–242.