A Comparative Study on Serum Levels of Iron, Magnesium, Calcium and Phosphorus during First & Second Trimesters of Pregnancy at a Tertiary Referral Hospital in South India

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ABSTRACT

BACKGROUND

Multiple micronutrient deficiencies commonly coexist in pregnancy and may play a role in the pathogenesis of many high-risk events in pregnancy. Mineral estimation during the first and second trimester may help in the identification of high-risk pregnancies predisposed to pre-eclampsia, preterm birth or low birth weight. Body mass index (BMI) is an independent risk factor influencing the serum levels of micronutrients like iron, calcium, phosphorous and magnesium. The purpose of this study was to compare the serum levels of iron, magnesium, calcium and phosphorus during first & second trimesters of pregnancy and their correlation with body mass index and adverse pregnancy outcomes.

METHODS

This was a longitudinal descriptive study, done over a period of twelve months, from April 2017 to March 2018. 94 low risk pregnant women were selected in the first trimester, and they were followed up for the entire duration of pregnancy. 2 ml of blood is collected at first and second trimester and analysed for the serum levels of iron (µg/dl), magnesium (mg/dl), calcium (mg/dl) and inorganic phosphorus (mg/dl). Mean values of these elements were noted and BMI was measured. Patients were followed up to observe the pregnancy outcome, especially development of hypertension, preterm birth and low birth weight. The data were subjected to suitable statistical analyses. P value less than 0.05 is considered as statistically significant.

RESULTS

Mean values of Ca, Mg, inorganic P and Fe were 9.3 mg/dl ± 0.52, 1.79 mg/dl ± 0.28, 3.59 mg/dl ± 0.54 and 83.7 µg/dl ± 25.36 respectively in first trimester and 8.9 mg/dl ± 0.47, 1.62 mg/dl ± 0.16, 3.17 mg/dl ± 0.44 and 76.17 µg/dl ± 21. 69 respectively in the second trimester. Hypocalcaemia was noted in 12.7 % and 38.3 % in the first and second trimester respectively. Hypomagnesaemia was seen in 51.1 % and 80.9 % in the first and second trimester respectively. Hypophosphatemia was seen in 3.2 % and 14.8 % in the first and second trimester respectively. These findings were statistically significant. There was no significant change in the iron levels in the present study. 19.1 % were underweight. 56.4 % had normal BMI. 19.2 % were overweight. 5.3 % were obese.

CONCLUSIONS

There is no correlation between serum values of trace elements and the BMI of study subjects. Even though significant hypocalcaemia, hypophosphatemia and hypomagnesaemia was noted in second trimester compared to first, there was no predisposition to the development of adverse outcomes like pre-eclampsia, preterm birth or low birth weight. This may be the effect of dietary and pharmacological supplementation during pregnancy.

KEYWORDS

Micronutrients, Pregnancy Complications, Calcium, Phosphorus, Magnesium, Iron

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BACKGROUND

Pregnancy is a physiological process associated with many complex and inter-related biochemical, physiological and anatomical alterations occurring in the body.[1] Pregnancy lasts for about nine months, and is conventionally divided into three trimesters. First trimester is a time of basic cell differentiation when the organs develop and hence it is a particularly vulnerable period. Deficiencies or excess of micronutrients in this period can have far reaching consequences to the newborn[2] and hence is a subject of intense research. Second trimester is a period of rapid growth and maturation of the body system of fetus. Third trimester marks the final stage of fetal growth and the development of fetal fat store. Imbalances in maternal nutrition and fetal demands in pregnancy can produce catastrophic complications to mother as well as newborn.[3,4] Serum levels of trace elements like iron, calcium, phosphorous and magnesium in pregnancy plays an important role in fetal homeostasis, fetal growth and development and is thought to have a role in the pathogenesis of high risk pregnancy events like gestational hypertension, fetal growth restriction, anaemia, postpartum haemorrhage, preterm birth, low birthweight, still birth, congenital malformations, reduced immune competence and abnormal organ developments.[5,6] Body mass index of the mother is another important factor influencing the growth and development of the unborn baby.[7] Serum levels of these elements are thought to be influenced by maternal nutrition, especially her BMI and supplementation during pregnancy. BMI less than 19.9 is underweight, between 20 - 24.9 is normal, between 25 - 29.9 is overweight and > 30 is obese. Both high and low BMI are associated with alterations in trace element status and adverse pregnancy outcomes.

Calcium, phosphorus and magnesium are critical to human physiology and are also needed for skeletal mineralisation.[8] Skeleton is the major reservoir of these elements. Lower levels of these elements in pregnancy can predispose to bone fractures.[9] The level of calcium, phosphorus, and magnesium tends to decrease as the pregnancy progress. This is due to the increasing demand of these minerals for the developing fetus, increasing haemodilution of pregnancy, hypoalbuminemia, and some of the inhibitory effects of hormones on homeostatic mechanisms.[10] Magnesium deficiency is associated with preterm delivery and low birth weight.[11] A small percentage of magnesium in serum is shared to the fetus. Therefore, the level of magnesium in pregnancy needs to be maintained as it could lead to metabolic disorders as well as bone demineralization of the fetus.

Iron is an essential nutrient at every stage of life. It is a critical component of proteins such as enzymes and haemoglobin. During pregnancy, women need more iron to support the increased maternal red blood cell mass. This supplies the growing fetus and placenta, and supports normal brain development in the fetus. In the third trimester of pregnancy, the fetus builds iron stores for the first six months of life.[12] Iron deficiency results in reduced oxygen carrying capacity, affects immunity, growth and development.

Calcium and iron levels are lower in patients with fetal growth restriction, miscarriage and preterm delivery. Variations in calcium, magnesium and phosphorus are also associated with bone-diseases.

Most Indian studies regarding nutrient deficiencies in pregnancy are on deficiencies of folate and Vitamin D. There is paucity of literature on serum levels of trace elements during pregnancy in our setting, thereby necessitating this study. Association between trace minerals and pregnancy, studied previously, are based on third trimester changes, when the variations are more marked. Aetio-pathogenesis of several conditions like pre-eclampsia and low birth weight points to insults in first or even second trimester. So, in this longitudinal study, we have examined the serum levels of certain trace elements in the first and second trimesters and tried to assess its relation with maternal BMI and adverse outcomes in pregnancy.

Aim

To compare the serum levels of iron, magnesium, calcium and phosphorus during first & second trimesters of pregnancy and its correlation with body mass index and adverse pregnancy outcomes.

Objectives

1. To assess the correlation between serum levels of iron, calcium, phosphorous and magnesium levels in first and second trimesters and body mass index.
2. To assess the correlation between serum levels of iron, calcium, phosphorous and magnesium levels in first and second trimesters and adverse pregnancy outcomes.

METHODS

This was a longitudinal descriptive study, and study duration was 12 months from April 2017 to March 2018, after institution human ethics committee approval. HEC number 06 / 13 / 2017 / MCT. Pregnant women with pre-existing cardiovascular disease, multiple pregnancy, liver disease, diabetes mellitus on insulin therapy, thyroid dysfunction and renal disease were excluded from the study. Pregnant women with already known risk factors for pre-eclampsia, preterm birth and fetal growth restriction were also excluded from the study.

Sample size was calculated by the formula:

$$N = \frac{(Z_{1-\alpha/2} + Z_{1-\beta})^2}{\Delta 2} + \frac{z^2_{1-\alpha/2}}{2} \quad N = 94$$

The selected population for this study was 94 low risk pregnant women attending the antenatal out-patient (OP) of SAT Hospital, which is a tertiary referral maternity wing of Govt. Medical College, Thiruvananthapuram. They were selected in the first trimester of pregnancy, based on willingness and after obtaining a written informed consent.

Once recruited, the BMI was calculated based on pre-pregnancy weight and they were followed up for the entire duration of pregnancy. 2 ml of blood is collected from the pregnant women during the first and second trimester and analysed for the serum levels of iron (µg/dl), magnesium (mg/dl), calcium (mg/dl) and inorganic phosphorus (mg/dl) at the clinical biochemistry laboratory, SATH. They were followed up to observe the pregnancy outcome, especially development of hypertension, preterm birth and low birth weight. Collected blood was poured in to metal free plain tubes and was allowed to clot at room temperature. Plain tubes were centrifuged for 10 min at 3500 rpm and the serum was separated, analyzed for calcium, phosphorus, magnesium within 30 min. The rest over serum sample is kept in trace elements free vials for awaiting analysis such as iron in freezer for 7 days only. Calcium, inorganic phosphorus and magnesium were analyzed using fully automatic analyser OLYMPUS AU 400. Iron was analyzed by MISPA VIVA Semi automated analyser. The demographic details and antenatal history of the pregnant women were obtained through a questionnaire. It also included details of drug intake, anthropometric measurements and laboratory investigations.

Statistical Analysis

The data that was elicited from different schedules were fed to statistical package for social sciences (SPSS) version 17.0. All data were subjected to suitable statistical analyses. Mean, standard deviation and percentages were calculated wherever necessary and tables prepared. Probability and t test were used to analyse the results. P value less than 0.05 is considered as statistically significant.

RESULTS

Demographic and Obstetric Profile of Patients

In the present study, the mean age of subjects was 25.47 ± 4.56 and the age range was 18 - 40. 37.2 % pregnant women belonged to above poverty level and 62.8 % were in the below poverty level. College level education was present in 47.5 % pregnant women whereas 35.1 % were educated up to high school and 19.1 % had primary school education. 88.3 % of the subjects were house makers and 100 % of them were non-vegetarians. 42.6 % of study subjects were primigravida and 57.4 % were multigravida. 80 % of the multigravida did not have any complications in previous delivery and all of them had term delivery in previous pregnancy. All the study subjects were compliant to universal iron and calcium prophylaxis in pregnancy.

Profile of Serum Calcium, Magnesium, Phosphorous and Iron in First and Second Trimester

Mean values of Ca, Mg, inorganic P and Fe were 9.3 mg/dl ± 0.52, 1.79 mg/dl ± 0.28, 3.59 mg/dl ± 0.54 and 83.7 µg/dl ± 25.36 respectively in first trimester and 8.9 mg/dl ± 0.47, 1.62 mg/dl ± 0.16, 3.17 mg/dl ± 0.44 and 76.17 µg/dl ± 21.69 respectively in the second trimester. The findings are summarized in the table 1

Trace Mineral	Reference Values	First Trimester Mean ± SD	Second Trimester Mean ± SD	t	P
Ca (mg/dl)	8.8 - 10.2	9.3 ± 0.52	8.9 ± 0.47	12.122	< 0.001
Mg (mg/dl)	1.8 - 3	1.79 ± 0.28	1.62 ± 0.16	8.569	< 0.001
P (mg/dl)	2.7 - 4.5	3.54 ± 0.54	3.17 ± 0.44	8.711	< 0.01
Fe (µg/dl)	60 - 150	83.7 ± 25.36	76.17 ± 21.69	25.0	0.334

Table 1. Profile of Serum Calcium, Magnesium, Phosphorous and Iron in First and Second Trimester

Serum Calcium Level in First and Second Trimester

86.2 % of the pregnant women in the first semester reported normal calcium level. In the second trimester, only 61.7 % had serum calcium in the normal range. Hypocalcaemia was noted in 12.8 % patients in first trimester and 38.3 % in second trimester. That is, three times higher the number of pregnant women reported low calcium levels in second trimester compared to first trimester. There is significant variation of calcium level between first (9.3 ± 0.52) and second trimester (8.9 ± 0.47). t value is 12.122 and P value shows < 0.001. There is significant P value in the calcium level. (Refer figure 1). In the first trimester median calcium is 9.35 (25th percentile 8.98 & 75th percentile 9.70) and in the second trimester 8.90 (25th percentile 8.60 & 75th percentile 9.20). There is significant P value (< 0.001).

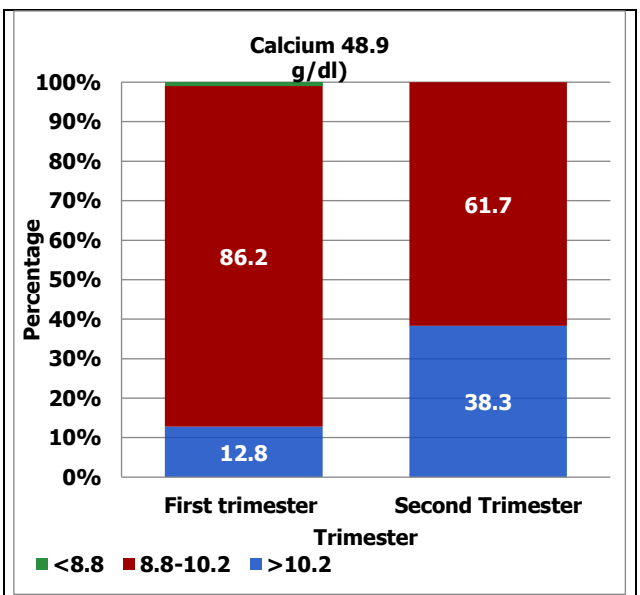

Figure 1. Serum Calcium Level in First and Second Trimester

Serum Inorganic Phosphorus Levels in First and Second Trimester

Normal inorganic phosphorus level is 2.7 - 4.5 g/dl. 92.5 % of study population had normal serum inorganic phosphorous levels in first trimester, whereas in second trimester, only 85.2 % only had serum inorganic phosphorous in the normal range. Those in hypophosphatemia range rose from 3.2 % in first trimester

to 14.8 % in second trimester. 4 patients (4.25 %) had hyperphosphatemia (> 4.5 g/dl) in first trimester. (Refer figure 2).

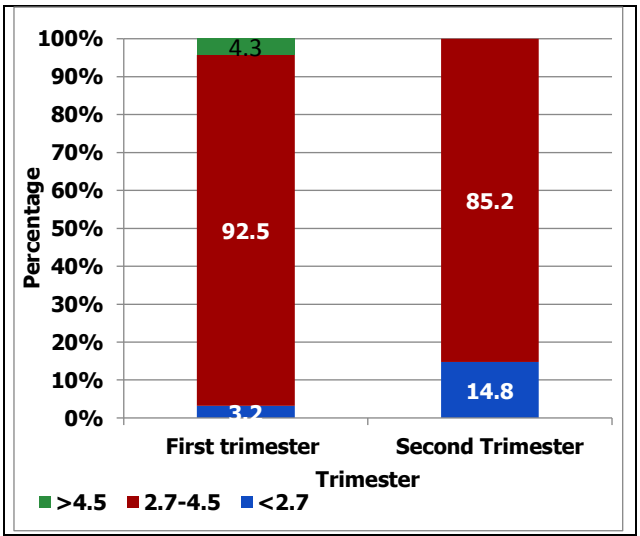

Figure 2. Serum Inorganic Phosphorous Level in First and Second Trimester

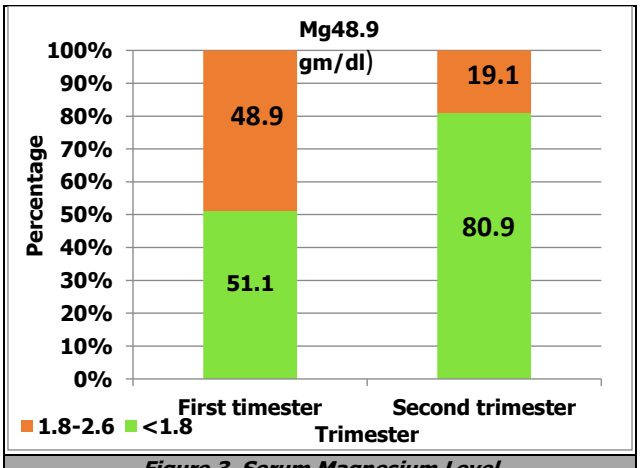

Figure 3. Serum Magnesium Level between First and Second Trimester

During first trimester the mean value is 3.54 ± 0.54 SD and in second trimester the mean value is 3.17 ± 0.44 SD. The median value in the first trimester is 3.55 (25th percentile 3.20 & 75th percentile 3.90) and in the second trimester is 3.20 (25th percentile 2.88 & 75th percentile 3.60).

Serum Magnesium Level in First and Second Trimester

48 pregnant women in first trimester showed low magnesium level with 46 numbers in normal magnesium level. The ratio is almost 1 : 1 in first trimester. In second trimester magnesium levels showed a drastic change from first trimester were the number of low magnesium value increased to 76 with a percentage of 80.9 and in normal cases the number decreased to 18 from 46 pregnant women. (Refer figure 3)

The mean value of Mg is 1.79 ± 0.28 in the first trimester and 1.62 ± 0.16 in the second trimester. T value is 8.569, there is significant P value < 0.001. During first trimester, the median value is 1.78 (25th centile 1.60 & 75th centile 2.00) and in the second trimester 1.60 (25th centile 1.50 & 75th centile 1.74). There is significant P value in the serum magnesium level between first and second trimester < 0.001.

Serum Iron in First and Second Trimester

In the current study, the serum iron levels both in first and second trimester pregnant women, the values were parallel without any change. In first trimester, 94 cases have mean value of 83.7 ± 25.36 and in the second trimester, 94 cases have mean value of 76.17 ± 21.69. Our finding is in contrast to the Iranian study by Tabrizi FM et al.[15] who found a significant lowering of iron levels in second trimester in their study. Comparison of serum iron level between first and second trimester with different age group showed a small decrease in mean values (first trimester 86.9 ± 24.8 and in second trimester 78.9 ± 21.8). The P value was 0.344 and 0.333 in the first and second trimester respectively. The P value was 0.354 in the level of iron in comparison with BMI which is not significant.

BMI and Serum Calcium, Magnesium, Phosphorous and Iron in First and Second Trimester

In the present study, 19.1 % (BMI 16.88 ± 1.07) were underweight. 56.4 % (BMI 21.82 ± 1.74) had normal BMI. 19.2 % (BMI 28.68 ± 3.22) were overweight. 5.3 % (BMI 23.8 ± 1.5) were obese. There is no increased predisposition to abnormal values of trace elements in this group. There is no correlation between serum values of trace elements and the BMI of study subjects (Refer table 2)

Pregnancy Outcome

95.7 % of the study subjects delivered at term, that is, after 37 weeks. There were no cases of gestational hypertension or pre-eclampsia. Only 4 preterm births were noted. The mean birth weight was 3.012 kg.

BMI	%	Calcium		Inorganic Phosphorous		Magnesium		Iron	
		1st TM	2nd TM	1st TM	2nd TM	1st TM	2nd TM	1st TM	2nd TM
Underweight	19.1	9.2 ± 0.41	9.23 ± 0.38	3.37 ± 0.63	3.41 ± 0.65	1.69 ± 0.33	1.75 ± 0.31	84.3 ± 21.9	84.1 ± 23.6
Normal	56.4	9.3 ± 0.57	9.3 ± 0.58	3.52 ± 0.5	3.46 ± 0.51	1.79 ± 0.27	1.74 ± 0.29	85.5 ± 27.3	86.9 ± 26.1
Overweight	19.2	9.41 ± 0.53	9.35 ± 0.49	3.67 ± 0.59	3.66 ± 0.54	1.90 ± 0.19	1.92 ± 0.18	74.9 ± 22.0	75.9 ± 24.6
Obese	5.3	9.28 ± 0.34	9.26 ± 0.54	3.82 ± 0.22	3.91 ± 0.31	1.77 ± 0.36	1.77 ± 0.31	94.0 ± 26.0	88.6 ± 24.7
P value		0.694	0.914	0.249	0.097	0.131	0.079	0.354	0.299

Table 2: Profile of BMI and Serum Calcium, Magnesium, Phosphorous and Iron in First and Second Trimester

DISCUSSION

Maternal serum calcium, magnesium, phosphorous and iron levels are found to vary in different trimesters.[13] In the present study, 12.7 % (8.37 ± 0.23) in the first trimester and 38.3 % (8.43 ± 0.25) in the second trimester were hypocalcaemic. 86.2 % (9.24 ± 0.38) in the first trimester and 61.4 % (9.19 ± 0.3) in the second trimester had normal calcium level. The mean value in the whisker 's plot shows significant P value in the calcium level between the first (9.30 ± 0.52) and second (8.90 ± 0.47) trimester. (P < 0.001; t = 12.122). There is significant variation of calcium level between first (9.3 ± 0.52) and second trimester (8.9 ± 0.47). t - value is 12.122 and P value shows < 0.001. There is significant P value in the calcium level. Sultana MS et al.[14] in their study noticed similar changes. Our finding is in contrast to the studies by Reitz et al.[13] Tabrizi FM,[15] who reported no significant change in the serum calcium level during the different trimesters. There is no correlation between the serum calcium levels and BMI score. The P value is 0.694. Also, no association could be demonstrated with low calcium levels in first or second trimester and development of pre-eclampsia, preterm birth or low birth weight.

In the current study 3.19 % (2.2 ± 0.11) in the first trimester and 14.8 % (2.44 ± 0.18) in the second trimester were having hypophosphatemia. Normal cases were 92.5 % (3.53 ± 0.44) in the first trimester and 85.1 % (3.29 ± 0.33) in the second trimester. The mean value in the whisker 's plot shows 3.54 ± 0.54 and 3.17 ± 0.44 during first and second trimester. There is slight statistical significance in the P value of phosphorous (p = 0.01; t = 8.711). Our finding is similar to studies by Bezzera et al.[10] Sultana MS et al.[14] in their study did not notice any change in phosphate levels between first and second trimesters. Serum inorganic Phosphorous levels with BMI score shows no significant change in P value.

In the present study, 51.1 % (1.57 ± 0.15) in the first trimester and 80.8 % (1.56 ± 0.12) in the second trimester were hypomagnesaemic. 48.9 % (2.0 ± 0.17) in the first trimester and 19.15 % (1.86 ± 0.06) in the second trimester reported normal magnesium level. The mean value of magnesium in the whisker 's plot shows 1.79 ± 0.28 in the first trimester and 1.62 ± 0.16 in the second trimester. P value shows significance (< 0.001) and t = 8.569. This disagrees with the studies of Tabrizi FM.[15] Their study did not observe any significant change in serum magnesium level during three trimesters. Comparison of serum magnesium levels with BMI levels are not significant.

In the current study hypocalcaemic (< 8.8) cases have normal inorganic phosphorous level in the first and second trimesters (3.38 ± 0.33 and 3.11 ± 0.38). Hypocalcaemic cases also significantly showed hypomagnesemia in the first trimester and second trimester (1.65 ± 0.23 and 1.58 ± 0.15).

In the current study, the serum iron levels, both during first and second trimester pregnant women, the values were parallel without any change. Our finding is in contrast to the Iranian study by Tabrizi FM et al.[15] who found a significant lowering of iron levels in second trimester in their study. There was no correlation between iron levels and maternal BMI. This finding is in contrast to the study by Rebecca L. Wilson et al.[16] who observed a low level of serum iron in obese pregnancies. This finding highlights the impact of correctly practiced universal iron supplementation programme during pregnancy in Kerala. It appears that food fortification and adequate weight gain during pregnancy mitigates the effect of abnormal prenatal weight and abnormal levels of trace elements in first and second trimester. The present study did not show any predisposition to the development of adverse outcomes like pre-eclampsia, preterm birth or low birth weight.

CONCLUSIONS

Multiple micronutrient deficiencies commonly coexist in pregnant women, thus mineral evaluation is necessary during pregnancy. Mineral estimation during the first and second trimester may help to identify the underlying pathogenesis of pregnancy problems.

There is no significant change in the iron levels in the present study. Even though significant hypocalcaemia, hypophosphatemia and hypomagnesaemia was noted in second trimester compared to first, there was no predisposition to the development of adverse outcomes like pre-eclampsia, preterm birth or low birth weight. This maybe the effect of dietary and pharmacological supplementation during pregnancy.

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