Original Article

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Biochemistry Section

The Micronutrient Levels in the Third Trimester of Pregnancy and Assessment of the Neonatal Outcome: A Pilot Study

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ABSTRACT

Context: During pregnancy, an adequate intake of vitamins and minerals is recommended, to prevent the occurrence of adverse effects in the mother and the foetus.

Aim: In our study, we aimed to study the levels of the micronutrients like iron, zinc and copper in the third trimester of pregnancy and to assess the neonatal outcome in them.

Material and Methods: Fifty pregnant women who were aged 20-30 years, who had completed 24 weeks of gestation, who were on regular antenatal checkups, were included. The collected venous blood samples were used for the estimation of haemoglobin, serum ferritin, zinc and copper. They were followed up till their deliveries and the neonatal outcomes were noted. The gestational ages, weight of the babies, the lengths of the babies and their head circumferences and any complications which had occurred during and after the deliveries, were noted in the proforma.

Results: The serum ferritin levels did not significantly correlate with the other study parameters. The zinc levels decreased with an increase in the parity ($p<0.05$). The copper levels decreased with an increase in the BMI ($p<0.05$). With an increase in haemoglobin, there was an increase in the levels of zinc and ferritin ($p<0.05$). With an increase in the parity, there was a decrease in the neonatal birth weight.

Conclusion: Our study clearly brought out a correlation between the microminerals such as iron, zinc and copper during late pregnancies. An improvement in the iron status brings out a positive effect on the ferritin and zinc levels, thus indicating better outcomes of the pregnancies.

Key words: Micronutrients, Pregnancy, Neonatal outcome, Zinc, Copper

INTRODUCTION

Pregnancy is a period of increased metabolic demands, with changes taking place in the womens' physiologies and in the growing foetuses [1]. Hence, during pregnancy, an adequate intake of vitamins and minerals is recommended, to prevent the occurrence of adverse effects in the mother and the foetus. Vitamins and minerals, which are referred to collectively as micronutrients, have an important influence on the health of pregnant women and on that of the growing foetuses. A micronutrient deficiency, whether it is clinical or sub–clinical, may have adverse effects on the mother, such as anaemia, hypertension, the complications of labour and even death [2]. The foetuses can be affected, resulting in stillbirths, pre-term deliveries, intrauterine growth retardation, congenital malformations, a reduced immunocompetence and abnormal organ development. Micronutrient deficiencies often co–exist. Though the importance of few micronutrient deficiencies such as that of iodine has already been recognized, the roles of others have only recently been appreciated. Iron deficiency results in anaemia, which may increase the risk of death from a haemorrhage which may occur during a delivery [3]. Also maternal anaemia is an independent risk factor for a preterm delivery and low birth weight. A zinc deficiency has also been associated with the complications of pregnancy and delivery [4]. However, only little direct evidence of a mild to moderate zinc deficiency is available. A copper deficiency is also associated with the complications of pregnancy, childbirth and foetal development [5]. The best casual evidence for the micronutrients and the adverse outcomes of pregnancy come from randomised controlled trials, and to date, these have largely been done for the individual vitamins or minerals. Any attempt to introduce a micronutrient supplementation programme during pregnancy must be based on adequate data on the prevalence of the micronutrient deficiencies, their adverse effects and the potential for reversing these through supplementations. However, in our study, we aimed to study the levels of the micronutrients like iron, zinc and copper in the third trimester of pregnancy and also to assess the neonatal outcome.

MATERIAL AND METHODS

The Type of Study

Prospective study.

The Study Population

This study was conducted in a tertiary referral maternity centre which conducted approximately 2000 deliveries per year. We focused on pregnant women who were in the third trimester of pregnancy. The institutional ethical committee's approval was taken before the commencement of the study and written consents were taken from the pregnant women before drawing the blood.

Inclusion Criteria

Pregnant women who were aged 20-30 years, who had completed 24 weeks of gestation, who were on regular antenatal checkups, were included.

Exclusion Criteria

The pregnant women with chronic diseases, gestational diabetes mellitus, pregnancy induced hypertension, multiple pregnancies or any pregnancy induced complications at the time of the sample collection, were excluded.

The Sample Size

The recruitment of the subjects into the study was for a period of 1 month. 50 subjects were included in the study.

The Study Design

About 3 ml of venous blood was collected from about 50 pregnant women by using aseptic precautions. At the same time, the data with regards to their ages, occupations, socioeconomic statuses, parities, weight gains during their present pregnancies, histories of diabetes and hypertension, previous histories of abortions and their dietary histories and haemoglobin levels, were noted in a preformed proforma.

The blood samples were centrifuged after 30 minutes and the separated sera were used for the estimation of serum ferritin, zinc and copper. Serum ferritin was assayed by a two–site sandwich immunoassay by using the direct chemiluminometric technology in an Siemens ADVIA Centaur CP immunoassay system. Zinc was estimated by a colourimetric method. The zinc which was present in the sample was chelated by the 5–Br–PAPS 2–(5–bromo–2–pyridylazo)–5–(N–propyl–N–sulfopropylamino)–phenol which was present in the reagent. The formation of this complex was measured at a wavelength of 560 nm. At a pH of 4.7, the copper which was bound to ceruloplasmin, was released by a reducing agent. It then reacted with a specific colour reagent, 3.5–Di–Br–PAESA 4–(3,5–Dibromo–2–pyridylazo)–N–Ethyl–N–(3–sulphopropyl) aniline, to form a stable, coloured chelate. The intensity of the colour was directly proportional to the amount of copper which was present in the sample. Both zinc and copper were calibrated and this was done on a fully automated RANDOX Daytona analyser. The quality control was performed as per specifications.

Iron deficiency anaemia was defined if the haemoglobin concentration was < 12.0 g/dl and if the serum ferritin levels were < 12μg/L. A zinc deficiency was defined if the serum zinc concentration was < 13.8 μmol/L. A copper deficiency was defined if the serum copper concentration was < 10μmol/L.

The 50 pregnant women who were included in the study were followed up till their deliveries and the neonatal outcomes were noted. The gestational ages, weights of the babies, the lengths of the babies and their head circumferences and any complications which had occurred during and after the deliveries, were noted in the proforma.

STATISTICAL ANALYSIS

In order to estimate the level of outcome of the parameters in different exposure categories, the mean and the standard deviation were worked out. To compare the quantitative parameters, the Student's 't' test was applied at a 5% level of significance. The Chi–square test was used to test the significance of the association of the qualitative outcome parameters with the exposure parameters. The data entry and all the statistical analyses were done by using the statistical softwares, Microsoft Excel and SPSS, version 11.0.

RESULTS

Fifty patients were included in our study and of these, forty eight patients were followed up till their deliveries. Two cases were lost during follow up, as they had delivered at other centres. The pregnant women were grouped into 3 groups, based on their serum ferritin levels i.e. those with serum ferritin levels of 0–12 mcg/dl as Group 1, those with levels of 12–20 mcg/dl as Group 2 and those with levels of 20–200 mcg/dl as Group 3. None of the above mentioned variables showed any statistical significant difference in the 3 groups [Table/Fig-1]. Though the levels of haemoglobin and serum zinc increased with the increase in the ferritin levels, this was not statistically significant.

The optimal level of serum zinc was 13.8-22.9 μmol/L. The pregnant women were grouped into 2 groups, based on their serum zinc levels i.e. those with serum zinc levels of < 13.8 μmol/L as Group 1 and those with levels of > 13.8 μmol/L as Group 2. Most of the primigravidas had serum zinc levels of > 13.8 μmol/L as compared to the multigravida whose levels were < 13.8 μmol/L. The mean gravida was 2.1 ± 1.2 and 1.5 ± 0.6 in the two groups respectively and the difference was statistically significant. The mean haemoglobin levels were 11.7 ± 1.0 gm/dl and 12.7 ± 0.7 gm/dl in the two groups and the difference was statistically significant. Though the serum levels of copper and ferritin had increased in Group 2 as compared to those in Group 1, this was not statistically significant [Table/Fig-2].

The normal serum copper levels ranged from 12.5-17.5 μmol/L. The pregnant women were grouped into 2 groups, based on their serum copper levels i.e. those with serum copper levels of < 10 μmol/L as Group 1 and those with levels of > 10 μmol/L as Group 2. The body mass index in Group 1 was 25.2 ± 3.3 and that in Group 2 was 21.2 ± 8.9 and this difference was statistically significant. None of the other parameters showed any statistically significant difference [Table/Fig-3].

The normal range of haemoglobin in females was 12.0-15.2 gm/dl. The pregnant women were grouped into 2 groups, based on their haemoglobin levels i.e. those with haemoglobin levels of < 12 gm/dl as Group 1 and those with levels of > 12 gm/dl as Group 2. The serum ferritin levels were 13.7 ± 8.2 mcg/dl and 26.6 ± 16.8 mcg/dl in the groups 1 and 2 respectively and the difference was statistically significant. Similarly, the serum zinc levels in the two groups were 11.3 ± 1.4 μmol/L and 16.1 ± 4.0 μmol/L respectively, which were also statistically significant. The rest of the parameters did not show any statistically significant difference [Table/Fig-4].

DISCUSSION

Micronutrient deficiencies in women of the reproductive age group have been recognized as a major public health problem in many developing countries. Several factors contribute to the widespread prevalence of micronutrient deficiencies. These include a low dietary intake, a low bioavailability and a poor utilization due to environmental factors such as poor hygiene, that lead to increased infections and infestations, adverse nutrient-nutrient interactions [4] and genetic causes [5]. Among the 48 subjects who were included in our study, 21 were primigravidas and 27 were multigravidas, with 8 subjects having been pregnant more than twice and 1 subject being Gravida 5. This indicated that some groups of people were still unaware of the Govt. norms of 2 children and of the risk of the undue complications in the mother and the foetus with each successive pregnancy. However, all the pregnant women were on iron supplementation.

	0-12 mcg/dl		12-20 mcg/dl		20-200 mcg/dl	
	Group 1 (n=18)		Group 2 (n=10)		Group 3 (n=21)	
VARIABLES	Mean	SD	Mean	SD	Mean	SD
AGE (years)	23.500	3.3998	23.800	4.1312	23.304	3.7832
BIRTH WEIGHT(gms)	2803.889	458.9264	2985.000	464.3095	2822.500	403.1047
BMI	22.833	6.6532	27.400	4.4272	24.609	2.7092
GRAVIDA	2.056	1.2113	1.700	0.9487	1.739	0.8100
HEADCIRCUMFERANCE (cms)	33.708	1.2515	33.667	1.8028	33.475	1.6422
HEMOGLOBIN (gm/dl)	11.938	1.3597	12.330	0.8908	12.570	0.7462
LENGTH (cms)	47.333	3.4989	47.833	3.1425	47.550	2.7429
SERUM COPPER (μmol/L)	7.330	2.8646	5.744	2.7611	6.543	3.5796
SERUM FERRITIN (mcg/dl)	8.694	1.7326	15.780	2.3237	37.504	12.9301
SERUM ZINC (μmol/L)	13.444	3.7176	14.170	2.7240	16.209	4.6472

[Table/Fig-1]: Mean and standard deviation (SD) of different variables in the three groups based on the serum ferritin levels

VARIABLES	< 13.8 µmol/L		> 13.8 µmol/L	
	Group 1 (n=24)		Group 2 (n=24)	
	Mean	SD	Mean	SD
AGE (years)	23.417	3.6939	23.519	3.6833
BIRTH WEIGHT(gms)	2892.083	440.3850	2806.667	432.1601
BMI	25.000	3.4135	24.111	6.0341
GRAVIDA	2.125	1.2270	1.593*	0.6360
HEADCIRCUMFERANCE(cms)	33.625	1.7004	33.548	1.4134
HEMOGLOBIN(gm/dl)	11.755	1.0932	12.784*	0.7320
LENGTH(cms)	48.150	2.6413	46.976	3.2499
SERUM COPPER(µmol/L)	6.277	2.6841	7.008	3.5859
SERUM FERRITIN(mcg/dl)	21.292	16.7943	24.663	15.4291
SERUM ZINC(µmol/L)	11.325	1.3267	17.952	3.1684

[Table/Fig-2]: Mean and standard deviation of different variables in the two groups based on the serum zinc levels
*p < 0.05.

	< 10 µmol/L		> 10 µmol/L	
	Group 1 (n=39)		Group 2 (n=9)	
VARIABLES	Mean	SD	Mean	SD
AGE (years)	23.262	3.6961	24.444	3.4681
BIRTH WEIGHT (gms)	2851.795	424.9946	2838.889	497.3541
BMI	25.238	3.3844	21.222*	8.9132
GRAVIDA	1.881	0.9927	1.667	1.0000
HEADCIRCUMFERANCE (cms)	33.686	1.4198	33.000	2.1909
HEMOGLOBIN (gm/dl)	12.178	1.0742	12.867	0.7106
LENGTH (cms)	47.557	2.9425	47.500	3.5637
SERUM COPPER (µmol/L)	5.631	2.4350	11.483	1.1105
SERUM FERRITIN (mcg/dl)	21.833	15.2066	28.878	19.2756
SERUM ZINC (µmol/L)	14.412	4.1505	16.800	3.7232

[Table/Fig-3]: Mean and standard deviation of different variables in the two groups based on the serum copper levels
*p < 0.05

	< 12 mg/dl		> 12 mg/dl	
	Group 1 (n=14)		Group 2 (n=34)	
VARIABLES	Mean	SD	Mean	SD
AGE (years)	23.214	4.3709	23.568	3.4038
BIRTH WEIGHT (gms)	2955.000	444.5698	2805.882	428.3097
BMI	24.857	4.3652	24.405	5.2039
GRAVIDA	1.857	1.0271	1.838	0.9864
HEADCIRCUMFERANCE (cms)	33.462	1.6515	33.643	1.5145
HEMOGLOBIN (gm/dl)	11.007	0.7385	12.789	0.6570
LENGTH (cms)	48.538	2.3670	47.089	3.1741
SERUM COPPER (µmol/L)	6.234	2.1031	6.826	3.5188
SERUM FERRITIN (mcg/dl)	13.714	8.2273	26.619*	16.8734
SERUM ZINC (µmol/L)	11.329	1.4866	16.159*	4.0610

[Table/Fig-4]: Mean and standard deviation of different variables in the two groups based on the hemoglobin levels
*p < 0.05.

When the study group was divided, based on the ferritin levels, the pregnant women did not show any significant differences in the 3 groups. Though the levels of haemoglobin and zinc increased with increased levels of ferritin, this was not statistically significant. This indicated that iron supplementation had helped in maintaining the ferritin levels. The iron requirement increases from 0.8 mg/day in the first trimester, to 6 to 7mg/day in the second half of the pregnancy. Overall, a pregnant woman needs about 2 to 4.8 mg of iron per day [6]. There were no significant changes in the maternal and foetal characteristics. The maternal ferritin levels are primarily a reflection of the iron statuses of the mothers [7]. Thus, the rationale of the routine iron supplementation, even in non-anaemic pregnant women, might be justified.

When the maternal zinc levels were compared, it was found that most of the women who had normal levels were primigravidas and that deficient levels were mostly seen in the multigravidas. This indicated that the zinc levels decreased with an increase in the parity of the women. Therefore, a zinc supplementation for multiparous women should be taken into account. The haemoglobin levels had also decreased significantly in the zinc deficient groups. The prevalence of low birth weight had increased significantly in the women who had serum zinc levels in the lowest quartile. This suggested that there was a threshold for the serum zinc concentration, below which the occurrence of the adverse pregnancy outcomes could increase significantly. A zinc deficiency has been associated with the complications of pregnancy and delivery, such as hypertension, a premature rupture of the membranes, placental abruption, prolonged labour, haemorrhage, infections, intrauterine growth retardation, low birth weight, congenital anomalies, increased neonatal morbidity and a poor neurobehavioural development [8]. Trials which had been undertaken in the developing countries have found that the babies whose mothers had been given zinc supplements in pregnancy, had improved immune functions and a reduction in the diarrhoea and the respiratory illnesses which occurred in infancy, thus suggesting their effects on the immune competence that persisted beyond birth [9].

The maternal copper levels, when they were compared with other parameters, showed a characteristic change with regards to the BMI of the pregnant women. The body mass index in the deficient group was 25.2 ± 3.3 and in the subjects with normal levels of copper, it was 21.2 ± 8.9 and the difference was statistically significant. This indicated that with an increase in the BMI, the copper levels had decreased. A copper deficiency is less common than an iron deficiency during pregnancy. However, copper is essential for the foetal development, and a maternal dietary copper deficiency can have both short- and long-term consequences [10]. A severe copper deficiency can lead to a reproductive failure, early embryonic death and gross structural abnormalities in the foetuses, whereas a moderate or a mild copper deficiency has little effect on either the number of live births or on the neonatal weight [11].

When the maternal haemoglobin levels were compared in the study population, it was found that the ferritin levels increased with increased levels of Hb, which was found to be statistically significant. This indicated that the ferritin levels were the correct indicators of the iron statuses of the patients and that the ferritin levels should be correlated with the Hb and other indices, to know the anaemic statuses of the patients. Though there is still some controversy concerning the optimal stage of the pregnancy at which the iron supplementation must be started, several studies have now shown that the iron stores at conception are strong predictors of the maternal iron status and the risk of anaemia in the later part of the pregnancy [7]. In our study, even the zinc levels had increased with the increasing Hb levels and they were found to be statistically significant. The women with mild anaemia could go through pregnancy and labour without facing any adverse consequences. The women with moderate anaemia had substantial reductions in their work capacities. They were more susceptible to infections and the recovery from the infections was prolonged. Premature births were common and they resulted in infants with lower birth weight and high perinatal mortality [12].

A significant number of subjects (30 out of 48) had developed complications during or after their deliveries, but none of the variables showed any significant differences when they were compared with those of the subjects who did not have any complications.

CONCLUSION

Serum microminerals play an important role in pregnancy and its outcomes. A supplementation with minerals reduces the risk which is associated with the complications of pregnancy. Our study clearly brought out a correlation between the micro minerals such as iron, zinc and copper during late pregnancies. An improvement in the iron status brings about a positive effect on the ferritin and the zinc levels, thus indicating better outcomes of the pregnancies. The future directions for research would include a replication of this study in a larger population, with serial measurements being made at different trimesters of the pregnancies.

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