Association of serum copper level with fasting serum glucose in south Indian women with gestational diabetes mellitus

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Abstract

Background and Aim: Prevalence of gestational diabetes mellitus (GDM) is higher in southern region compared to other parts of India. Deficiency of certain trace elements has been indicated in the progression of type 2 diabetes mellitus. Therefore, an inadequate supply of micronutrients in pregnancy may compromise the health and growth of both mother and fetus. Though copper and magnesium have an important role in glucose tolerance, they are not routinely checked in pregnancy. Therefore, this study was designed to determine the serum level of copper and magnesium in GDM patients and compare it with that of euglycemic pregnant women with similar gestational age.

Methods: Serum was collected at 0, 1st and 2nd h from the blood drawn from pregnant ladies as part of their screening test for oral glucose tolerance test (OGTT), after obtaining informed consent. Serum copper and magnesium was estimated from fasting serum sample.

Results: This prospective study showed a significant increase in copper level without significant change in magnesium level in GDM patients compared with euglycemic pregnant women. The increase in serum copper level was positively correlated with the rise in fasting serum glucose. Linear regression demonstrated association of serum copper level to fasting blood glucose (beta = 0.265, P = 0.017).

Conclusion: Rise in serum copper level in GDM is linked to the level of fasting blood glucose. Therefore, women, who are diagnosed with GDM and found to have higher copper level, should be monitored carefully for ill-effects of higher copper level as it is linked to their hyperglycemic status.

Key words: Copper, gestational diabetes mellitus, magnesium

INTRODUCTION

Diabetes mellitus (DM) is one of the most common endocrine disorders in India.[1] It is characterized by hyperglycemia resulting from an absolute or relative deficiency of insulin. Gestational diabetes mellitus (GDM) is a condition of glucose intolerance which sets in or is recognized during pregnancy. Prevalence of GDM has been found to range from 3.8% to 21% with an average value at 13.9% in India.[2] Moreover, it was more prevalent in the urban area compared to semi-urban and rural areas.[3] However, the prevalence is little higher in South India. It was found to be around 16.2% in Chennai alone.[3] Women diagnosed as GDM as well as children born to these women, are at increased risk to develop the future DM, especially type 2 DM.[4] This disease exerts adverse metabolic effects for both pregnant women and fetuses.[5]

Though macro-elements are important nutrients for the body as they are required in larger quantity, trace elements also perform an essential role in various metabolic processes despite their less requirement. They are essential for the metabolism, nerve conduction,
Obtained from all the participants of the study groups as approved by the research council and human ethics committee of PIMS. Written informed consent was obtained from all the participants of the study groups prior to their enrollment into this study.

**Sample collection**

Blood samples, from pregnant women, which was withdrawn for OGTT during screening for GDM, was used for this study. Serum was extracted by centrifugation (at 3000 rpm for about 5 min) and analyzed for glucose level. The fasting serum samples were stored at −40°C till the estimations of copper and magnesium.

**Selection process**

According to the new ADA criteria for the OGTT levels of the pregnant women, our subjects were asked to report to clinical biochemistry blood collection center in the morning in a fasting condition. Their fasting blood sample was drawn, and then they were provided with 75 g of glucose powder dissolved in 250 mL of water and they were asked to drink it within 5 min. Then their blood sample was collected at 1st and 2nd h interval. The pregnant ladies were kept under observation for any feeling of discomfort throughout this test as per the guidelines of OGTT. Based on the glucose values the subjects were divided into following two groups:

- **Control** ($n = 91$): Pregnant women with normal OGTT values for all three following reading
  - Fasting $<92$ mg/dl; at 1 h $<180$ mg/dl and at 2 h $<153$ mg/dl
- **GDM** ($n = 85$): Pregnant women with abnormal OGTT values (glucose values exceeding any one out of three following
  - Fasting $\geq 92$ mg/dl; at 1 h $\geq 180$ mg/dl and at 2 h $\geq 153$ mg/dl.

**Glucose estimation**

Serum glucose was estimated by Hexokinase method (Roche Diagnostics, Indianapolis) in the autoanalyzer (Integra 400 plus, Roche Diagnostics, Indianapolis). Hexokinase catalyzes phosphorylation of glucose present in the sample with the help of adenosine triphosphate in presence of Mg$^{2+}$ provided in the reagent, to give glucose 6 phosphate and adenosine diphosphate. Glucose 6 phosphate further gets oxidized by glucose 6 phosphate dehydrogenase enzyme in the presence of nicotinamide adenine dinucleotide (NAD) to produce 6 phosphogluconate and reduced form of NAD (NADH). The amount of nicotinamide adenine dinucleotide (NAD) produced is directly proportional to the glucose concentration in the sample. NADH is measured by measuring the increase in absorbance at 340 nm.

**Copper estimation**

Serum copper was estimated by colorimetric assay using 3,5-DiBr-PAESA method using commercial kits from Futura system (Parecchi scientific diagnostic, Italy). The copper gets extracted from its protein complex by acidification with reaction buffer. This is followed by its reduction to cuprous ion which when combines with...
the chromogen 3,5-DiBr-PAESA, produces a colored complex which is measured at 580 nm.

**Magnesium estimation**

Serum magnesium was estimated by xylidyl blue method using commercial kits from Beacon.[15] In this test, magnesium reacts with the chromogen xylidyl blue in the presence of glycoetherdiaminotetraacetic acid to form a purple color complex. The intensity of color is directly proportional to concentration of magnesium present in serum. It was measured at 520 nm.

**Statistical analysis**

All parameters are expressed as mean ± standard deviation. Statistical analyses were performed using the IBM SPSS program version 16 (SPSS Software Inc., Chicago, IL, USA). Normality of the data distribution was checked by Smirnov–Kolmogorov test. Significance of the differences between control and test groups was done by Student’s t-test. The association between fasting serum glucose (FSG) and copper was done by Pearson correlation analysis. The link of copper level with FSG was assessed by linear regression. P < 0.05 were considered as significant.

**RESULTS**

The comparison of serum glucose at different interval and serum copper and magnesium values are included in Table 1. The fasting serum copper level was significantly higher in GDM groups compared with the control group while there was no difference in magnesium level [Table 1]. There was 19% increase in copper level while only 3% decrease in magnesium level in GDM group in comparison to control group as depicted in Figure 1.

There was a significant positive association between fasting serum copper level and FSG in GDM group [Table 2]. The copper level had an independent contribution toward raised FSG level in the GDM group, but not in the control group [Table 3].

**DISCUSSION**

Copper is an essential cofactor for many enzymes with important roles in the human body such as catalase, cytochrome oxidase, superoxide dismutase, etc.[16] In case of pregnancy, apart from nutritional copper supplement to fetus, it also has an interactive role in placental iron transport even though the mechanism is not yet clear.[17] Therefore, altered levels of copper in maternal serum could affect fetal health.

The normal level of serum copper in nonpregnant women ranges from 80 to 155 µg/dl. Alterations in serum trace elements have been reported before. However, reports are controversial.[9,18] Loven et al. reported...
no significant difference in the copper level between healthy pregnant women and women with GDM. In another work by Wang et al., an increased level of copper and decreased zinc content in GDM compared to normoglycemic pregnant ladies was reported. Hence, our result corroborates with the previous report of Wang et al. The increased copper level found in our study could be due to pregnancy induced rise in ceruloplasmin levels which in turn could be linked to an elevated estrogen levels. In pregnancy, the levels of copper and ceruloplasmin rises from the 6th week onwards, reaching high levels during the third trimester and then it returns to normal, 6 weeks following delivery. Studies have shown that altered maternal copper levels may contribute to restriction in fetal growth or may lead to preterm delivery or premature rupture of fetal membrane. Therefore, despite controversy in the level of ceruloplasmin in GDM, its analysis in maternal serum in GDM cases deemed pertinently. There was a significant positive association between fasting serum copper level and FSG in GDM group [Table 2]. This indicates that increased copper level could be directly linked to decreased insulin sensitivity.

Moreover, the intensity of the relationship between of raised copper level and serum glucose level in the GDM group is further highlighted by its significant independent contribution towards the higher FSG level in linear regression model Table 3 (P = 0.017).

Magnesium is also known as an essential cofactor for many enzyme systems. It is an essential ion involved in glucose homeostasis. A complex interplay exists between magnesium and glucose metabolism. The normal level of serum magnesium in nonpregnant women ranges from 1.9 to 2.5 mg/dl. Hypomagnesemia has been previously reported in normal pregnancy as well as in GDM cases. However, in our study there was no difference in serum magnesium level between control and GDM group. Our sample size was larger compared to the previous study. Another study from India shows that the magnesium levels are unaltered in DM patients. The percentage increase in copper was more in GDM and GDM group. Our sample size was larger compared to the previous study.

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CONCLUSION
Copper level was found to be lower in women with GDM during routine OGTT screening test, and it was significantly associated with an increase in FSG level. It is inferred that rise in fasting glucose is more affected by increased copper level compared to any alteration in magnesium level. Therefore, women with GDM the status of trace element such as copper, also should be checked and if associated with a higher level of copper, suitable diet modification and regular check-up for insulin resistance should be advised.

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