Original Article

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

Received: 11th September, 2014; ***Revised:*** 22nd October, 2014; ***Accepted:*** 31st October, 2014

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]

Access this article online	
Quick Response Code:	Website: www.ijcep.org
	DOI: 10.4103/2348-8093.149766

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,

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muscle contractions, regulation of membrane potential, immune regulation, various enzyme reactions, etc.[6] Therefore, an inadequate supply of these micronutrients will compromise the health and growth of both mother and conceptus, which can be detrimental to the fetus.[7]

Pregnancy is a phase in a woman's life with an increased metabolic demand which is also associated with changes in physiology and the growth of the fetus.[8] The metabolism of women during pregnancy undergoes great alterations[5] and so does the trace elemental levels in the serum.[9] It is only iron, which is regularly checked in pregnancy for all women. However, as variation in macro-elements and trace elements are indicated in the progression of type 2 diabetes,[6] the same may also play a role in either causation of GDM or in its progression into DM at a later stage. As pregnancy *per se* puts an increased demand of nutrients on the mother, therefore, it would be beneficial to assess the level of these elements, especially in women diagnosed with GDM so that any deficiencies detected can be corrected at the earliest.

Iron levels are often checked in pregnancy due to adverse health risk of anemia in both mother and fetus. However, trace elements such as copper and magnesium which play an important role in glucose tolerance are not checked regularly in pregnancy.[10,11] Therefore, it is important to assess their serum levels at least during pregnancy in India. The objective of our study was to determine the serum level of copper and magnesium in GDM patients and compare it with that of euglycemic pregnant women of similar gestational age (more than 20 weeks of gestation).

MATERIALS AND METHODS

Setting and participants

The recruitment of subjects was done by randomized case control technique. Pregnant ladies were recruited from the out-patient Department of Obstetrics and Gynecology, Pondicherry Institute of Medical Sciences (PIMS), for this study. A total of 176 pregnant ladies with a gestational age more than 20 weeks and within the age range of 18–40 years, were taken for this study. Out of these, 91 were normoglycemic, and 85 were GDM based on their OGTT, which was based on new American Diabetes Association criteria 2011 (ADA 2011).[12] The normoglycemic pregnant ladies were selected after matching their age with GDM cases. All those pregnant women with the previous history of diabetes, hypertension, and other endocrine disorders were excluded from this study. The study was 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 ≥92 mg/dl; at 1 h ≥180 mg/dl and at 2 h ≥153 mg/dl.

Glucose estimation

Serum glucose was estimated by Hexokinase method (Roche Diagnostics, Indianapolis) in the autoanalyzer (Integra 400 plus, Roche Diagnostics, Indianapolis).[13] 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 adinine dinucleotide (NAD) to produce 6 phosphogluconate and reduced form of NAD (NADH). The amount of nicotinamide adinine 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).[14] 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 glycoletherdiaminotetracetic 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 FBG 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].

Table 1: Comparison of various parameters in subjects of control (healthy pregnant women) group and GDM group

Parameters	Control (*n*=91)	GDM (*n*=85)	*P*
Age	26.75±4.39	28.04±4.50	0.056
FSG (mg/dL)	80.48±6.84	96.35±15.60	0.000
1-h (mg/dL)	133.63±22.41	173.54±31.97	0.000
2 h (mg/dL)	103.59±19.30	137.38±31.73	0.000
Copper (µg/dL)	123.13±113.98	147.11±139.78	0.028
Magnesium mg/dL)	1.73±0.91	1.67±0.99	0.661

Data is expressed as mean±SD. FSG: Fasting serum glucose, GDM: Gestational diabetes mellitus, SD: Standard deviation, NR: Normal range. Norrmality of the data was checked by one sample Kolmogorov-Smirnov test. Analysis was done by student's *t*-test for parametric data and by Mann-Whitney test (for copper) for nonparametric data. *P*<0.05 was considered significant

Table 2: Correlations of serum copper with other biochemical parameters in the control (healthy pregnant women) group and GDM by Pearson correlation analysis

Parameters	Control (*n*=91)		GDM (*n*=85)	
	r	*P*	*r*	*P*
FSG	−0.037	0.731	0.251	0.021

P<0.05 was considered significant. GDM: Gestational diabetes mellitus, FSG: Fasting serum glucose

Table 3: Linear regression of serum copper level (as dependent variable) on FSG (independent variable) in control (healthy pregnant women) and GDM group

	B±SE	Beta	95% CI	*P*
Control	−0.002±0.006	−0.037	−0.015-0.010	0.731
GDM	0.028±0.012	0.265	0.004-0.052	0.017

FSG: Fasting serum glucose, GDM: Gestational diabetes mellitus, *B*: Unstandardized coefficients, SE: Standard error of B, Beta: Standardized coefficients, CI: Confidence interval of B. *P*<0.05 considered significant: **P*<0.05

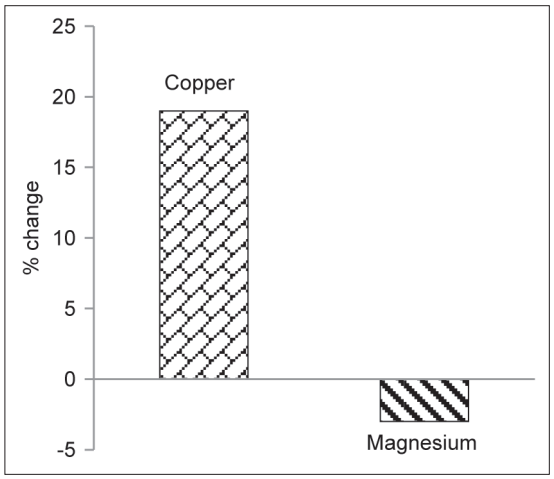

Figure 1: Percentage change in serum copper and magnesium the GDM (gestational diabetes mellitus) compared to control (healthy pregnant women) group

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.[19] 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.[19] 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.[20] Therefore, despite controversy in the level of ceruloplasmin in GDM,[18,21] 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.[22] A complex interplay exists between magnesium and glucose metabolism.[23,24] 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.[22,25] 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.[25] Another study from India shows that the magnesium levels are unaltered in DM patients.[26] The percentage increase in copper was more in GDM group of ladies compared to the decrease in magnesium level [Figure 1]. Therefore, it may be inferred that rise in FSG may be more affected by rise in copper level rather than alteration in magnesium level among South Indian women with GDM.

Limitations of the study

Due to limited funding we could not assess fasting insulin and hence insulin resistance in the present study. The absence of data on insulin is a limitation in the present study.

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.

REFERENCES

1. Wild S, Roglic G, Green A, Sicree R, King H. Global prevalence of diabetes: Estimates for the year 2000 and projections for 2030. Diabetes Care 2004;27:1047-53.
2. Seshiah V, Balaji V, Balaji MS, Paneerselvam A, Kapur A. Pregnancy and diabetes scenario around the world: India. Int J Gynaecol Obstet 2009;104 Suppl 1:S35-8.
3. Seshiah V, Balaji V, Balaji MS, Paneerselvam A, Arthi T, Thamizharasi M, *et al*. Prevalence of gestational diabetes mellitus in South India (Tamil Nadu) – A community based study. J Assoc Physicians India 2008;56:329-33.
4. Seshiah V, Balaji V, Balaji MS. Scope for prevention of diabetes – Focus intrauterine milieu interieur. J Assoc Physicians India 2008;56:109-13.
5. Gabbay-Benziv R, Baschat AA. Gestational diabetes as one of the "great obstetrical syndromes" – The maternal, placental, and fetal dialog. Best Pract Res Clin Obstet Gynaecol 2014;[Epub ahead of print].
6. Siddiqui K, Bawazeer N, Joy SS. Variation in macro and trace elements in progression of type 2 diabetes. ScientificWorldJournal 2014;2014:461591.
7. King JC. The risk of maternal nutritional depletion and poor outcomes increases in early or closely spaced pregnancies. J Nutr 2003;133:1732S-6.
8. Pipkin FB. Maternal physiology. In: Edmonds KD, editor. Dewhurst's Textbook of Obstetrics and Gynaecology. Oxford, UK: Blackwell Publishing; 2007.
9. Wang Y, Tan M, Huang Z, Sheng L, Ge Y, Zhang H, *et al*. Elemental contents in serum of pregnant women with gestational diabetes mellitus. Biol Trace Elem Res 2002;88:113-8.
10. Fields M, Ferretti RJ, Smith JC Jr, Reiser S. Impairment of glucose tolerance in copper-deficient rats: Dependency on the type of dietary carbohydrate. J Nutr 1984;114:393-7.
11. Paolisso G, Scheen A, D'Onofrio F, Lefèbvre P. Magnesium and glucose homeostasis. Diabetologia 1990;33:511-4.
12. American Diabetes Association. Standards of medical care in Diabetes – 2011. Diabetes Care 2011;34 Suppl 1:S11-61.
13. Tietz NW, editor. Clinical Guide to Laboratory Tests. 4th ed.. Philadelphia: WB Saunders; 2006. p. 444-51.
14. Abe A, Yamashita S, Noma A. Sensitive, direct colorimetric assay for copper in serum. Clin Chem 1989;35:552-4.
15. Endress DB, Rude RK. Mineral and bone metabolism. In: Burtis CA, AShwood ER, editors. Tietz Textbook of Clinical Chemistry. 3rd ed.. Philadelphia: WB Saunders; 1999. p. 1395-457.
16. Liu J, Yang H, Shi H, Shen C, Zhou W, Dai Q, *et al*. Blood copper, zinc, calcium, and magnesium levels during different duration of pregnancy in Chinese. Biol Trace Elem Res 2010;135:31-7.
17. McArdle HJ, Andersen HS, Jones H, Gambling L. Copper and iron transport across the placenta: Regulation and

interactions. J Neuroendocrinol 2008;20:427-31.
18. Loven A, Romem Y, Pelly IZ, Holcberg G, Agam G. Copper metabolism – A factor in gestational diabetes? Clin Chim Acta 1992;213:51-9.
19. Al-sarrag NF, Rajab TMA, Altai WF. Estimation of ceruloplasmin activity, and copper, iron levels in sera of normal pregnant. Ibn-Al-Haitham J Pure Appl Sci 2007;20:1-10.
20. Li M, Huang CY, Wang XD, Liu XH. Analysis of the concentration of plasma copper and collagen in fetal membrane of the preterm's mothers. Wei Sheng Yan Jiu 2006;35:453-6.
21. Dey P, Gupta P, Acharya NK, Rao SN, Ray S, Chakrabarty S, *et al*. Antioxidants and lipid peroxidation in gestational diabetes – A preliminary study. Indian J Physiol Pharmacol 2008;52:149-56.
22. Kimura Y, Murase M, Nagata Y. Change in glucose homeostasis in rats by long-term magnesium-deficient diet. J Nutr Sci Vitaminol (Tokyo) 1996;42:407-22.
23. Emila S, Swaminathan S. Role of magnesium in health and disease. J Exp Sci 2013;4:32-43.
24. Takaya J, Higashino H, Kobayashi Y. Intracellular magnesium and insulin resistance. Magnes Res 2004;17:126-36.
25. Goker Tasdemir U, Tasdemir N, Kilic S, Abali R, Celik C, Gulerman HC. Alterations of Ionized and Total Magnesium Levels in Pregnant Women with Gestational Diabetes Mellitus. Gynecol Obstet Invest 2014;[Epub ahead of print].
26. Zargar AH, Shah NA, Masoodi SR, Laway BA, Dar FA, Khan AR, *et al*. Copper, zinc, and magnesium levels in non-insulin dependent diabetes mellitus. Postgrad Med J 1998;74:665-8.

How to cite this article: Asha D, Nanda N, Daniel M, Sen SK, Ranjan T. Association of serum copper level with fasting serum glucose in south Indian women with gestational diabetes mellitus. Int J Clin Exp Physiol 2014;1:298-302.

Source of Support: Nil, **Conflict of Interest:** Nil.