

Serum levels of copper, zinc and magnesium in pregnant women with Impaired Glucose Tolerance test: a case-control study

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Summary. *Objective:* Gestational Diabetes Mellitus (GDM) is a type of diabetes that begins during pregnancy. This problem causes many complications for mother and foetus. Mineral levels in diabetic patients change and since the levels of these elements have not received much attention in patients with Impaired Glucose Tolerance test (IGT), in this study serum concentration of copper (Cu), zinc (Zn), and magnesium (Mg) were analysed in singleton pregnant women with IGT compared with euglycemic pregnant women. *Methods:* The subjects of this case-control study have been selected from pregnant women who referred to Rohzنده health and therapeutic center in northwest of Iran from December 2013 - April 2014. 46 pregnant women with IGT and 35 euglycemic women were selected. The levels of hemoglobin, hematocrit, zinc, copper and magnesium in the blood samples were measured. *Results:* The findings of this study indicated that the difference of hemoglobin and hematocrit and magnesium levels was not significant between two groups. The difference of copper concentration in healthy pregnant women and women with IGT was statistically significant ($P < 0.001$) which indicated a high level of copper in healthy pregnant women. Interestingly, in pregnant women with IGT plasmatic zinc level was lower than healthy women ($P = 0.028$). *Conclusions:* Pregnancy is a condition that can affect the mineral status and to achieve better results, further researches are needed with larger sample size.

Key words: copper, gestational diabetes mellitus, magnesium, zinc

Introduction

Gestational Diabetes Mellitus (GDM) is one of the most common complications of pregnancy that causes adverse outcomes to the mother and foetus (1). The undesirable effects of gestational diabetes include premature delivery, shoulder dystocia, fetal macrosomia, neonatal hypoglycemia, hyperbilirubinemia, hypercalcemia and increased embryonic mortality (2). The incidence of gestational diabetes has increased during

the last decades and reported approximately 1-14% (3). The prevalence of GDM in Iran is about 1.3-18.6 % which demonstrates high prevalence rate among Iranian pregnant women (4, 5). The role of mineral elements in metabolic processes and their relation to human health and disease has been studied in recent years. Minerals have catalytic, structural and regulatory functions (6). Disturbances in some mineral status and their role in oxidative stress in diabetic patients play essential role in insulin resistance and diabetes progression (7).

Zinc (Zn) is an essential trace element that is needed for cell function and it is one of the major components of many enzymes (6). Antioxidant feature of zinc (8) protects insulin and pancreatic cells against free radicals (9). Zinc is effective for insulin synthesis, storage and secretion (8). It is also effective in insulin function by regulating insulin tyrosine kinase receptors and increasing the phosphorylation of tyrosine kinase (10). Copper (Cu) is another essential trace element with a particular role in the structure of superoxide dismutase (6). Serum copper concentration in pregnant women becomes almost double (11). Magnesium (Mg) is the second intracellular cation which plays an important role in many biological reactions (12). Magnesium deficiency can lead to serious clinical symptoms (13). It was known as a cofactor for many enzymes that involved in the oxidation of glucose and insulin release from pancreatic beta cells (14).

Various studies have measured the levels of minerals in diabetic patients. The pioneering studies showed that low serum zinc levels in diabetic patients may be associated with the pathogenesis of diabetes mellitus (15). In addition, low levels of magnesium in diabetic patients have been reported previously. (12). The research findings on serum copper status are contradictory (6, 16). Women with impaired glucose tolerance, i.e. suffering high amount of blood glucose level, are in the high risk of perinatal complications although they lack GDM (17, 18).

As far as we know, the status of minerals in pregnant women with impaired glucose tolerance has not been studied yet. To fill this research gap, serum levels of copper, zinc and magnesium in women with impaired glucose tolerance compared with healthy pregnant women have been investigated.

Materials and Methods

In this case-control study, we selected our subjects from singleton pregnant women who referred to Rohzende maternal and child healthcare centre in Shabestar, North West of Iran, during the period of December 2013 to April 2014. Every woman visited there for the 24-28 weeks screening in order to receive the oral glucose challenge test (OGCT). Each subject

was administered 50 gram glucose orally in fasting state and blood glucose measurements were taken after one hour. Based on criteria of the American Diabetes Association (ADA) if 1 hour blood glucose levels were ≥ 130 mg/dL, impaired glucose tolerance (IGT) were diagnosed and oral glucose tolerance test with 75 gram glucose was performed as follows in order to distinguish those with GDM.

Values of glucose considered as:

- FBS (Fasting Blood Sugar) ≤ 92 mg/dL
- 1 h Blood Sugar ≤ 180 mg/dL following a 75 g oral glucose load
- 2 h Blood Sugar ≤ 153 mg/dL following a 75 g oral glucose load.

During this period, 92 pregnant women were diagnosed with IGT and at the same time, 150 euglycemic pregnant women were chosen. Among these participants, 46 pregnant women with IGT and 35 healthy euglycemic pregnant women were selected based on inclusion and exclusion criteria and enrolled as our case and control groups respectively. These pregnant women were matched based on gestational age and pre-pregnancy body mass index (BMI).

The informed written consent was obtained from all women, with ethical permission for the study obtained from the ethics committee of Tabriz University of Medical Sciences. The inclusion criteria were pre-pregnancy body mass index (BMI=21-25 Kg/m²), age 25-35 years, singleton pregnancy and gestational age 24-28 weeks.

The exclusion criteria included pregnant women with a history of diabetes before pregnancy and women with a history of active infection, chronic disease or drug therapy or a history of smoking or alcohol consumption. Five ml venous blood sample was taken after a 12 hour fasting by experts. The level of hemoglobin, hematocrit, zinc, copper and magnesium in the blood samples were measured. Levels of hemoglobin, hematocrit were measured from the whole blood samples by cell counter (Sysmex KX21N, Japan). To measure zinc, copper and magnesium, blood samples were centrifuged after clotting for 5 min at 3000 rpm and the serum samples were separated and poured into metal-free plastic tubes. Then the levels of minerals from fresh serum samples were examined by Photometric Technique (Hitachi 911; kit Pars Azmoon for zinc and for copper and magnesium with through kit ZiestChem).

Statistical analysis

Kolmogorov-smirnov goodness of fit test was used to normalize the data distribution. Due to normality of data the mean \pm SD (standard deviation) were reported. Independent sample t-test was used to compare two groups of tests. The statistical software SPSS version 21 (SPSS Inc. IL, Chicago, USA) has been employed for data entry and analysis.

Results

In the present study 35 healthy pregnant women with the mean age 29.31 ± 4.99 and 46 pregnant women with positive 50 gr Glucose Challenge Test with the mean age 31.24 ± 4.81 were selected based on inclusion and exclusion criteria. The age difference between the two groups was not statistically significant ($P=0.083$). The mean BMI based on pre pregnancy weight was 24.1 ± 5.71 and 23.8 ± 5.60 in IGT and healthy group respectively. The mean hemoglobin levels were 11.92 ± 1.07 in healthy pregnant women and 11.85 ± 1.18 in IGT group. The hemoglobin and BMI difference between two groups were not significant ($P>0.05$). The mean hematocrit level in non IGT group was 36.12 ± 2.31 and in IGT group was 36.33 ± 2.96 ($P=0.740$). The mean magnesium concentration was similar in both groups ($P=0.949$) (Table1). The mean serum zinc level in pregnant women with IGT was ($61.00 \mu\text{g/dL}$) which was less than the lower limit of normal range for women ($75\text{-}110 \mu\text{g/dL}$). Also the mean copper

concentration in those pregnant women was ($47.56 \mu\text{g/dL}$) which was less than the lower limit of normal range for women ($80\text{-}155 \mu\text{g/dL}$).

The difference between copper and zinc levels was significantly different which indicates a high level of copper in healthy pregnant women and low levels of zinc in pregnant women with IGT (Fig.1).

Discussion

To the best of our knowledge, this is the first study to compare serum concentration of copper, zinc and magnesium between pregnant women with IGT and healthy pregnant women.

A number of studies on the association between diabetes and metabolism of minerals have been reported. In

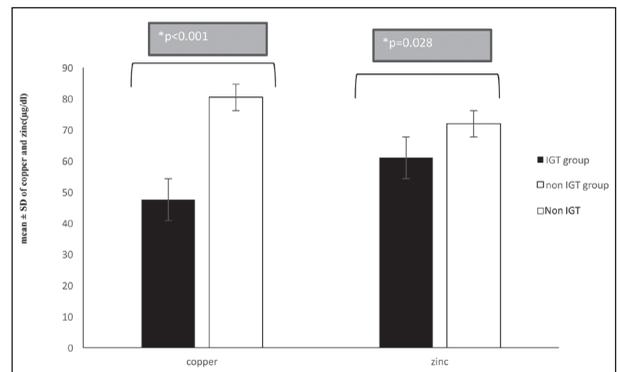


Figure 1. Serum levels of Zinc and copper in study groups, X-axis represents the study groups. Y-axis shows Blood levels of Zinc and copper. Statistical analysis was done by Independent Sample T test. * $p<0.05$ statistically significant

Table 1. The mean \pm SD for Age, BMI, Hemoglobin, Hematocrit, copper, magnesium and zinc in impaired glucose tolerance (IGT) and non IGT women.

| Parameter | IGT (n=46) | non IGT (n=35) | *P value |
|--------------------------------------|-------------------|-------------------|----------|
| Age (year) | 31.24 ± 4.81 | 29.31 ± 4.99 | 0.083 |
| Prepregnancy BMI (Kg/m^2) | 24.1 ± 5.71 | 23.8 ± 5.60 | 0.341 |
| Hemoglobin | 11.85 ± 1.18 | 11.92 ± 1.07 | 0.813 |
| Hematocrit | 36.33 ± 2.96 | 36.12 ± 2.31 | 0.740 |
| Copper ($\mu\text{g/dL}$) | 47.56 ± 18.79 | 80.47 ± 38.60 | <0.001 |
| Magnesium (mg/dL) | 2.20 ± 0.27 | 2.25 ± 0.21 | 0.949 |
| Zinc ($\mu\text{g/dL}$) | 61.00 ± 20.39 | 71.97 ± 30.05 | 0.028 |

*: based on independent sample – T test, BMI: body mass index, $p<0.05$ statistically significant

many studies, impaired insulin secretion, insulin resistance and glucose intolerance have been associated with impaired levels of elements such as chromium, magnesium, selenium, vanadium, zinc and copper. Since the literature on the levels of these elements in patients with IGT is limited, here we have investigated the serum concentration of copper, zinc and magnesium in pregnant women with IGT compared with euglycemic pregnant women.

The results of our study showed that serum magnesium, hemoglobin and hematocrit levels were not significantly different between the two groups. However, copper level in healthy euglycemic pregnant women was significantly higher than IGT group. Furthermore, zinc levels in IGT group were significantly lower than healthy group.

Wang et al. reported that copper levels in women with GDM and IGT compared with healthy pregnant women has increased although serum zinc levels in women with GDM and IGT compared with healthy pregnant women has decreased (19). The result of our study with respect to Zn is similar to Wang et al. while the result on Cu shows a contrasting status with them. The difference in results may be due to differences in sample size or difference in weeks of pregnancy at the time of sampling.

Basaki et al. demonstrated that serum copper and zinc levels in type 2 diabetic patients were lower than the healthy control group and this results are harmonic with our findings (7). Zargar et al. showed a significant increase in copper levels of non-insulin dependent diabetic patients compared with the healthy control group but changes in zinc and magnesium levels in the two groups were not significant (6). In Al-Saleh study no difference in serum zinc and copper levels in women with gestational diabetes compared with healthy pregnant women were observed (20). In another study, Al-Saleh reported that serum zinc levels in obese pregnant women with GDM compared with healthy obese women were lower, but copper levels were similar in both groups (21). Decreased serum magnesium levels and increased urinary excretion in patients with diabetes type 1 and 2 are shown in a number of studies (22-24). Similar to our results, difference in magnesium levels between diabetic patients and healthy subjects were not observed by Walter et al (16).

Enhanced Zn requirements during pregnancy and lactation increase the risk of Zn deficiency. The interaction among Zn and glucose metabolism is complex and zinc deficiency might interfere with insulin storage and its ac-

tivation. Zinc deficiency is related with insulin resistance, alteration in insulin secretion and impaired of glucose metabolism. It has been revealed that disturbance of Zn homeostasis seems to be related with emerging diabetes (23).

Copper is one of the essential trace elements for human and is necessary for Cu-dependent enzymes like SOD1 (Superoxide dismutase 1) as well as glyucose-6-phosphate dehydrogenase in pentose phosphate pathway (24). Beyond this Cu ion may also play a protective role in the cumulating of human islet amyloid peptide, which is one of the major components of amyloid sediment in pancreatic β -cells of type 2 diabetic patients; however due to contradictory results whether or not Cu have a protective role in the etiology of type 2 diabetes is not defined (25,26).

In contrast with some studies (27, 28) our finding showed that Mg level was normal and there were no differences in this element among the two groups. The hypomagnesaemia in patient diagnosed having IGT compared to whom free of IGT and the importance of magnesium in the appearance of insulin resistance may encourage more trials to explain the exact role of magnesium in the pathogenesis of IGT.

This area of research welcomes more studies on this element. The pregnant women are exposed to noticeable changes in the level of minerals and any further study on their mineral condition would be of great importance. The limitations of our study are the low sample size and dietary differences between groups were not considered. By removing the limitations of our study may achieve better results in this field.

Conclusion

In conclusion, the findings suggest that serum levels of some minerals may associated with gestational hyperglycemia. If other studies confirm these results, it seems to be a beneficial intervention approaches in prevention and controlling of gestational diabetes.

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