A Study on the Association of Copper and Zinc Serum Levels with Insulin Resistance Indices in Gestational Diabetes

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ABSTRACT

Objectives: The levels of serum Zinc (Zn) and Copper (Cu) between pregnant women with GDM (gestational diabetes mellitus) have been compared to non-GDM ones, then the association between insulin resistance indices with the above two elements has been investigated. **Methods**: 460 pregnant women (210 with GDM and 250 without diabetes) referred to Central Kashmar Laboratory between September 2018 and May 2020, participated in this study. Mann-Whitney U and Independent sample t-test were used to compare zinc, copper, and insulin resistance indices between the two groups. The relationship between insulin resistance indices with serum Zn and Cu in the two above groups was examined using a linear regression test. **Results**: Differences in the levels of Cu (P <0.001) and Zn (P=0.03) between the two groups were significant. Correlations between plasma Cu levels and IR indices were not significant (QUICKI (β= 1.534, P =0.234), HOMA-IR (β = 0.608, P=0.155), HOMA-B (β = -0.039, P=0.457), 1/Insulin (β = -1.982, P=0.237), G/I (β = 0.868, P=0.295)); in addition, no significant correlations between plasma Zn levels and IR indices were observed (QUICKI (β= -1.930, P =0.14), HOMA-IR (β = -0.783, P=0.071), HOMA-B (β = 0.041, P=0.442), 1/Insulin (β = 2.270, P=0.181), G/I (β = -1.062, P=0.206)) by linear regression test taking into account the effect of age and group. **Conclusion**: Plasma levels of zinc and copper are not directly affected by insulin resistance. It can also be found that increased insulin resistance cannot alter serum copper and zinc levels in pregnant women with gestational diabetes.

Key Words: *Gestational diabetes mellitus, GDM, Copper, Zinc, Insulin resistance indices.*

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INTRODUCTION

Gestational Diabetes Mellitus (GDM) refers to any amount of glucose resistance that is detected during pregnancy [1]. An increase in insulin secretion in pregnant women is a normal reaction to keep a normal range of plasma glucose levels in them. Gestational Diabetes Mellitus will occur in women who are not able to increase their insulin levels enough [2].

Zinc is an essential trace element for all living things as the function of more than 300 enzymes and 1000 transcription factors require the presence of this element. After iron, zinc is the second most abundant and the only metal in humans which participates in all enzyme classes activity [3]. The antioxidant properties of zinc, protect insulin and pancreatic cells against free radicals [4] and are effective in the synthesis, storage, and secretion of insulin [5].

Copper is another trace element that is essential to the proper functioning of organs and metabolic processes in human; such as incorporation into a variety of proteins and metalloenzymes which perform essential metabolic functions [6]. Serum copper concentrations almost double in pregnant women [7].

1/Insulin, Glucose/Insulin, HOMA-IR, HOMA-B, QUICKI (insulin resistance indices) were used for insulin resistance evaluation [8, 9].

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One of the roles of insulin in glucose homeostasis is to increase glucose uptake by muscles and adipose tissue; Insulin also inhibits hepatic gluconeogenesis. Tissue resistance to insulin function is one of the factors contributing to the development of the metabolic syndrome. This phenomenon, known as insulin resistance (IR), is the reduction of the metabolic effects of insulin in increasing glucose uptake into tissues and (or) suppressing glucose production by the liver (hepatic glucose production (HGP)) [10].

Given that insulin resistance occurs in patients much earlier than the onset of diabetes, many researchers believe that diabetes in a person can be predicted 10 to 20 years before it occurs by diagnosing insulin resistance in her/him [11].

Unlike difficult techniques such as "Euglycemic Clamp", simpler indicators for determining insulin resistance are provided, which are known as "fasting indices"[12], the same ones have been used in this study. One of these indices which is widely used to express IR is HOMA-IR. It is based on fasting glucose and fasting insulin levels [13, 14].

Another index for assessment of IR or insulin sensitivity is QUICKI. Katz et al. showed a significant relationship between QUICKI and the Euglycemic Clamp (r=0.78) [15].

Due to the importance of copper and zinc in metabolism and their role in the production and secretion of insulin, our hypothesis is whether the difference in zinc and copper concentrations is significant between healthy pregnant women (normal glucose tolerance (NGT)) and women with GDM? If so, is there a significant relationship between these changes and increased insulin resistance in women with GDM? As far as we know, this type of study has not been done yet.

MATERIAL AND METHODS

Subjects

Our study is a case-control analytical one that the exposure is pregnancy and outcome is plasma levels of zinc, copper, blood glucose, insulin, and insulin resistance (IR) indices.

The convenience model (accidental sampling) was used as a sampling method; all pregnant women who referred to the Kashmar Health Laboratories (Iran; Khorasan Razavi province) for the screening of gestational diabetes in the 24^{th} to 28^{th} week of pregnancy, participated in this study during 20 months (between September 2018 and May 2020). They were screened for GDM using OGTT (oral glucose tolerance test) and 250 non-diabetic pregnant women were selected accidentally and about all pregnant women with GDM (210) were studied during this period. All participants in the study signed the relevant consent form.

Measurements and Assessments

For all two groups pregnant women, OGTT, copper, zinc, and fasting insulin were evaluated in sera. OGTT was interpreted according to the IADPSG criteria (International Association of the Diabetes and Pregnancy Study Group); 75 grams of oral glucose syrup is given to a person after taking a sample of fasting blood, and two blood samples are taken one hour and two hours after drinking glucose syrup; Gestational diabetes is diagnosed with one of the following: Fasting blood glucose (FBG)>91 mg/dl; Blood glucose one hour after glucose intake: 180 mg/dl or more; Blood glucose two hours after glucose intake: 153 mg/dl or more [16].

Normal serum copper levels are 80-155 µg/dl in women, 118-302 µg/dl in pregnant women, and 14-170 µg/dl in men. These normal ranges for zinc are 70-141 µg/dl in women and 72.6-127 µg/dl in men.

Blood samples were drawn after a 6 to 8-hour overnight fast. A glucose oxidase method (Selectra XL, Vital Instrument Co. Netherlands; Bionik kit, Iran) was used for serum glucose evaluation. For insulin measurement, an enzyme immunoassay (ELISA) method (Autobio Plate Reader, China; DiaMetra kit, Italy) was used. A colorimetric method using 3,5-Di-Br-PAESA (3,5-Dibromo-2-pyridylazo)-N-ethyl-N-(3-sulphopropyl) aniline), and an essay based on 5-Br-PAPS (2-(5-Bromo-2-Pyridiylazo)-5[N-n-Propyl-N-(3-Sulfopropyl) amino] phenol) colorimetric method was used for measuring copper and zinc in sera, respectively (Selectra XL, Vital Instrument Co. Netherlands; BIOREXFARS kit, Iran).

For evaluation of insulin resistance, some indices (HOMA-IR, QUICKI, HOMA-B, 1/Insulin, G/I) were calculated using the mathematical formula as follows:

HOMA-IR (Homeostasis Model Assessment of Insulin Resistance) = (Fasting Glucose, mmol/l× Fasting Insulin, µIU/ml)/22.5 [17, 18].

QUICKI (Quantitative Insulin Sensitivity Check Index) = 1/[log (Fasting insulin, µU/ml) +log (Fasting glucose, mg/dl)][15] .

HOMA-B (Homeostasis Model Assessment for estimation of index β-cell secretion; the index of insulin secretion capacity)= (360×insulin, micro-unit/lit)/Glucose, mg/dl -63) [19].

1/Insulin (1/fasting insulin) and G/I (fasting glucose/fasting insulin) were two other calculated IR indices.

A study on the significance of the differences between insulin resistance (IR) and increased levels of copper and decreased levels of zinc in GDM and healthy non-GDM patients was assessed by statistical analysis.

Statistical Analysis

To study our hypothesis, after using the Kolmogorov-Smirnov test for examining the data distribution, the

Mann-Whitney U test was used to evaluate the significance of the difference in measured and calculated parameters between the two groups. Given that none of the study data had a normal distribution, a nonparametric test (Mann-Whitney U) was used for statistical analysis. Eventually, for investigating the relationship between IR indices with Cu and Zn variables, a linear regression test was used. By SPSS version 16.0 for Windows software, data were analyzed and p-values less than 0.05 were considered to indicate statistical significance.

RESULTS

A summary of the results in this study is presented in Table 1. The fasting insulin and copper levels were significantly higher in the GDM group than in the NGT group, while the zinc level in the GDM group is significantly less than the other.

Table 1- Summary of Measured and Calculated Parameters (Interquartile Range and Median)

Parameter	NGT (n = 250)	GDM (n = 210)	p value
FBS	79 (74.19-84)	92 (86-96)	< 0.001
GTT1 (BS-1Hr)	123 (105.11-141)	194 (182-199)	< 0.001
GTT2 (BS-2Hr)	102.18 (91.16-121.57)	156 (138-164)c	< 0.001
Zinc	83.93 (74.4-101.87)	90.17 (68.59-98.64)	0.03
Copper	98.37 (85.07-127.28)	119.57 (102.52-138.89)	< 0.001
Insulin	9.51 (7.25-12.58)	22.7 (13.7-25.4)	<0.001
HOMA-IR	1.87 (1.41-2.4)	4.96 (3.09-5.83)	< 0.001
1/Insulin	0.1 (0.07-0.13)	0.04 (0.039-0.072)	< 0.001
QUICKI	0.34 (0.33-0.36)	0.3 (0.29-0.32)	< 0.001
HOMA-B	229.96 (154.13-340.38)	263.42 (217.54-353.46)	0.14
FBS/Insulin	8.49 (6.17-10.62)	4 (3.66-6.06)	< 0.001

Differences of all IR indices values between the two above groups were statistically significant, except HOMA-B. As shown in Table 1, the median of the 1/insulin, G/I, and QUICKI indices was lower in the GDM group compared to the NGT group, whereas HOMA-IR in diabetic pregnant women was significantly higher than healthy ones. However, the difference between other indexes (HOMA-B) between NGT and GDM groups was not significant (P-value=0.14).

The result of the correlation between IR indices and two other variables (serum Zn and Cu), in GDM and NGT groups, showed no significant relationship between them.

DISCUSSION

According to our latest information, this is the first study about the relationship between IR Indices with serum zinc and copper in pregnant women with GDM.

Disorders in some minerals and their role in oxidative stress in diabetic patients play an important role in insulin resistance and the progression of diabetes [20].

Copper and zinc are essential trace elements that play a key role in the structure and function of many enzymes and are involved in important metabolic pathways.

In this study, the relationship between the changes of these two elements in pregnant women with GDM compared to healthy pregnant women, and also the relationship between these changes and insulin resistance in these people have been investigated.

Zinc is effective in the synthesis, storage, and secretion of insulin [5]. It is also effective in insulin function by regulating tyrosine kinase insulin receptors and increasing phosphorylation of tyrosine kinase [21].

Enhanced zinc needs during pregnancy and lactation increase the risk of zinc deficiency. The interaction between zinc and glucose metabolism is complex, and zinc deficiency may interfere with insulin storage and activation. Zinc deficiency is associated with insulin resistance, changes in insulin secretion, and impaired glucose metabolism. Impaired zinc homeostasis appears to be associated with emerging diabetes [22].

Numerous studies have examined plasma levels of zinc in pregnant women with GDM, but some are associated with conflicting results. Some authors show that serum zinc levels in pregnant women (28-24 weeks gestation) with an abnormal glucose tolerance test, are lower than in the normal pregnancy group [23-25].

In a study by Mariana, correlation analysis showed that plasma glucose was negatively correlated with plasma zinc concentration (r = -0.13) in GDM [26].

The results of all these studies are similar to the findings of our present study. But they contradict the research results of Behrashi et al. that did not find a statistically significant difference in serum zinc levels between the GDM and NGT groups [27].

Zinc is involved in the production, storage, and secretion of insulin monomer (Wiernsperger et al., 2010), as well as its conversion to a dimeric form for storage and secretion as crystalline insulin. The important role of zinc in the stabilization of insulin hexamers and pancreatic storage of insulin is well known and can also enhance insulin binding to liver cell membranes [28, 29].

The complexity of the relationship between zinc and glucose metabolism has been proven. Theoretically, a decrease in Zn ion concentration may harm insulin storage and activity. In other words, zinc deficiency is associated with insulin resistance and impaired insulin secretion and glucose metabolism. However, a clinical trial on pregnant

women with insulin resistance in Iran showed that zinc supplementation for 8 weeks had no significant effect on improving insulin resistance, despite an increase in serum zinc levels compared with the placebo group [30], similar to the results of our study that no association was found between insulin resistance and decreased plasma zinc levels in pregnant women with gestational diabetes.

Copper is an essential trace element for humans and is essential for copper-dependent enzymes such as SOD1 (superoxide dismutase 1) as well as glucose-6-phosphate dehydrogenase (G6PD) in the pentose phosphate pathway [31].

In Reza Didedar's study [32], similar results to those of Woods S. et al. were obtained [33], copper was lower in pregnant women with GDM than in healthy pregnant women, but this difference was not statistically significant. In Al-Saleh's study, no difference in serum zinc and copper levels was observed in women with GDM compared to healthy pregnant women [34]. All these results conflict with the results of our study. Basaki et al. showed that serum copper and zinc levels in type 2 diabetic patients were lower than in the healthy control group [20].

However, similar to our results, Wang et al. reported that copper levels increased in women with GDM compared with healthy pregnant women, although serum zinc levels in women with GDM decreased compared with healthy pregnant women [35]. Also in the other studies [36, 37], the amount of copper in the GDM group was calculated higher than in the NGT group.

A study in Bangladesh showed that serum copper levels in GDM cases were significantly higher than non-diabetic women in both the second and third trimesters of pregnancy. They suggested that the possible causes of high serum copper concentrations in GDM cases could be due to hormonal, metabolic, and enzymatic changes in pregnancy as well as decreased insulin sensitivity in GDM [38].

Employing different statistical methods, Zheng et al. and Li et al. found consistent evidence of higher copper associated with higher gestational glucose levels [39, 40].

Choi points out in his study that copper absorption increases in pregnant women due to the increased need for copper-containing enzymes, including cytochrome c oxidase and superoxide dismutase [41].

Furthermore, copper ion may play an important role in the accumulation of amyloid peptide in pancreatic islets in type 2 diabetic patients. However, given the conflicting results, it is not yet clear whether copper has a protective role in the etiology of type 2 diabetes [42].

Nevertheless, it is not yet possible to determine with certainty whether high serum copper in pregnant women with gestational diabetes is a risk factor for GDM or, under physiological and disease conditions, the amount of copper absorbed through the intestine is increased or (and) the amount of copper excreted in the urine or bile is decreased.

To study diabetes or its severity, the use of insulin resistance indicators can be valuable, especially for people who have not yet developed diabetes and who have a normal glucose tolerance test. However, these indicators are not yet widely used in laboratory diagnosis [9].

Kirwan's simultaneous use of glucose tolerance test and insulin resistance indices (to predict diabetes in women before and during pregnancy) showed that glucose tolerance test was better than fasting insulin and G/I indices for this purpose [43].

HOMA-IR, among the insulin resistance indices, was found to have the greatest ability to predict GDM in pregnant women even a few weeks before routine screening for gestational diabetes, while it seems that HOMA-B cannot be considered a reliable indicator for this prediction or screening of GDM.

According to the good correlation of QUICKI with results of the standard Euglycemic clamp method and the results of this study, it may be used in the early screening of GDM diagnosis for all pregnant women.

For Korean women with a history of GDM, a mean HOMA-IR of 2.2 to 3.2 was reported by Kwak [44]. In the Sae Jeong Yang study, this index has been calculated 2.3 that was not significantly different from the values obtained by Kwak [45].

The lack of correlation between decreased zinc levels and increased copper in the plasma of diabetic pregnant women with insulin resistance indices may indicate that although the role of these two elements in metabolic processes and insulin production and secretion is largely known, their reduction or increase cannot cause tissue resistance to insulin. However, it can be seen from the results of this study that attributing the apparent changes in plasma copper and zinc levels of pregnant women with gestational diabetes to the increase in insulin resistance in these patients seems unlikely.

CONCLUSION

Along with other diagnostic tests, and not exclusively, measurement of plasma levels of Zn and Cu can be useful in the screening of GDM. In a conclusion, evaluation of these trace elements can help study and understand the etiology of gestational diabetes rather than its diagnosis. However, we showed that insulin resistance could not be a factor in copper increase and zinc decrease in pregnant women plasma with GDM. Eventually, it can be concluded that the measurement of Zn and Cu levels in plasma cannot be used to determine insulin resistance and its severity in pregnant women with gestational diabetes and cannot be used in place of IR indices.

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Conflicts of Interest

The authors have no conflicts of interest.

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