

Original Research Article

Zinc and Insulin Resistance in Pregnancy Complicated with Gestational Diabetes

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ABSTRACT

Metabolism of certain trace elements, as zinc, was altered in gestational diabetes mellitus (GDM) with insulin resistance (IR) as a key factor in the pathophysiology. The aim of the present study was to investigate the relation between IR in GDM and plasma, and erythrocyte levels of zinc. Totally 35 pregnant women with normal glucose tolerance (NGT) and 37 pregnant with GDM were included. Zinc was measured by flame atomic absorption spectrophotometry. Positive correlations between erythrocyte zinc, plasma zinc and fasting blood glucose (FBG), insulin levels and HOMA-IR were found for healthy pregnant group. The correlations between zinc in erythrocytes and FBG, insulin and HOMA-IR were higher than those between plasma zinc and the same parameters with statistical difference for HOMA-IR ($P < 0.04$). Negative correlations between glucose and zinc levels in plasma ($r = -0.13$), between zinc in erythrocytes and FBG, insulin and HOMA-IR ($r = -0.12$; $r = -0.16$; $r = -0.16$; $p < 0.05$ and $p < 0.002$ for insulin and HOMA-IR) were established for GDM group. Our data indicate that hyperglycemia in GDM impacts negatively not only plasma zinc, but also intracellular zinc levels in erythrocytes. Possible role of erythrocyte zinc in development of IR during pregnancy with this pathology might be assumed.

Keywords: gestational diabetes; zinc in plasma, zinc in erythrocytes, insulin resistance.

INTRODUCTION

Gestational diabetes is carbohydrate intolerance that begins or is first recognized during pregnancy. ⁽¹⁾ The underlined mechanisms for GDM development are related to β -cell dysfunction and insulin resistance or decreased maternal insulin sensitivity. ⁽²⁾ During pregnancy with GDM the pancreatic β -cell function is insufficient to meet the body's insulin needs. Existing evidence suggests that β -cell defects in GDM are result from the same spectrum of causes that underlie hyperglycemia. ⁽³⁾

Zinc is required for normal glucose metabolism, and strengthens the insulin-induced transportation of glucose into cells by its effect on the insulin signaling pathway. ⁽⁴⁾ In gestational diabetes mellitus (GDM) like type 2 diabetes, in addition to insulin resistance, there is also a problem with insulin secretion with serum zinc levels being of importance on insulin function. ⁽⁵⁾

Many evidences underline association between hyperglycemia and metabolism of minerals. A part of the papers have provided data about impaired insulin secretion, and increased insulin resistance in

association with altered metabolism of many elements, particularly zinc. ⁽⁶⁾ Zinc deficiency may aggravate carbohydrate intolerance. Different studies indicate the role of zinc deficiency in glucose intolerance, diabetes mellitus, insulin resistance and cardiovascular disease. ⁽⁷⁾

Zinc is a micronutrient with requirements increased during pregnancy. Zinc deficiency is common in this specific physiological condition ⁽⁸⁾ and it has been associated with poor fetal outcomes. ^(9,10)

This study is intended to establish the relationship between zinc levels (plasma and intracellular) and insulin resistance in pregnant women with gestational diabetes.

MATERIALS AND METHODS

Study design

Totally 72 pregnant women between 24±4 gestational weeks (with NGT n=35, mean age 27.3±4.2 years) and with GDM (n=37, mean age 28.4±4.7 years) were included in the study. Written informed consent was obtained from all females. Ethical approval for the study protocol was obtained from Ethics Committee of Medical University-Sofia and the study was conducted in accordance with the ethical standards laid down in the Declaration of Helsinki of 1975, as revised in 1983.

Exclusion criteria for pregnant women were applied: chronic diseases, acute infection during pregnancy or at GDM diagnosis establishment, chronic illness, drugs affected the carbohydrate metabolism or interfered with insulin sensitivity, anemia, multiple pregnancies, known diabetes before pregnancy, fetal malformation, or other severe maternal illnesses, age <18 or >45 years, history of smoking or alcohol abuse.

All the tested pregnant women with no previously diagnosed diabetes were screened for GDM with a 2 h 75 g oral glucose tolerance test (OGTT) between 24 and 28 weeks of pregnancy. Diagnosis of GDM was according to the recommendations of the International Diabetes in Pregnancy Study Group: fasting

plasma glucose ≥ 5.1 mmol/L, 1 h ≥ 10.0 mmol/L, 2 h ≥ 8.5 mmol/L. ⁽¹¹⁾

The following data were collected for all pregnant women: age, pregnancy BMI at GDM diagnosis, gestational weeks. Blood was drawn just before, at 60 min and 120 min after ingestion of glucose for GDM diagnosis. Blood samples for insulin, glucose, zinc and hemoglobin measurements were collected from individuals in a fasting state 8.00-9.00 am, after a 12-hour fasting pause overnight. Plasma glucose was determined in the venous blood by the method of oxygen consumption (Analox GM9, Analox Instruments USA, reference range 2.8-6.1 mmol/L). Serum insulin concentration in the venous blood was analyzed by Electrochemiluminescence immunoassay (ECLIA) (Elecsys 2010, Roche Diagnostics, reference range 2.6-24.9 μ U/ml). The self-reported weight was expressed as kilograms (kg) and the height measured during the interview was expressed as squared meters (m^2) to calculate maternal BMI (kg/m^2). HOMA-IR values were calculated from the concentrations of insulin and glucose using the following formula: fasting serum insulin (μ U/ml) \times fasting plasma glucose (mmol/l)/22.5. ⁽¹²⁾

Blood for determination of plasma and hemolysate zinc levels was collected by evacuated tubes (Sarstedt Monovette, Germany, 7.5 ml, LH-Metall Analytic, anticoagulant lithium heparin). Hemoglobin in hemolysates was analysed by hematological analyzer ABX Micros 60 (Horiba, Kyoto, Japan).

Zinc measurements in plasma and hemolysate were done by flame atomic absorption spectrophotometry (Perkin Elmer AAnalyst 300, USA). ⁽¹³⁾

All laboratory assays are done at Central Clinical Laboratory, University Hospital "Alexandrovska"-Sofia, Bulgaria.

Statistical analysis

Shapiro-Wilk test is used to determine whether each variable has a normal distribution. Kruskal-Wallis test and

the U Mann-Whitney test are applied for comparison of the selected groups. All continuous variables are presented as mean values \pm SD (standard deviation). A $p < 0.05$ value is defined as significant for statistical difference. Comparisons between the subgroups are performed by one-way analysis of variance (ANOVA) with post-hoc analysis to locate the differences. Student t-test is used to study the

differences between zinc levels in the tested groups of patients and controls. Data are processed by Statistical software for Windows 13.0 SPSS-SPSS.

RESULTS

Clinical and metabolic characteristics of the participants with the data of statistical analysis were presented in Table 1,2,3,4.

Table 1: Clinical and metabolic characteristics of the participants

Characteristics	Pregnant with NGT (n ₁ =35)	Pregnant with GDM (n ₂ =37)	Statistical significance (P<0.05)
Age (years)	27.3 \pm 4.2	28.4 \pm 4.7	NS
BMI (kg/m ²)	25.1 \pm 3.0	30.8 \pm 4.0	NS
Gestational weeks	24 \pm 4	24 \pm 4	NS
Fasting glucose (Glu ₀) (mmol/l)	4.41 \pm 0.28	6.32 \pm 1.1	p<0.004
HOMA-IR	1.95 \pm 1.03	3.31 \pm 3.05	p<0.01
Fasting insulin μ U/l	8.13 \pm 5.3	14.9 \pm 8.1	p < 0.02
Zinc in plasma (μ mol/L)	11.02 \pm 2.11	12.05 \pm 2.02	NS
Zinc in erys (μ mol/g Hb)	0.63 \pm 0.11	0.78 \pm 0.15	NS

Table 2: Correlation coefficients between fasting blood glucose, fasting insulin levels, HOMA-IR and Zn in plasma and Zn in erythrocytes in pregnant women with NGT

Parameters	Zn in plasma	Zn in erythrocytes	Statistical significance (P<0.05)
Fasting glucose (mmol/l)	r=0.180	r=-0.240	P=0.26
Fasting insulin μ U/l	r=0.09	r=0.200	P=0.08
HOMA-IR	r=0.140	r=0.235	P<0.04

Table 3: Correlation coefficients between fasting blood glucose, fasting insulin levels, HOMA-IR and Zn in plasma and Zn in erythrocytes in pregnant women with GDM

Parameters	Zn in plasma	Zn in erythrocytes	Statistical significance (P<0.05)
Fasting glucose (mmol/l)	r= -0.13	r= -0.12	P=0.76
Fasting insulin μ U/l	r= 0.06	r= -0.16	P<0.05
HOMA-IR	r= 0.02	r= -0.16	P<0.002

Table 4: Correlation coefficients between baseline parameters, Zn levels in plasma and in erythrocytes in pregnant women with NGT and GDM

Parameters	Pregnant women with NGT		Pregnant women with GDM		Statistical significance (P<0.05)
	Zn in plasma	Zn in erythrocytes	Zn in plasma	Zn in erythrocytes	
Fasting glucose (mmol/l)	r=0.180*	r=0.240•	r=-0.13*	r=-0.12•	P=0.26* P<0.003•
Fasting insulin μ U/l	r=0.09 **	r=0.200••	r=0.06**	r=-0.16••	P=0.42** P=0.28••
HOMA-IR	r=0.140•	r=0.235••	r=0.02•	r=-0.16••	P<0.004• P=0.08••

Legend: *-correlation coefficient- zinc in plasma and FBG between two pregnant groups

** - correlation coefficient - zinc in plasma and insulin between two pregnant groups ; • - correlation coefficient - zinc in erythrocytes and FBG between two pregnant groups; •• - correlation coefficient - zinc in erythrocytes and insulin between two pregnant groups; • correlation coefficient- zinc in plasma and HOMA-IR between two pregnant groups; •• correlation coefficient- zinc in erythrocytes and HOMA-IR between two pregnant groups.

DISCUSSION

None of the variables show any statistically significant difference between the two groups on the baseline characteristics as age, BMI, gestational weeks. Significant statistical changes ($p < 0.02$, $p < 0.004$; $p < 0.01$) were observed for serum levels of insulin, FBG and HOMA-IR respectively. No significant difference in

plasma and intracellular (erythrocyte hemolysate) zinc level between NGT and GDM pregnant women is established in this study. These results are compatible with the results from our own previous study. (13)

Numerous studies examine plasma and intracellular zinc levels in GDM pregnant women, but with contradictory results. Some authors (Bo et al., 2005) show

that zinc serum levels in pregnant women (24-28 weeks of gestational age) with abnormal glucose tolerance test is lower than in normal pregnancy group. ⁽¹⁴⁾ According to Wang et al. (2002) serum zinc of pregnant women with GDM is decreased compared to that of normal pregnant women. ⁽¹⁵⁾ Lower levels of serum zinc in GDM pregnant women in comparison to normal pregnant women in third trimester as results of monitoring, is reported by Hussein (2005). ⁽¹⁶⁾ Behrashi et al. (2011) do not establish statistically significant difference between GDM and NGT pregnant group regarding serum zinc levels. ⁽¹⁷⁾

Increased insulin resistance is often considered as one of the major factors leading to GDM. Insulin resistance increases during pregnancy, especially during the last trimester, due to increased maternal adipose tissue, placental hormones and increased insulin clearance by the placenta. ⁽¹⁸⁾

Several mechanisms have been suggested to explain the association between zinc and insulin resistance. Zinc is involved in the synthesis, storage, and secretion of monomeric insulin, ⁽¹⁹⁾ as well as conversion to a dimeric form for storage and secretion as crystalline insulin. Zinc is known to play a major role in the stabilization of insulin hexamers and in the pancreatic storage of insulin because it can enhance insulin binding to hepatocyte membranes. ^(20,21)

Zinc regulates insulin function via stimulation of insulin tyrosine kinase receptors and increasing the phosphorylation of tyrosine-kinase. ⁽²²⁾ Zinc is essential in insulin action and carbohydrate metabolism. ⁽²³⁾ Additionally, zinc is a cofactor of antioxidant enzymes such superoxide dismutase (SOD) and catalase, and is involved in the protection of pancreatic beta cells ⁽²⁴⁾ and insulin against free radicals. ⁽²⁵⁾

Positive correlations between zinc in erythrocytes and plasma zinc and FBG, insulin and HOMA-IR for healthy pregnant females are found in the present study.

Interestingly, the correlations between zinc level in erythrocytes and FBG, insulin and HOMA-IR are higher than the correlation coefficients between zinc in plasma and the same parameters with statistical difference for HOMA-IR $P < 0.04$.

Pregnant women with GDM show opposite results—most of correlation coefficients are negative (especially between erythrocyte zinc and FBG, insulin and HOMA IR) and negative correlation between plasma zinc and FBG with significant difference for HOMA-IR and fasting insulin ($P < 0.002$; $P = 0.05$, respectively). The detailed analysis of the results for both pregnant groups depicts statistical difference between correlation coefficients of glucose and zinc in erythrocytes ($P < 0.003$). Intriguingly, significant correlation of HOMA-IR and zinc in plasma ($P < 0.004$) is observed and at the same time lack of such a significant difference in terms of the correlation between insulin and zinc in both groups of pregnant females.

In our study correlation analysis shows that plasma glucose is negatively correlated with plasma zinc concentration ($r = -0.13$) in GDM. The result is similar to this of Ugwuja et al. (2010) ⁽²⁶⁾ and in consonance with the report of Esfahani et al. (2011) with finding of a significant negative correlation between zinc and glycemic control ⁽²⁷⁾ According to the present data, a negative correlation exists only between zinc in plasma and glucose level, but negative correlations are seen between Zn in erythrocytes and glucose, fasting insulin and HOMA-IR in GDM group. Obviously, hyperglycaemia in pregnancy complicated with gestational diabetes impacts negatively plasma zinc status. This fact could reflect the antioxidant properties of zinc. ⁽³⁰⁾ The significantly negative correlation between plasma glucose and plasma zinc corroborates earlier studies. ^(28,29) Some authors suggest two possible roles for zinc: an inhibition of the post insulin receptor intracellular events with results of a decreased glucose tolerance and of a

relatively decreased insulin secretion. Zinc is an important component of intracellular signaling pathway and of transporters thus providing essential role in regulation of insulin and glucose homeostasis. ⁽³¹⁾

According to Roshanravan et al. (2015) zinc supplementation increases serum zinc significantly and decreases fasting glucose, insulin and HOMA-IR but with no statistically significant effect. ⁽³²⁾ Some other researchers have found that serum zinc concentration increases significantly, but FBG does not change after zinc supplementation in the healthy obese adults ⁽³³⁾ and in diabetic patients. ⁽³⁴⁾

Zinc and insulin concentrations in the pancreas change concomitantly in a variety of physiological and pathological changes in humans. ⁽³⁵⁾

Australian researchers have found improvement of fasting glucose with zinc supplementation over 6 month and statistically significant improvements in beta-cell function, insulin resistance and insulin sensitivity in patients with pre-diabetes. Zinc is necessary in β -cells for insulin crystallization in hexamers. Moreover, it is co-secreted with insulin and exerts insulinomimetic and antioxidant actions and participates in the regulation of β -cell mass. ⁽³⁶⁾

Serum zinc homeostasis during pregnancy has been linked to placental hormones. ⁽³⁷⁾ Failure to use gestational-appropriate standards for evaluating the plasma zinc concentrations leads to erroneous conclusions about maternal zinc status. ⁽³⁸⁾ Obviously, these various reasons are not able to explain apparently complex character of the pathology event during pregnancy.

CONCLUSION

We did not manage to find enough experience and publications to explain a negative correlation between zinc in erythrocytes and some parameters of the insulin resistance such glucose, insulin or HOMA-IR. Perhaps further studies are needed to elucidate this phenomenon and its

role in connection to insulin resistance. Future studies could explore the relationship between zinc in erythrocytes and metabolic risk factors including insulin resistance not only in pregnant women with GDM.

Finally, this research points that hyperglycaemia in pregnancy complicated with GDM impacts negatively not only plasma zinc status, but also intracellular zinc in erythrocytes. Possible participation of erythrocyte zinc in insulin resistance during GDM might be considered.

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