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RESEARCH ARTICLE

PLASMA AND INTRACELLULAR ERYTHROCYTE ZINC LEVELS DURING PREGNANCY IN BULGARIAN FEMALES WITH AND WITHOUT GESTATIONAL DIABETES

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Abstract

Maternal zinc deficiency is often seen during pregnancy. Zinc is a micronutrient whose requirements increase with pregnancy, but Zinc deficiency during pregnancy is common.

The purpose of the study was to estimate plasma and intracellular (erythrocyte hemolysate) zinc in pregnant with/without gestational diabetes mellitus (GDM) women, compared to healthy non pregnant.

Patients and Methods: The study involved pregnant with GDM (n=45), healthy pregnant women with normal glucose tolerance (NGT, n=45) consecutively and fasting non-pregnant healthy women (n=45). Blood samples were obtained in the third trimester in both pregnant groups. The blood samples were analyzed for plasma and intracellular zinc by Flame Atomic Absorption Spectrophotometry (FAAS). Results: Plasma zinc level in normal pregnant, compared to non-pregnant controls were (11.40±2.51 μmol/l vs 15.55±2.48 μmol/l, $p<0.0001$; zinc in erythrocytes 0.65±0.12 μmol/l/g Hb vs 0.56±0.09 μmol/l/g Hb, $p<0.0001$) and GDM women, compared to non-pregnant controls (11.81 ± 2.42 μmol/l vs.15.55±2.48 μmol/l, $p<0.0001$; zinc in erythrocytes 0.66±0.13μmol/l/g Hb vs. 0.56±0.09 μmol/l/g Hb, $p<0.0002$). The study founded positive correlation between plasma zinc levels and pro-insulin in GDM pregnant ($r=0.225$) and expected negative in healthy pregnant women ($r=-0.119$).

Conclusion: The survey did not identify statistically significant differences between healthy pregnant women and those with GDM in the level of plasma and hemolysate zinc. Both pregnant groups had higher level of intracellular erythrocyte zinc in comparison to non-pregnant.

INTRODUCTION

Zinc is an essential constituent of over 200 metalloenzymes participating in carbohydrate and protein metabolism, nucleic acid synthesis, antioxidant functions, and other vital functions (21), including growth, bone formation, reproduction, fetal development, immune mechanisms (36, 45). It is known that zinc has insulin-like functions in lipogenesis (13), glucose transport (29) and leptin production in humans (12). Zinc is indispensable in the proper maintenance of pregnancy (34). Frequently pregnancy may be associated with disorders of glucose metabolism resulting in gestational diabetes. The definition GDM as any degree of glucose intolerance with onset or first recognition during pregnancy is largely accepted. The underlined mechanisms for the development of GDM are related to β-cell dysfunction and insulin resistance or decreased maternal insulin sensitivity. Pregnancy is normally attended by progressive insulin resistance that begins near mid-pregnancy and progresses through the third trimester to levels that approximate the insulin resistance typical for the type 2 diabetes (3). There is also secretion problem of insulin. In this regard, due to importance of zinc serum levels on insulin function, the role of zinc deficiency in pregnancy is remarkable (1, 38).

Women with GDM are at a higher risk for preeclampsia during pregnancy and fetal growth restriction (31), complications for mother and fetus during delivery and at high risk of developing of diabetes mellitus type 2 (DM2) later in life (5). It has been known for decades that a physical chemical relationship exists between insulin and zinc. It is clear that the predominant effect on zinc homeostasis of diabetes is hypozincemia which may be the result of hyperzincuria or decreased gastrointestinal absorption of Zn or both (11).

Plasma zinc concentration is commonly used as an indicator of zinc status, but the assessment is more difficult during pregnancy because plasma zinc concentrations decline in proportion to the increase in plasma volume (40). Zinc has been routinely determined in plasma and in erythrocytes as the advantages and disadvantages of using erythrocytes for this element are reviewed. Erythrocyte zinc has potential application in detecting of zinc deficiency related to development of neural tube defects in the early stages of pregnancy (42). Zinc concentration in erythrocytes appears to be a more sensitive index to detect cellular deficiency (42%) than plasma zinc (32%) (26).

MATERIAL AND METHODS

Study design

Totally 80 pregnant women between 24±4 gestational weeks (NGT n=45, mean age 27,3±4,2 years and GDM n=45, mean age 28,4±4.7 years) were included in the study. Control group of non-pregnant women consisted of 45 female volunteers, clinically healthy, mean age 26,1±4.8 years. Written informed consent was obtained from all subjects. Ethical approval for the study protocol was obtained from Ethics Committee of Medical University-Sofia. Selected anthropometric, clinical, and pathophysiological parameters were evaluated in all of them.

Exclusion criteria for pregnant women were 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 21 and 28 weeks of pregnancy. Diagnosis of GDM was in accordance 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 (20).

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 μU/ ml). Serum for pro-insulin was frozen at -20°C and pro-insulin levels were measured by ELISA method (Mercodia, AB Company, Uppsala, Sweden, reference range 3,3–28 pmol/l). Internal Quality Control Scheme in applied by control materials "Control L" 10.4 (8.55–12.3 pmol/l) and „Control H„ 40.8 (34.7-46.9 pmol/l). The measurement was performed on ELISA Microplate Multimode Detector Zenith 3100 (Anthos).

The self-reported weight is expressed as kilograms (kg) and the height measured during the interview is expressed as squared meters (m^2) to calculate maternal BMI (kg/m^2).

Health status of non-pregnant female controls was evaluated on the base of the following laboratory tests: WBC were determined by hematological analyzer ABX Micros 60 (Horiba, Kyoto, Japan), fasting serum glucose, serum creatinine, GFR, serum enzymes AST and ALT, serum total cholesterol and triglycerides (Cobas Integra 400, Roche).

Zinc quantitative determination in plasma and hemolysate

Blood for plasma and hemolysate zinc was collected by evacuated tubes (Sarstedt Monovette, Germany, 7.5 ml, LH-Metall Analytic, anticoagulant lithium heparin). Plasma was separated by centrifugation (20 min, 3000 rpm) up to 1 hour after blood collection and plasma zinc concentration were analysed. After removing of the separated plasma, fresh erythrocytes were washed 3 times with ice cold saline solution (NaCl 0.015 mol/L). Saline was added to erythrocyte mass to restore the initial tube volume of 7.5 ml. After every washing, the samples were centrifuged and the washing solution was removed. The washed erythrocytes were lyses by adding of ice cold bidistilled water (1+1, sample+water by volume). The samples were refrigerated (2-4 ^{0}C) for 24 hours. Then, zinc and hemoglobin in hemolysates were measured. Hemoglobin in hemolysates was analyses 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). The instrumental parameters of the spectrophotometer are presented in Table 1. The measurements were done after method calibration by standard calibration solution with known zinc concentration (zinc stock standard Titrisol, 1000 mg Zn as $ZnCl_2$ in 0.06% HCl, Merck KGaA, Darmstedt, Germany). The analyses were done by validated method for zinc measurement in biological fluids. Scheme for Internal Quality Control was applied as component of Quality Assurance System for zinc.

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

Statistical analysis

The Shapiro-Wilk test was used to determine whether each variable had a normal distribution. The Kruskal-Wallis test and the U Mann-Whitney test were used to compare selected groups. All continuous variables are presented as mean values +/− SD (standard deviation). A $p<0.05$ value was defined as significant. Comparisons between the subgroups were performed by one-way analysis of variance (ANOVA) with post-hoc analysis to locate the differences. Student t-test was used to find out the significant differences between zinc levels for the tested groups of patients and controls. Data were analyzed using Statistical software for Windows 13.0 by SPSS – SPSS.

RESULTS

Clinical and metabolic characteristics of the participants with the data of statistical analysis are presented in Table 2.

Tabl. 1. Instrumental parameters of flame atomic spectrophotometer Perkin Elmer AAnalyst 300

Wavelength (nm)	213.9
Spectral Bandwidth (Slit) (nm)	0.7
Light Source	Electrodeless Discharge Lamp (EDL)
Light Current (W)	6
Atomizer	Flame: air 2L/min-acetylene 10 L/min
Preliminary sample preparation	Proper dilution of plasma and hemolysate with bidistilled water

Tabl. 2. Clinical and metabolic characteristics of the participants

Characteristics	Pregnant with NGT (n_1=45)	Pregnant with GDM (n_2=45)	Non-pregnant women (n=45)	Statistical significance
Age (years)	27.3 ±4.2	28.4 ±4.7	26.1±4.8	NS
BMI (kg/m^2)	25.8±3,5	26.2 ±3.9	25.4±2.2	NS

Gestational weeks	24±4	24±4		NS
Fasting glucose (mmol/l)	4.62 ± 0.28	5.9 ± 1.07		p<0.0001
Glucose at 60 min (mmol/l)	6.95± 1.37	8.49 ± 2.29		p< 0.001
Glucose at 120 min (mmol/l)	6.14 ± 1.21	7.49 ± 2.04		p<0.005
Proinsulin at 0 min (pmol/l)	3.11 ± 2.78	7.59 ± 5.27		p=0.006
Fasting insulin μIU/l	9.15 ± 5.1	12.95 ± 8.5		p= 0.02
Zinc in plasma (μmol/L)	11.4±2.51 ▫	11.81±2.42 *	*15.55±2.48▫	p<0.0001▫ p<0.0001*
Zinc in erys (μmol/g Hb)	0.65±0.12 ▪▪	0.66±0.13 **	**0.56±0.09▪▪	p<0.0001▪▪ p<0.006**

BMI — body mass index; NS=Not significant (p>0.05).

DISCUSSION

The results of the present study revealed that there was a statistically significant decrease in zinc for pregnant women–NGT and GDM, when compared to non-pregnant healthy females as revealed by plasma levels. Significantly lower serum zinc levels in pregnancy have been reported in some studies for different parts of the world (21, 28, 2, 15). Earlier studies also showed a decrease in serum levels of zinc during pregnancy (40, 8, 18). According to Kirksey et al.,(1994) and other researches, plasma zinc level decreases as pregnancy progresses (25, 21, 27). In normal pregnancies and in the three periods of the third trimester, zinc concentration in plasma of the mother is significantly lower than in non-pregnant women (34). Ejezie and Nwagha (2011) established that the plasma zinc level decreased as gestation progressed, with the lowest concentration of serum zinc obtained during the third trimester (15). Brito et al. (2013) established that during pregnancy, the plasmatic zinc decreased by 20% to 30% from the third month, with decreasing related to the progress of pregnancy (9). The decreasing plasma Zn concentrations during pregnancy may be due to the expansion of plasma volume seen in nearly all pregnant women, but it is not exactly clear to what extent plasma volume expansion is responsible for decreasing Zn concentrations (41). Another important aspects in the reduction of this mineral in the plasma are increased urinary excretion (39), the transfer of this mineral from mother to the growing fetus (9, 24), the effect of hormones, in particular increased levels of estrogen (22). High levels of corticosteroids are also related to this common reduction at the end of a normal pregnancy (33, 37). The decrease in plasma Zn could be associated with reduced plasma levels of albumin during pregnancy (17). Serum zinc homeostasis during pregnancy has been linked to placental hormones (15). On the other hand, it is important to note that failure to use gestational-appropriate standards for evaluating the plasma zinc concentrations leads to erroneous conclusions about maternal zinc status (24). Obviously, these reasons are not able to explain this apparently complex event in pregnancy.

Despite of certain evidence, there are studies that show a lack of difference in plasma zinc concentrations between pregnant and non-pregnant women (30). According to Reyes et al. (2000) for normal pregnancies studied in 1994-1995, zinc levels showed no difference compared to that in non-pregnant women (35). Borella et al. (1990) found a slight, but significant, increase in plasma zinc in pregnant women affected by gestational diabetes, probably as a result of reduced zinc uptake by the fetus (7).

The observations in the present study suggested that this variability in zinc levels during pregnancy could be also due to influence of more complex factors including also altered rate of intestinal absorption and changed dietary habits during this special physiological state.

No significant difference in plasma and intracellular (erythrocyte hemolysate) zinc level between NGT and GDM pregnant women was established in this study. Furthermore, the erythrocyte zinc levels in both pregnant groups were significantly higher than in non-pregnant controls. We also established plasma zinc levels lower for both pregnant groups as compared to non-pregnant controls. The reduction of this mineral in the plasma could be also related to certain redistribution from the fluid compartment into the erythrocytes because of increased activity of zinc dependent erythrocytes enzymes (9). Some authors have noted that erythrocytes actively absorb trace elements required for enzymatic reactions or bound to membrane proteins. So, during 120 days life span, erythrocyte levels can be be useful index for evaluation of nutritional or disease status (26). High concentration of zinc in the erythrocytes could demonstrate adequate nutritional status of the mineral. Zinc level in erythrocytes is a parameter reflected long-term nutritional status as it is changed only when severe deficiency occurs (16).

Numerous studies were performed to determine plasma and intracellular zinc levels in GDM pregnant women, but with contradictory results. Other authors, (Bo et al., 2005) showed that zinc serum levels in pregnant women (24-28 of gestational age) with abnormal glucose tolerance test were lower than in normal pregnancy group (6). Another study, conducted by Wang et al. (2002) showed serum zinc of pregnant women with GDM decreased compared to normal pregnant women (43). Lower levels of serum zinc in GDM pregnant in comparison to normal pregnant women in third trimester as results of monitoring, were reported by Hussein (2005) (19). Behrashi et al. (2011) did not establish statistically significant difference between GDM ant NGT pregnant group regarding serum zinc levels (4).

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 (10). Zinc is involved in the processes insulin synthesis, as secretory vesicles transfer pro-insulin from the endoplasmic reticulum to the Golgi apparatus with rich of zinc and calcium environment favours formation of soluble zinc-containing pro-insulin hexamers and retention of the activity under storage condition and during mobilization. Pro-insulin and zinc typically comprise no more than 6% of the islet cell secretion (14). According to the presented in this study observations, it could be suggested that zinc deficiency would lead to reduce insulin's effect. The reduced insulin activity, associated with high blood glucose and fat deposition in obese subjects with history for hereditary predisposition and metabolic dysfunctions, leads to functional failure of the islet β-cells. Women with GDM tend to be obese; adipose tissue affects carbohydrate metabolism by different metabolic pathways. Progressive β-cells dysfunction has been reported in women with GDM that, over years, leads to progressive hyperglycemia and diabetes after pregnancy (44). Pro-insulin levels increase together with insulin concentrations in insulin resistance (32). Increased pro-insulin concentrations are specific for GDM and might thus serve as marker and potentially even identify subjects at high risk for the development of type 2 diabetes (23). The important feature in the results of this study was that the mean levels of pro-insulin were significantly higher during pregnancy in GDM pregnant than NGT ($p=0.006$). The correlation analysis in the present study showed that pro-insulin was positive correlated with plasma zinc concentration in GDM pregnancy ($r=0.225$) and negatively in pregnant with NGT ($r=-0.119$).

In conclusion, we established that the survey did not identify statistically significant differences between healthy pregnant women and those with GDM in the level of zinc both for plasma and intracellular content. The tested pregnant groups presented higher hemolysate zinc levels in comparison to non-pregnant group.

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