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Research Paper

Plasma and Intracellular Erythrocyte Magnesium Levels in Healthy Pregnancy and Pregnancy with Gestational Diabetes

Medical Science

KEYWORDS : Gestational Diabetes Mellitus, Normal Pregnant, Plasma Magnesium, Magnesium In Erythrocytes Hemolysate

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ABSTRACT

The aim of the study was to estimate plasma and intracellular hemolysate magnesium in pregnant with/without gestational diabetes mellitus (GDM) women in the third trimester, compared to non pregnant. Patients and Methods: The study involved GDM pregnant, n=40, healthy pregnant women with normal glucose tolerance (NGT) n=40, and fasting nonpregnant (n=40). The blood samples were analyzed for plasma and intracellular magnesium by Flame Atomic Absorption Spectrophotometry (FAAS). Results: NGT pregnant, compared to nonpregnant controls (plasma magnesium 0.695±0.06 mmol/L vs 0.78±0.079 mmol/L, p<0.0036; magnesium in erythrocytes 6.54±1.28 <mol/l/g Hb vs 5.54±0.76<mol/l/g Hb, p<0.0002) and GDM women, compared to non pregnant controls (plasma magnesium 0.70±0.06 mmol/L vs.0.78±0.079 mmol/L, not significant), magnesium in erythrocytes 6.7±2.22<mol/l/g Hb vs. 5.54±0.76<mol/l/g Hb, p<0.006). It was detected that healthy pregnant women had lower levels of Mg in the plasma compared to healthy non-pregnant, but not in pregnant with GDM. The level of intracellular Mg is higher in both groups of pregnant women in comparison to non-pregnant. Conclusion: The survey did not identify statistically significant differences between healthy pregnant women and those with GDM in the level of magnesium both in plasma and intracellularly.

INTRODUCTION

Magnesium is the second most abundant intracellular cation (after potassium) and its plasma concentration is remarkably constant in healthy subjects. Magnesium is directly involved in numerous important biochemical reactions, intracellular Mg is a critical cofactor for several enzymes in carbohydrate metabolism, in regulating insulin action, insulin-mediated-glucose uptake (3). Magnesium deficiency may result in disorders, affecting the activity of the insulin receptor and related to the development of a postreceptorial insulin resistance, and thus to decreased cellular glucose utilization (15). In vitro studies have shown that Mg has an important role in insulin action (17). GDM is defined as carbohydrate intolerance that begins or is first recognized during pregnancy (18). The underlined mechanisms for the development of GDM are related to β-cell dysfunction and insulin resistance (IR) or decreased maternal insulin sensitivity. Pregnancy is normally attended by progressive IR that begins near mid-pregnancy and progresses through the third trimester to levels that approximate the insulin resistance typical for the type 2 diabetes (5). Women with GDM are at a higher risk for preeclampsia during pregnancy, complications for mother and fetus during delivery and at high risk of developing of diabetes mellitus type 2 (DM2) later in life (7). It is known that plasma Mg falls in pregnancy because of accumulation of ion in the placenta and fetus. Bardicef et al.(1995) have come to the conclusion that pregnancy is in fact a state of extracellular depletion of magnesium deposits (6). Pregnant women are prone to lose magnesium. Some researchers have suggested that this depletion was more pronounced in women affected by GDM. In this connection, it is assumed that the elevation in female hormones and magnesium deficiency during pregnancy impairs insulin sensitivity and these disturbances may act synergistically (20). Nair et al. (2012) hypothesized that pregnant women who have an altered magnesium levels handling are particularly prone to severe impairment of glucose tolerance or GDM (20). Insulin-resistant patients have an impaired insulin-mediated erythrocyte Mg accumulation that correlates with a decrease in insulin sensitivity (23). Some research studies on magnesium status during pregnancy are conducted, sometimes with contradictory results. The great result variety might be explained by the use of different methodological approaches (various study designs; consideration of different factors, affecting magnesium balance; analytical methods for quantitative magnesium measurement in biological samples not always with guarantee quality of the results) (9). Total magnesium does not always reflect the real magnesium status, whereas the intracellular fraction is biologically active and reflects magnesium status in better way (32).

The aim of the present study was to assess levels of plasma and intracellular (in hemolysates) magnesium in pregnant with/without GDM women, compared to non pregnant controls.

Material And Methods

Study design

A total of 80 pregnant women between 24±4 gestational weeks (40 GDM and 40 with NGT) were included in the study. Written informed consent was obtained from all subjects. Ethical approval for the study protocol was obtained from the 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 establishing of diagnosis, drugs affected the carbohydrate metabolism or interfered with insulin sensitivity, multiple pregnancies, known diabetes, fetal malformation, or other severe maternal illnesses, age < 18 or > 45 years.

All the tested pregnant women with no previously diagnosed diabetes went trough screening for GDM with a 2 h 75 g oral glucose tolerance test (OGTT). Diagnosis of GDM was in accordance to the recommendations of the International Association of Diabetes and Pregnancy Study Groups (IADPSG) - fasting plasma glucose ≥5.1 mmol/L, 1 h ≥10.0 mmol/L, 2 h ≥8.5 mmol/L (13).

The following data were collected for all women: age, pregnan-

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cy body mass index [BMI] at GDM diagnosis, gestational weeks. Samples were drawn just before, at 60 min and 120 min after ingestion of glucose for GDM diagnosis.

Blood samples for insulin, glucose and magnesium measurements were drawn from individuals in a fasting state between 8.00 am and 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_electrochemiluminescent immunoassay (ECLIA) (Elecsys 2010, Roshe Diagnostics, reference range 2.6 – 24.9 mU/ ml).

Blood for total plasma and erythrocyte magnesium was collected by evacuated tubes (Sarstedt Monovette, Germany, 7.5 ml, LH-Metall-Analytic, dry lithium heparin as anticoagulant). After centrifugation within 1 hour after blood collection, plasma was separated and magnesium plasma concentrations were analysed.

Preparation of hemolysates

After removing the separated plasma, the fresh erythrocytes were washed 3-times with ice cold saline solution (NaCl 0.15 mol/L). Saline was added to erythrocyte mass to restore the initial total volume of the tube (7.5 ml). The samples were centrifuged after every washing and the washing saline solution was removed. The washed erythrocyte samples were lysed by adding ice cold bidistilled water (1 + 1, sample + water by volume). Hemolysates were refrigerated (2-4^{0}C) for 24-hours before the same quantitative determination of total erythrocyte magnesium. Hemoglobin in hemolysates was determined by hematological analyzer Cobas Micros 60 ABX (Roshe Diagnostics).

Magnesium quantitative determination

Magnesium measurements in biological specimens were done by flame atomic absorption spectrophotometry (atomic absorption spectrophotometer Perkin Elmer AAnalyst 100, USA). The instrumental parameters of the spectrophotometer are presented in table 1. Measurements were done after method calibration by standard calibration solutions with known magnesium concentration (magnesium stock standard Titrisol, 1000 mg Mg as MgCl2 in 6% HCl, Merck, Germany). The analyses were done by validated method for magnesium in biological fluids. A scheme for Internal Quality Control is applied to guarantee the result quality.

All laboratory assays were performed at the Central Clinical Laboratory, University Hospital " Alexandrovska".

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 proportions of serum magnesium and levels between patients and controls. Data were analyzed using Statistical software for Windows 13.0 by SPSS – SPSS.

RESULTS

A total of 80 pregnant women were enrolled in the study (mean age 27.34, SD±4.4 years), where fasting glucose, glucose at 60 min, glucose at 120 min,

insulin at the basal state and HOMA-IR were statistically higher in GDM pregnant ($p<0.0001$, <0.001, $p<0.005$, $p=0.02$, $p<0.0001$). Statistically difference between plasma and erythrocytes magnesium in NGT women and non-pregnant controls was found ($p<0.0036$, $p<0.0002$), between erythrocytes magnesium GDM and controls ($p<0.006$). No significant differences between plasma magnesium in GDM and controls and between both groups of pregnant women regarding magnesium levels in plasma and erythrocytes were established.

Table 1. Instrumental parameters of flame atomic spectrophotometer Perkin Elmer Aanalyst 300

Wavelength (nm)	285.2
Spectral Bandwidth (Slit) (nm)	0.7
Light Source	Hollow Cathode Lamp (HCL)
Light Current (mA)	6 mA
Atomizer	Flame air-acetylene
Preliminary sample preparation	Proper dilution of plasma and hemolysate with 0.25% La(III)-chlorid-heptahydrat (Merck, Germany).

Table 2. Clinical and metabolic characteristics of the participants.

Characteristics	Pregnant with NGT (n_1=40)	Pregnant with GDM (n_2=40)	Non-pregnant women (n=40)	P Value
Age (years)	27.51 ±4.27	28.2 ±4.53	26.31±4.63	NS
BMI (kg/m^2)	25.83±3,54	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
Fasting insulin mIU/l	9.15 ± 5.1	12.95 ± 8.5		p= 0.02
HOMA-IR	1.79 ± 1.08	3.8 ± 3.05		p<0.0001
Mg in plasma (mmol/l)	0.69±0.06 ▫	0.70±0.06 *	*0.78±0.079▫	p<0.003▫ NS*
Mg in erys (mmol/l g Hb)	6.54±1.28 ▪▪	6.7±2.22 **	**5.54±0.76▪▪	p<0.0002▪▪ p<0.006**

HOMA-IR - homeostasis model assessment for insulin resistance; NS=Not significant (p>0.05).

DISCUSSION

Pregnant women have lower plasma magnesium levels compared to non-pregnant controls. First reports on magnesium levels in pregnant were made in 1923. Further, researchers found low serum magnesium levels in pregnant during the third trimester than in normal non-pregnant women (Arcella, 1965) (27). Lim *et al.* (1969) similarly reported significantly lower serum (and erythrocyte) levels in normal pregnant women in the third trimester than in normal non-pregnant women. The average value for erythrocyte Mg was also lower than in non-pregnant women (16). Researchers link these differences with the increasing needs of the growing fetus. When the dietary intake of magnesium is not sufficient to meet the demands of gestation, the maternal stores are mobilized and magnesium deficiency can develop (27).

Numerous studies in pregnant women to determine serum and intracellular magnesium were performed, but the results are contradictory (10). A subset of these researchers found that total and intracellular erythrocyte magnesium were significantly lower both normal pregnant and gestational diabetic individuals compared with non-pregnant women, using a nuclear magnetic resonance spectroscopy. These results support idea about magnesium depletion in pregnancy itself and to a greater extent in gestational diabetes. Bardicef *et al.*(1995) have come to the conclusion that pregnancy is in fact a state of extracellular depletion of magnesium deposits (6). Yamasaki (2012) found that blood levels of total Mg, ionized Mg and intracellular Mg of platelet were lowered in the 2nd trimester of gestation and then. At the same time urinary excretion of Mg does not change during the whole period of pregnancy (33). It is generally accepted that pregnant women have lower plasma magnesium levels compared to non-pregnant controls. Our observations about plasma magnesium levels in pregnant with NGT were in the same direc-

tion – statistical decreasing of plasma total magnesium in comparison to non-pregnant control group ($p<0.003$). Intriguingly, contrary to some opinions, not such statistically significant decreasing of plasma levels was observed for GDM pregnant group compared to non-pregnant control group. Plasma levels between both pregnant groups did not differ statistically and they were just below the lower limit of the reference interval of plasma magnesium for Bulgarian population (0.7 – 1.2 mmol/l) with no evidence for clinically manifested magnesium deficiency. Erythrocyte intracellular total magnesium was statistically increased during pregnancy (NGT and GDM in comparison to non-pregnant controls ($p<0.0002$; $p<0.006$)) with no evidence for statistical difference between intracellular magnesium for both pregnant studied groups; only very slight tendency for increasing of erythrocyte magnesium in GDM pregnant compared to pregnant with NGT was presented.

Our data, similar to those of other authors, suggest not significant depletion of total magnesium during normal pregnancy (9). Decreasing of plasma magnesium, observed by us, statistically expressed but not clinically relevant, might be result of possible hemodilution. Also, we did not find any data about hypomagnesaemia in pregnancy with GDM possibly because they were under proper control. Established by us significant increasing in total intracellular magnesium for both pregnant groups might reflect physiological adaptive mechanisms to keep intracellular energy balance during pregnancy. This physiological response could be associated with some redistribution and accumulation of magnesium in the erythrocytes.

The adaptive mechanism might be due to eventual elevation of magnesium levels in erythrocytes because of possible intracellular depletion of potassium (27).

From the other hand, increased erythrocyte magnesium levels in both pregnant groups could reflect the fact that pregnant women are usually encouraged to keep more balanced nutrition and to take vitamin and mineral supplements.

Only slight, not statistically expressed increasing of erythrocyte magnesium in pregnant women with GDM, could be due from one hand to disordered insulin secretion and insulin resistance and from the other hand due to good clinical management of risky pregnancy with GDM.

Our observations with data for statistical elevation of erythrocyte magnesium during pregnancy but with no statistical difference between both pregnant groups (only slight tendency for increasing of intracellular erythrocyte magnesium in GDM pregnant) imply that physiological adaptive mechanisms during pregnancy could prevalence upon possible magnesium intracellular accumulation due to disordered glucose balance.

This study did not find data about pronounced magnesium deficiency during pregnancy –normal and even diagnosed with GDM-on the base of plasma magnesium. Present study underlined once more considerable controversy in the results for magnesium both under physiological and pathological conditions. The variety in reported results is probably related to variety both in biological specimens (serum, plasma, erythrocytes or other mononuclear cells) and in analytical methods for quantitative magnesium measurements. Not all analytical methods guarantee the result quality. Flame atomic absorption spectrophotometry is a reference clinical laboratory method for determination of total magnesium in biological fluids. This analytical platform provides proper control of certain interferences thus assuring results with very high quality. Definitely, measurement of total magnesium both in plasma and erythrocytes by atomic absorption principle is one underlined advantage of the presents study. Determination of hemolysate magnesium by atomic absorption could present one routine laboratory approach for assessment of intracellular magnesium concentration.

Plasma and intracellular Mg concentrations are tightly regulated by several factors. Among them, insulin seems to be one of the most important (4, 12, 22, 25). Highly specific insulin receptors have been identified on human erythrocytes. These available human erythrocyte has both specific insulin binding sites and binding characteristics similar to other human cell types (14). It was also observed that insulin enhances intracellular magnesium uptake (12) and this in turn mediates diverse effects ascribed to insulin (1). Insulin also modulates the activity of the ion transport mechanism of cells, such as erythrocytes, platelets and rat uterus cells (30). Furthermore, insulin partially regulates intracellular magnesium accumulation (24).

It is well known that pregnancy results in a state of insulin resistance (8). This condition during pregnancy mediated by the placental anti-insulin hormones estrogen, progesterone, human somatomammotropin; the pituitary hormone prolactin; and the adrenal hormone, cortisol. If the maternal pancreas cannot increase production of insulin to sustain normoglycemia despite these anti-insulin hormones, gestational diabetes occurs (21).

Homko *et al.* (2001) reported that patients with GDM during late pregnancy were more insulin resistant than controls (11). According to the International Diabetes Federation criteria, the HOMA-IR cut-off point to differentiate between low and high insulin resistance is 2.38, several previous studies performed on smaller populations have demonstrated that HOMA-IR index assessed at diagnosis of GDM ranged from 1.6 to 25 (28). Subjects with type 2 DM are more insulin resistant and this impairs the ability of insulin to stimulate Mg and glucose uptake (2). In this regard insulin-resistant patients have an impaired insulin-mediated erythrocyte Mg accumulation that correlates with a decrease in insulin sensitivity (24). These results may explain established by the authors relationship between high degree of IR and Mg metabolism disturbance (30). In our research, it was specified that HOMA-IR values in pregnant women were in range 0.7-6.8. We hypothesed that the higher level of intracellular magnesium in pregnancy might be result of insulin resistance grade.

Our data for total erythrocyte and plasma magnesium levels both in NGT and GDM pregnancy suggest that modulation of nutritional status by changed dietary habits or supplementation would be critical for maintain magnesium balance in extreme physiological and even in pathological situations. Also, our findings indicate that good pregnancy management is especially important for adequate magnesium body balance assessed by plasma and intracellular magnesium.

In fact, there is no simple, rapid and accurate laboratory test to indicate the total body magnesium status. The total serum magnesium concentration is not the best method to evaluate magnesium status as changes in serum protein concentrations may affect the total concentration of magnesium (29). The red blood cell magnesium **should be the clinical practice "Gold Standard" for the diagnosis of normomagnesemia because it is truly measures latent and intracellular magnesium deficiency** (19). **M**agnesium deficiency in pregnant women is frequently observed due to inadequate or low magnesium intake (34-31), there are some disagreements about magnesium depletion in patients with gestational diabetes (34). The haemodilution during the last trimester of pregnancy could be another contributing factor leading to a higher prevalence of deficiency of magnesium (26). A change in diet and the type of food intake are probably another reason for hypomagnesemia in pregnant women.

Molecular mechanisms of hypomagnesemia in pregnancy and also its clinical complications, associated with insulin resistance and GDM, are not sufficiently studied. There is need for more detailed and comprehensive studies with very carefully considered study design and special attention to many different important factors: physiological, nutritional, clinical manifestation of magnesium disorders, standartisation of pre-analytical phase and use of highly reliable analytical methods for laboratory evaluation. Only such complex approach would elucidate in more details complicate picture of magnesium metabolism thus providing better base for treating of magnesium disorders.

Conclusion: The findings of the present study indicate accumulation of intracellular magnesium during normal and GDM pregnancy compared to non-pregnant controls. No data for underlined hypomagnesaemia during normal pregnancy and GDM pregnancy were observed. Increased total intracellular magnesium for both pregnant groups might reflect physiological adaptive mechanisms during pregnancy with some redistribution and accumulation of magnesium in the erythrocytes. There is a need for conducting of more comprehensive studies on magnesium balance during pregnancy with monitoring of the levels even in the postpartum period, especially for women with a history of GDM.

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