PREGNANCY OUTCOMES IN NORMOGLYCEMIC WOMEN WITH HYPERINSULINEMIA TREATED WITH METFORMIN BEFORE AND DURING PREGNANCY – A CASE-CONTROL STUDY BIGUANID IN PREGNANCY OF HYPERINSULINEMIC WOMEN

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Summary. The aim of this study was to evaluate the effects of metformin on maternal and neonatal pregnancy outcome among normoglycemic hyperinsulinemic women with one previous spontaneous abortion (SA). A prospective, two-year case-control study was performed including sixty-six pregnant women with normal carbohydrate tolerance before pregnancies and one SA. A 75-gram Oral Glucose Tolerance Test (OGTT) was performed before pregnancy. The levels of blood glucose (BG) and immunoreactive insulin (IRI) were measured at 0 min, 60 min and 120 min. Women with hyperinsulinemia were treated with metformin (0.75 g/day) before and during pregnancy. OGTT was repeated at 12 and 36 gestational weeks (g.w.) in all pregnant women. Pregnant women were divided into two groups: non-treated with metformin (Group 1; $n_1 = 32$) and treated with metformin (Group 2; $n_2 = 34$). The changes in BG, IRI levels during OGTT, live births and SA rates and newborn's body weight were recorded. Statistical comparison was performed between treated and non-treated pregnant women. Logistical regression analysis was used to assess the effect of hyperinsulinaemia and metformin on the risk of pregnancy loss. No statistically significant differences in the mean values for age, body mass index (BMI) and BG levels were found. The IRI was significantly higher in women of n_1 compared to n_2 in early and in late pregnancy. Eleven (34.4%) pregnant women of n_1 and five (14.7%) of n_2 presented impaired glucose tolerance (IGT) at late pregnancy (P=0.03). There was no case of gestational diabetes (GD) in n_2, whereas

four (12.5%) of n_1 developed GD in late pregnancy. There were no maternal complications and no birth defects in the group of patients treated with metformin. The body weight of the newborns was similar for both groups. The rate of miscarriage was 18.7% in Group 1, and 8.8% in Group 2. Pregnant women with IRI levels over 50 mIU/ml at 12 g.w. showed a significantly higher risk for SA in comparison with those with normal level of IRI, proved by the model of logistic regression (OR = 4.9; CI. 2.1-19.3). There was no statistically significant effect of metformin on the logistic regression model. Conclusion of our study was that the treatment with metformin during pregnancy was safe, improved metabolic markers and significantly reduced spontaneous miscarriage rates.

Key words: *hyperinsulinemia, insulin resistance, gestational diabetes, metformin, pregnancy outcome, spontaneous abortion*

INTRODUCTION

Puberty and pregnancy are two physiologic conditions associated with decreased sensitiveness of peripheral tissues to biological action of insulin. Insulin resistance (IR) results from hormonal changes and its severity may be variable. Glucose balance is supported, in normal range, because pancreatic β-cells react by adequate raise in insulin secretion and this way compensates the decreased biological effect of the insulin action. During pregnancy, hormones which decrease peripheral insulin sensitiveness are increased and glucose homeostasis is supported by balanced changes in insulin secretion. This mechanism enhances simultaneously with progress of pregnancy. Hyperinsulinemic IR before pregnancy could be a precondition for the occurrence of pancreatic β-cells dysfunction later [1, 2]. During pregnancy, IR is a risk factor for undesirable maternal-fetal complications. Gestational Diabetes (GD) is the pathological condition of a carbohydrate intolerance of variable severity, which occurs as a result of inadequate β-cell insulin production in response to tissue resistance [3, 4].

The biguanid metformin (dyametyl guanyl guanidine) is usually used in type 2 diabetes (T2D). It reduces plasma insulin levels by reducing hepatic gluconeogenesis and stimulating peripheral glucose uptake. Treatment with metformin improves the defects of the insulin activity, decreases the insulin secretion, reduces the ovarian steroidogenesis and slows the progress of GD in women with polycystic ovarian syndrome (PCOS) [6, 7]. Metformin decreases the weight, preserves β-cell reserves and decreases the risk of future development of T2D in women with PCOS [8, 9]. Metformin is generally considered safe and it has received a pregnancy category B. Glueck at al. reported improvement of the pregnancy outcome after metformin treatment, without teratogenic or other side effects concerning both the physical, the motor as well as the social development of the newborn [10]. Metformin decreases fibrinolysis and enhanced thrombogenesis, resulting from IR by suppressing the action of the tissue plasminogen activator-1 (PAI-1) and de-

creasing the concentration of plasminogen activator type 1 (PAI-1) inhibitors [11]. Jakubowiez at al showed that the frequency of miscarriages decreased after treatment with metformin in pregnant women with PCOS [12].

OBJECTIVE

Assuming that IR may be one of the probable key mechanisms for the miscarriage, we supposed that the therapy with metformin before and during pregnancy decreases the frequency of SA in normoglycemic, hyperinsulinemic women with one previous SA.

STUDY DESIGN

This was a prospective, observational, case-control study type, comparing the pregnancy outcome in metformin treated group versus non-treated group in regard to miscarriages. The study population was ethnic Bulgarian patients, who achieved pregnancy for the second time after one previous SA. All patients attended the Clinical Institute of Reproductive Medicine, Pleven, Bulgaria, from May 2006 to May 2008 (n = 66).

SUBJECTS AND METHODS

Cases and controls. Before the beginning of the diagnostic procedure, all women were informed about the study and signed an informed agreement. The study was approved by the Local Ethic Board. The participants' selection was done according to the following including criteria: age (range 18-35), BMI ≤ 25 kg/m^2, presence of a second, planed pregnancy with a previous history of one SA, defined as a pregnancy interruption before 12 weeks' gestation. There were not disturbance of glucose tolerance before pregnancy, hyperandrogenemia, and PCOS. Excluding criteria were: PCOS, hyperglycemia before pregnancy, hormonal treatment of infertility, inherited or acquired thrombophilia, hyperandrogenemia, hormonal or immunological signs of thyroid autoimmune disease. Pregnant women were divided into two groups: non treated with metformin (Group 1; n_1 = 32) and treated with metformin (Group 2; n_2 = 34).

Diagnostic criteria. All women underwent a 75 g OGTT before pregnancy. The levels of BG in venous plasma and IRI in serum at 0, 60, and 120 min after glucose loading were measured. The BG results were interpreted according to the criteria of WHO [13]. The values of IRI during OGTT were used for diagnosing concomitant hyperinsulinemia. IRI levels at 0 min ≥ 20 mUI/ml, at 60 min ≥ 50 mUI/ml, at 120 min ≥ 25 mUI/ ml, were considered a manifestation of IR. All women with normal glucose tolerance and with IR were treated with metformin in a daily dose of 750 mg, administered in three applications of 250 mg. The metformin treatment was performed until the end of the pregnancy. All participants were given dietetic

instructions for food intake with low rapid carbohydrate and high protein contents. All women took folic acid (1 mg per day) before and during pregnancy until delivery. During pregnancy, the pregnant women were advised to keep the dietetic instructions similar to the ones given before pregnancy.

All pregnancies were planed in advance applying a multidisciplinary team approach – obstetrician, immunologist and endocrinologist. Before the pregnancy, hormonal, immunologic and genetic examinations were performed to exclude the most frequent causes for pregnancy loss. These examinations include a thyroid and sex hormones analysis, including testosterone, anti-phospholipids and anti-thyroid antibodies, as well as DNA mutations, associated with inherited thrombophilia. The 75 g – OGTTs were repeated twice during pregnancy: between the 10–12 g.w. and the 34-36 g.w. Final OGTTs were performed in the women, whose pregnancies were terminated with miscarriage at the moment of pregnancy interruption. The change of BG and IRI during pregnancy, the frequency of miscarriages and the birth weight of the newborns were recorded. BG measurements were performed immediately after blood taking using the glucooxydases method in mmol/l on a gluco-analyzer: the Beckman's apparatus, at a referent range of 3.7-5.5 mmol/l. The level of IRI was determined using electrohemoluminiscence method (ECLICA) by the analyzer Roche Elecsys 1010 for imunnoassays, in a referent range of 5.0-15 mUI/l. This test showed 0.05% from the crossed reactivity towards the intact human proinsulin and insulin.

All statistic analysis was done with the statistic panel SPSS for Windows version 11.0.1. The results are presented as an average value and their standard deviations (SD), which are to be signed as "mean ± SD" or n (%). The difference between the groups is determined by the two tailed Student,s t test and the Mann-Whithey's test. ANOVA was applied for multiple comparison. Multivariate logistical regression analysis was use to assess the effect of IR, metformin and other risk factors, including age, BG and BMI. Adjusted odds ratio (OR) and its 95% confidenence interval (CI) was calculated on the analysis. Statistical significance level of $P < 0.05$ was used.

RESULTS

The mean age of the pregnant women was 28.5 yrs, ranging from 23 to 34 yrs without significant difference between both groups. No women were overweights or obeses before pregnancy. The mean BMI in early pregnancy was 21 kg/m^2 ranging from 19 to 24 kg/m^2 similar for both groups. Fourteen patients of g_1 (43.8%) and eighteen of g_2 (52.9%) had a family history of type 1 diabetes or type 2 diabetes mellitus in the first and/or in the second generation.

Twenty-six (81.2%) women of g_1 and thirty-one (91.2%) women of g_2 carried their full term pregnancy. The metabolic characteristics evaluating the change in the BG level and IRI before and during pregnancy of women in the first group are

shown in Table 1. The mean values of BG at the end of first trimester were similar to those before pregnancy. The value of IRI increased significantly at beginning of the pregnancy, in 11 women (34.3%) IR was established in early pregnancy. The mean values of BG levels at the end of pregnancy were significantly higher in comparison with those in a non-pregnant state. Fifteen pregnant women (46.8%) have had different stage of carbohydrate intolerance: 11 of them (34.4%) were with IGT and 4 (12.5%) with GD. The IRI values in the late pregnancy increased significantly in all pregnant women compared to the early pregnancy. IR at the end of the pregnancy was proved in 26 pregnant women (81.2%); all of them had history for diabetes.

Table 1 shows also the changes in the mean values of BG and IRI for women of the second group, before and during pregnancy. In early pregnancy, there were no changes in the carbohydrate tolerance or in the IRI concentration in any of the pregnant women. The mean values of BG and IRI in early pregnancy were similar to the levels in a non-pregnant state. At the end of pregnancy, evidence for IGT and increased IR was established in five (14.7%) pregnant women, despite the metformin therapy. There was no case of GD.

The difference in metabolic parameters in the first and in the second group during pregnancy are shown in Table 2. There were no significant differences in BG levels after glucose loading in early and late pregnancy, but the concentrations of insulin were significantly higher in first group until pregnancy. There was significant difference in frequency of carbohydrate intolerance disturbances between both groups – 46.8% n_1 women vs. 14.7% n_2 women (P=0.03).

Table 2. Changes in metabolic parameters of patients of first and second Group

	Early pregnancy g_1= 32	Early pregnancy g_2= 34	Significance	Late pregnancy g_1= 26	Late pregnancy g_2= 31	Significance
BG-0 min.	5.1 ± 0.6	4.3 ± 1.2	NS	6.5 ± 1.2	5.5 ± 0.4	NS
BG-60 min.	7.9 ± 1.6	6.5 ± 1.8	NS	8.8 ± 1.9	8.1 ± 0.8	NS
BG-120min.	7.4 ± 1.2	6.1 ± 0.6	NS	7.9 ± 1.3	7.4 ± 0.9	NS
IRI-0 min.	16.2 ± 8.5	11.4 ± 3.2	NS	22.2 ±12.9	17.2 ± 9.1	P=0.051
IRI-60 min.	56.8 ±12.8	23.3± 12.4	P=0.02	93.8 ± 15.5	48.4 ±13.5	P=0.001
IRI-120 min.	37.3 ±10.3	15.3 ± 9.3	P=0.04	85.6 ± 23.7	31.5 ± 17.8	P=0.001

BMI rised in all of the pregnant women during pregnancy. Compared to the first group, the women in the second one had a significantly lower BMI in late pregnancy (g_1 = 31.5 ± 4.2 кg/m^2 vs. g_2 = 26.8 ±3.6 кg/m^2; P = 0.01). The highest weight gain during pregnancy was observed in pregnant women with IGT which belong to first group. The observed differences in the BMI mean values between the pregnant women with IGT in both groups were significant (g_1 = 30.6 ± 2.1 кg/m^2 vs. g_2 = 28.3 ± 2.4 кg/m^2; P = 0.02).

Of all sixty-six observed pregnancies 57 (86.4%) were successfully terminated. There were no observed obstetrical complications as pregnancy induced hypertension, preeclampsia, preterm deliveries or infections in any one of them. The estimated rate of live births of newborns was 86.4%. There was no case of prenatal death or congenital anomalies in both groups. The mean weight of the newborns was 3.580 ± 381.3 g. There was no difference between the weight of newborns in both groups (g_1 = 3.600.9 ± 343.5 g.; g_2 = 3.460.3 ± 408.9 g.; P = 0.6). There was no newborn with macrosomia (> 4.000 g).

The overall SA rate was 13.6% (9 out of 66).There was a significant difference in miscarriage rate between the two groups: 18.75% (6 out of 32) in the first group and 8.82% (3 out of 34) in the second group (P=0.02). Women with miscarriages had equal levels of BG and IRI at the moment of fetal lost (table 3). There was no significant difference in BG levels in early pregnancy between women with miscarriages and women with successfully terminated pregnancy, but the concentrations of insulin measured at 60 and 120 min after glucose loading were significantly higher in women with miscarriages comparing to those without miscarriages [(IRI measured at 60 min in SA group = 72.7 ± 6.6 mIU/ml vs. IRI at 60 min in non SA group = 40.0 ± 12.6 mIU/ml; P=0.001) and IRI measured at 120 min in SA group = 48.5 ± 12.6 mIU/ml vs. IRI at 120 min in non SA group = 26.3 ± 9.8 mIU/ml; P=0.001)].

Table 3. The Mean levels of BG and IRI in women with miscarriage

	Miscarriage g_1= 6	Miscarriage g_2= 3	Significance
BG-0 min.	5.6 ± 0.4	5.2 ± 0.2	NS
BG-60 min.	8.1 ± 0.3	8.8 ± 0.7	NS
BG-120min.	7.1 ± 0.6	6.9 ± 0.4	NS
IRI-0 min.	12.8 ± 6.2	10.3 ± 2.7	NS
IRI-60 min.	76.6 ± 8.3	68.5 ± 6.3	NS
IRI-120 min.	49.7 ± 9.5	48.2 ± 4.1	NS

Risk factor analysis of the miscarriage occurrence did not determine relationship among the age, the BG levels and the BMI in early pregnancy. A relationship was determined only between the IRI measured at 60 and 120 min in early pregnancy. Pregnant women with IRI levels over 50 mIU/ml at 60 min had a significantly higher risk for SA in comparison with those who had normal level of IRI, proved by the model of logistic regression (OR = 4.9 ; CI. 2.1-19.3). Patients with IRI levels

over 40 mIU/ml at 120 min also have a significantly higher risk for miscarriages (OR =3.4; 95% CI, 1.7- 5.1).

DISCUSSION

All pregnancies observed by us were preplanned for excluding the most frequent causes for miscarriages. Familial history for diabetes mellitus and absence of strong reasons for fetal loss established the implementation of OGTT. The results of this study indicated, that the risk for miscarriage is positively related to the IRI levels in early pregnancy. Pregnant women with IRI higher than 40 mIU/ml have a four times higher risk for SA that of those with IRI of 40 mIU/ml or less. IRI is an independent risk factor for pregnancy loss, no matter which BG level and weight gain during pregnancy. The finding that IR is a possible risk factor for SA within the Bulgarian population gave an important information and helped us to propose a new diagnostic and prophylactic approach. The criterion adopted by us for IR – levels of IRI higher than normal during OGTT – is less precise than the euglycemic clamp but is well standardized, economical, not very invasive and easily applicable for outpatients.

Although all the specific pathophysiologies of pregnancy losses in normal women remain unknown, we suggest that decreasing of the glucose-induced insulin secretion by insulin sensitizers such as metformin will reduce the rate of miscarriages among women with one previous SA. The obtained results revealed that the rate of SA could be reduced after medication to 8.8%.

Probably this is only the first of many reports showing the direct relationship between the exact IR levels and the risk of SA. A similar risk definition but with HOMA IR without using euglycemic clamp was performed by Tain Li et al in women with prior sterility and applied assistant reproductive technique [14]. The author reported that HOMA IR higher than 4.5 increased eight times the risk for miscarriage in women with overweight or PCOS.

Chronic hyperinsulinemia in women with PCOS is at the root of obesity, thrombosis of placental capillaries, abnormal endometrial growth and functioning during implantation. Apart of these mechanisms, IR decreases the production of two specific proteins, the glycodelin and the IGR- binding protein-1 (IGF-BP1), which are secreted by endometrium and play an important role in the endometrial receptivity during implantation and early pregnancy. Glycodelin inhibits the immunity response of the endometrium to the embryo by blocking the mixed lymphocyte reaction and the NK (natural killer) cell activity. IGF-1BP accelerates the process of adhesion and maternal-fetal interaction during the preimplantation period [15]. The pathophysiology of miscarriage in insulin resistant women without PCOS is unwell-studied. Hyperinsulinemia increases PAI-1 levels [16, 17] and may cause placental bed thrombosis in women with recurrent pregnancy loss without gene defects for thrombophilia [18].

In conclusion, IR plays a critical role in the etiology of SA in hyperinsulinemic women. Medication with metformin reduces hyperinsulinemia and decreases the frequency of miscarriages to 8.8% without side effects on the fetal growing. In the future, a large, randomized prospective study would be necessary to clarify the possible mechanisms on how IR may cause SA in hypeinsulinemic women without PCOS. Moreovers future studies are needed to determine the equality of different insulin sensitizing drugs in decreasing miscarriage in normoglycemic women without PCOS.

Acknowledgements
We thank all the mothers who collaborated in the study

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