PROINSULIN LEVEL IN WOMEN WITH GESTATIONAL DIABETES ACCORDING TO BODY MASS INDEX

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Diabetes mellitus is associated during pregnancy with pathologic changes both in mother and fetus, with an increased risk of morbidity and perinatal mortality, particularly associated with macrosomia. Our study included a group of 15 gestational diabetes women (GD) and 24 pregnant women without diabetes, aiming for a better understanding of the GD pathogenesis. The higher level of proinsulin found in GD patients could be related with a defect in posttranslational processing of secretory promolecules in endoplasmic reticulum (ER), leading to the production of immature secretory vesicles (SV). Such vesicles cannot be promptly and efficient exocyted, explaining the decompensation of blood glucose regulation. This defect could be also associated with overweight/obesity, which induces an overload of the pancreatic β cell function and is often associated with GD. A subliminal genetic defect in the pancreatic β cell function associated with the transient overload, specific for pregnancy, could explain the temporary decompensation of blood glucose regulation, specific for GD, which often disappears after delivery. However, gestational diabetes and macrosomia remain strong risk indicators for the later development of persistent diabetes mellitus.

Key words: Gestational diabetes; body mass index; proinsulin level.

INTRODUCTION

After the two major diabetes phenotypes (Type 1 – T1DM and Type 2 Diabetes –T2DM), the next phenotype (as importance and prevalence) is represented by gestational diabetes (GD). This has been studied in the context of the relationship between diabetes and pregnancy, a topic on which Priscilla White (1900–1989) published her first article in 1935[1]. The 27 pages chapter 19 ("Pregnancy and Diabetes") of the 1971 edition of *Joslin's Diabetes Mellitus*[2], has been written by her on the basis of an unique experience in this field that time: 1822 pregnant diabetics whose offspring reached the viable period of 28 weeks, seen and treated between January 1936 and March 1968. At that time, gestational diabetes was described as "*diabetes of any degree of severity appearing during pregnancy with remission after delivery and reappearing only in subsequent pregnancies*". From that time dates the old method of diagnosing gestational diabetes based on a preliminary oral glucose tolerance testing with 50 g of glucose followed in cases of incertitude by a 100 g oral glucose tolerance test. We think this has been an uninspired proposal that was repeatedly criticized during the last years[3,4]. Finally in 2010, following the proposals of the *International Association of Diabetes and Pregnancy Study Group* it has been agreed that the diagnosis of GD will be made using an oral glucose tolerance test with 75 g of glucose, but with slightly different cut-off values than for the other phenotypes of diabetes[5].

A less wise decision promoted in the last two classification of diabetes has been to include in the GD group any form of glucose alteration diagnosed for the first time during pregnancy, irrespectively if this alteration disappears or not after delivery. By this,

a great part of the cases classified as GD will be in fact genuine type 2 diabetes cases diagnosed during pregnancy. The distinction of GD from T2DM and T1DM can be suggested by a correct investigation of diabetes in pregnancy soon after its diagnosis. If in the first trimester of pregnancy blood glucose levels are clearly normal (below 90 mg/dl), but in the second part of gestation blood glucose increases progressively towards 100 mg/dl, a 75 g OGTT will settle the diagnosis of GD: fasting blood glucose > 92 mg/dl (7 mmol/l), 1 h > 180 mg/dL (10 mmol/l), and 2 h blood glucose > 153 mg/dL (8,6 mmol/l); any one abnormal value form these three is diagnostic for GD. If a defect in blood glucose regulation is detected in the first trimester of pregnancy (eventually associated with HbA1c > 7%), type 2 diabetes is probably the correct diagnostic. If the symptoms of diabetes are suggestive for severe alteration of blood glucose regulation (polydipsia, polyuria, weight loss) associated with high blood glucose levels, ketonuria/ketonemia and high HbA1c, T1DM can be the most probable diagnostic but requires for confirmation the detection of anti beta cell antibodies[3,4,6]. In fact, this is a pregnancy that occurred on the background of an ongoing anti beta cell autoimmunity, progressing towards overt T1DM.

Today, the pathogenesis of GD remains still unclear as in the time of Priscilla White[2], involving hypersecretion of some placentar hormones as well as maternal estrogens, progesterone and cortisol, all included among the insulin "hormonal anta-gonists"[1]. Fetal macrosomia, anatomo-histological alterations of placenta, as well as hyperplasia and hypertrophia of pancreatic islets of the infants with "fetal gigantism" are common complications of diabetic pregnancies. An important role in the excessive placento-fetal growth is attributed to IGF-I and the placentar growth factor II (paternally imprinted) which function to facilitate materno-fetal nutrient transfer in late pregnancy, a defect that may depend on fetal insulin levels[7].

The present paper intends to investigate the pathogenesis of GD taking into account it's frequent association with overweight/obesity, observed more and more frequently in pregnant women with and without GD.

PATIENTS AND METHODS

Inclusion and exclusion criteria: pregnant patients of 18–40 years old regardless of parity, with full term pregnancy spontaneous obtained and with only one foetus were selected for this study. Exclusion criteria included pre-existent pathology such as: inflammatory diseases, systemic lupus erythematosus, anemia with hemoglobin under 7 g/L, known diabetes mellitus prior to pregnancy, congenital and acquired thrombophilia known or diagnosed during current pregnancy, benign and malign tumoral pathology, foetal malformations suggestive of trisomy diagnosed in current pregnancy.

Informed consent has been obtained in written from all patients. The study was performed according to the international recommendations regarding human studies, fulfills the ethic standards for human experiments as specified in the Declaration of Helsinki and received the approval of the ethic local committee.

Study groups: The patient group included 15 women with gestational diabetes and a control group of 24 pregnant women with OGTT negative for GD. The diagnostic criteria used for GD were those proposed by the *International Association of Diabetes and Pregnancy Study Group* and currently accepted by ADA[5]. All patients were monitored during pregnancy at Dr. "I. Cantacuzino" Hospital, Bucharest, in 2009.

For all subjects the following parameters were recorded: age, gestation rank, gestational age, weight and BMI prior to pregnancy, weight gain during pregnancy, blood pressure and the presence of the other metabolic syndrome components. Obesity prior to pregnancy was defined as a BMI > 30 kg/mp. Genealogical trees for diabetes and obesity have also been obtained for all subjects from the two groups.

In order to evaluate the fetus development, for all neonates weight, gestational age and the Apgar score were recorded. The morphological evaluation of the placenta at birth was made by measuring its weight and the foeto-placental index – neonate's weight divided to placenta's weight (both in grams).

Collection of samples: For both study groups, samples of blood from the umbilical cord have been obtained immediately after birth after surgical clamp of the umbilical cord. In order to minimize the influence of blood hemolysis on insulin measurements, the samples were immediately transmitted to the laboratory and centrifuged in the next approximately 20 minutes. Plasma was frozen in 60 minutes. The collecting protocol for sampling has been available in all maternity rooms of the obstetrics clinic. The assessment of insulin and proinsulin (pg/ml) from the umbilical cord samples has been done using the Elisa method.

For both study groups, venous blood was drawn from the mothers during the first trimester and the following biochemical determinations were performed using automatic immuno-enzymatic methods: total hemoglobin, plasma glucose, triglycerides and cholesterol.

There was no antenatal administration of corticoids in the last 24 hours prior to delivery. All deliveries were on full term (over 37 weeks). The placenta was examined and weighed immediately after birth.

Statistical analysis: was made using the student's t test and the multiple regression analysis with the help of SPSS 17.0 software version. The statistical significance threshold was set for a p value < 0.05.

RESULTS

The main clinical characteristics of the women from the gestational diabetes group and the control group are given in the Table 1.

After analysis of the data, it comes out that the patients with GD had a body mass index and values of blood pressure significantly higher than the control group. As expected, the weight of newborns was significantly higher in the GD group. There are no differences regarding the mother's age or the gestational age, the rank of parity nor the gender of the neonates. In a first analysis, the placenta had a tendency of higher weight in GD (571.28 ± 83 *vs*. 693 ± 79, p > 0.001), but the foeto-placental index didn't show significant differences on patients with gestational diabetes. The Apgar score has not been different between the two groups (8.5±0.65 *vs*. 8.33±0.90, p = 0.50).

From the family history for diabetes and obesity (Table 2), a significantly higher frequency of these diseases in relatives of GD patients *vs*. controls can be noticed. The same tendency may also be observed in the case of personal history of macrosomia on prior deliveries, as well as for the metabolic syndrome presence in family.

Table 1

The main clinical characteristics of women from the two studied groups

	Control Group **n = 24**	**Gestational Diabetes** **n = 15**	**P[*]**
Age (years)	27.92 ± 5.9	26.8 ± 2.7	0.43
Parity			
1	12 (50)	8 (53.3)	
2	10 (41.7)	5 (33.3)	
>2	2 (8.3)	2 (13.3)	0.91
Gestational age at birth (weeks)	39.21 ± 1.53	39.27 ± 1.28	0.90
BMI (kg/mp)	22.9±4.3	30.9±2.8	<0.001
Systolic BP	120±10	139±5.7	<0.001
Gender new born(M/F)	13/11	8/7	0.96
Weight NB (grams)[‡]	3425.5 ± 458	4150 ± 469.4	<0.001
Male	3511±308	4425±486	<0.001
Female	3322 ± 590	3835 ± 149	0.005
Weight of placenta (g)	571.28 ± 96	693 ± 79	<0.001
Feto-placentar index	6.03 ± 0.41	5.99 ± 0.32	0.78

Table 2

Personal and heredo-collateral antecedents of GD *vs*. normal women

	Control Group n=24	**Gestational Diabetes n=15**	**P***
Diabetes heredity	4 (16%)	**9 (60%)**	**<0.05**
Obesity heredity	5 (20%)	**7 (46%)**	**<0.05**
Antecedent macrosomia	0 (0%)	**3 (20%)**	**<0.05**
Previous presence of metabolic syndrome	4 (16%)	**12 (80%)**	**<0.05**

* Hi^2 Test for non-parametric variable.

From Table 3 and Figure 1 it can be seen that both insulin and proinsulin are significantly increased in the umbilical cord of women with gestational diabetes *vs.* control group: insulin 81.3 pmol/l (53–121) *vs.* 42.29 pmol/l (15.25–64.58), **p** > 0.001 and proinsulin 13.4 (8–23) pmol/l *vs.* 8.71 pmol (4–18), **p** > 0.001). This corresponds also with a higher weight of the newborn from GD women vs. control group (Table 2). Dyslipidemia was also more prevalent in GD women in comparison with the control group.

The analysis of pregnant women according to the presence of obesity is interesting, suggesting that part of the biochemical disturbances associated with GD are similar with that encountered in obesity. Thus, from Table 4, we can notice a significant increase of plasma insulin and proinsulin in the umbilical cord of neonates from non-diabetic women with isolated obesity, which suggests that obesity can induce a significant overload of the fetus pancreatic β cell function.

In cases with obesity, systolic blood pressure and gestational age at birth have been significantly higher. It can be observed a tendency of growth regarding the weight of neonates and placenta, but the small number of cases did not allow reaching statistical significance. The Apgar score has not been significantly different between the two groups.
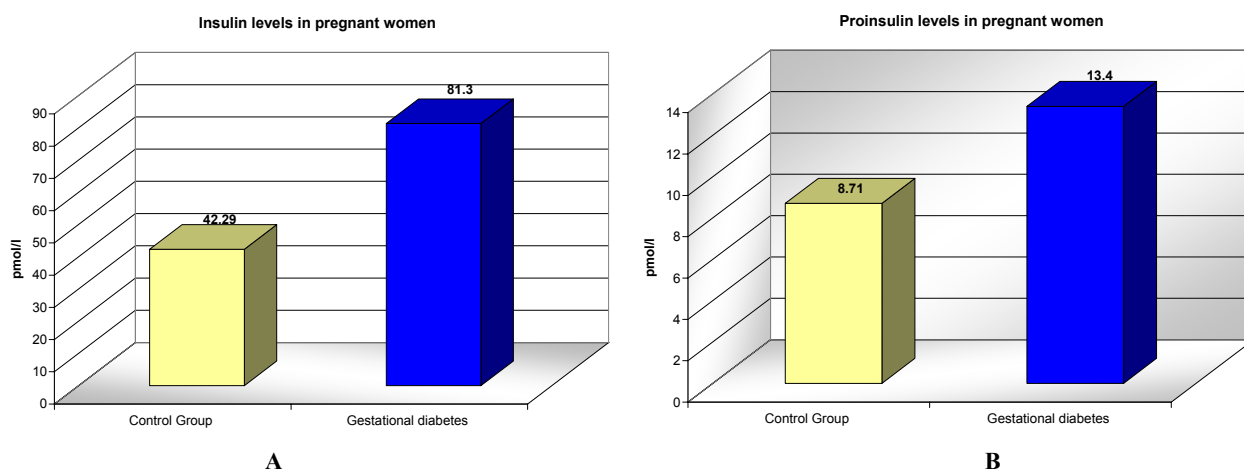

Fig. 1. The levels of insulin (A) and proinsulin (B) in the two groups of pregnant women.

Table 3

Umbilical cord blood	Control Group n = 24	Gestational Diabetes n = 15	p
Insulin (pmol/l)	42.29 (15.25–64.58)	81.3 (53–121)	<0.001
Proinsulin (pmol/l)	8.71 (4–18)	13.4 (8-23)	<0.001
Maternal blood			
Hemoglobin (g/l)	11.54±1.25	12.19 ±0.90	0.08
Blood glucose (mg/dl)	81.98± 12	124,73±44	<0.001
Cholesterol (mg/dl)	192±30	240±17.11	<0.001
Tryglicerides (mg/dl)	170.5±30	224.5±28	<0.001

Table 4

Various parameters according to presence or not of obesity

	Normal weight Controls	Obese Controls	*P**	GD	*P**
Number	20	4		15	
Age (years)	28.9 ± 5.6	23 ±5.8	0.06	26.8 ± 2.7	0.15
Gestational age at birth (week)	38.9 ± 1.48	40.75 ±0.5	0.024	39.27 ± 1.28	0.44
Systolic BP (mmHs)	117.5±8.9	135 ±5.7	0.001	139±5.7	<0.001
Weight new born (grams)[‡]	3347.50 ± 442	3812,50±366	0.06	4150 ± 469.4	<0.001
Weight of placenta (g)	555,85± 85	665 ±122	0.07	693 ± 79	<0.001
Foeto-placentar index	6.04 ± 0.4	5.94 ±0.5	0.64	5.99 ± 0.32	0.68
Umbilical cord parameters					
Number (n)	20	4		15	

Table 4 (continued)

Insulin (pmol/l)	39.73 ±11.5	55.1 ±7.44	0.019	81.3 (53–121)	<0.001
Proinsulin (pmol/l)	7.75 ±1.8	13.5 ±3.1	<0.001	13.4 (8-23)	<0.001
Maternal blood parameters	20	4		15	
Hemoglobin (%)	11.4±1.28	11.97 ±1.12	0.46	12.19 ±0.90	0.08
Plasma glucose (mg/dl)	83.19± 11.9	75.95 ±13.7	0.28	124,73±44	<0.001
Total Cholesterol (mg/dl)	187.9±26	212.5 ±45	0.14	240±17.11	<0.001
Triglycerides (mg/dl)	168.1±29	182.5 ±39.4	0.40	224.5±28	<0.001
New born *(n)*	20	4		15	
Apgar	8.4±0.68	9	0.1	8.33±0.90	0.50

DISCUSSION

Our data demonstrate that GD is associated with increased levels of proinsulin and insulin in fetal blood, confirming the idea that the fetal macrosomia encountered in this phenotype of diabetes is the consequence of metabolic disturbances which initially appear in the energy homeostasis of the mother and subsequently are reflected in the development of the fetus. This mother – fetus cross-relationship is so close that metabolic disturbances appearing in one of the two partners (mother/fetus) is reflected quickly in the metabolism of the other. The infants of mothers with various phenotypes of diabetes have a high rate of gaining weight, leading to fetal macrosomia with its potential consequences such as emergency Caesarian section, birth trauma and birth asphyxia. In addition, as Dabelea *et al.*[8] and others[9] demonstrated, the intrauterine exposure to diabetes conceives a high risk for obesity and diabetes in offspring. Maternal hyperglycemia increases the placental transfer of glucose which results in fetal hyperinsulinemia[10]. Indeed, elevated HbA1c level during the first trimester was the strongest predictor of macrosomia[11]. In human diabetic pregnancy, there is a strong association between weight and fetal insulin as assessed by amniotic and umbilical cord insulin and C peptide[12, 13].

Our data confirm previous data[14] reporting increased levels of proinsulin in the umbilical cord of women with gestational diabetes. The same increased levels of proinsulin were found by us in infants of obese women, suggesting a common mechanism operating both in gestational diabetes and in fat overload.

The increased levels of proinsulin in plasma obtained from the umbilical cord (which expresses the reaction of fetal β cells to mother's hyperglycemia) could be a clue in understanding the reaction of the β cell mass to an unusual load in a period of life in which the proliferation of various cells (including β cells) is very high. The most probable mechanism that could explain the higher insulin and proinsulin levels in fetal circulation is the increase in the fetal β cell mass since blood glucose is an important stimulator of β cell replication. β cell hyperplasia noted in the pancreas of a newborn from a diabetic mother (dead soon after birth) demonstrates that, in this early stage of development (fetal period), the proliferative capacity of the β cell is very high. Neonatal hypoglycemia associated with fetal hyperinsulinemia is a marker of such a mechanism. In this context, hyper-proinsulinemia found in the umbilical cord of fetuses from diabetic mothers (GD, T2DM or T1DM) could express an immaturity of the β cells developed rapidly after an unusual high blood glucose level.

It is important to known that the capacity of the β cells to replicate is limited to the first period of life. This is a very active process in the fetal period and also during childhood, puberty and adolescence. Around 20 years of age, the β cell arrives in a post-mitotic stage so that, after this age, the capacity for replication of this cell is almost totally lost[15–20]. That is why, type 2 diabetes is usually diagnosed after the age of 25 years and its incidence increases up to 60–65 years, affecting the percentage of population in which the "mature β cell mass" is already restricted by the presence of various genetically determined β cell defects.

The majority of GD patients are clinically more similar with T2DM subjects than with T1DM. A recent study genotyped the 17 loci identified by GWAs as associated with T2DM on a Korean population of 869 women with GD and two control groups[21]. A significant association with GD (with an OR > 1.2) has been found for 8 of these loci: CDKAL1 (rs 7756992 and rs 7754840); CDKN2A/28 (rs 10811661); HHEX (rs 1111875; rs 5015480; rs 7923837); SLC30A8 (rs 13266634) and TCF7L2 (rs 7903146 and rs 1255352). These data suggest a similar genetic background for GD and T2DM. It will be of interest to analyze also the women with GD according to the clinical phenotype of diabetes, suggesting T2DM and

respectively T1DM. Yet, more important genetic analysis might be carried out on "true GD", defined as those cases of diabetes "*which appear during pregnancy, with remission after delivery and reappearing in subsequent pregnancies*"[1]. Such cases (probably few) should be of great interest for genetic testing.

It is worthy of note that a normal pancreas exhibits a wonderful capacity of the β cells to adapt their size and function (in various animal models also their number) to a variety of physiological and pathological conditions[22,23]. Unfortunately, this wonderful capacity is not proved in humans due to the extremely limited access to the pancreas, not only in pregnant women, but generally in humans. An old study[24] carried out on pregnant women dead in car accidents (5 cases) suggested a proliferative capacity of the β cells. Recently, Butler *et al.*[23], analyzing postmortem material from18 pregnant and 6 postpartum women, found that the pancreatic fractional β cell area was increased by 1,4 fold in human pregnancy, with no change in the mean β cell size. They found also that in the pancreas of pregnant women there were more small islets rather than an increase in islets size and β cell size and number, suggesting that the adaptation can be made by the production of new small islets[23]. However, if such mechanism does exist, why this could not be stimulated after hemi-pancreatectomy in non-diabetic subjects, for instance?

In normal pregnancies, the basal need of insulin can be almost double[25] and this increase is relevant by its permanent character. Over it will be superimposed the daily physiologic postprandial secretory insulin demand. In this context, the "functional pancreatic reserve" will be exceeded in a low percentage of cases (1–2%) which is equal with the prevalence of DG in normal weight population. These subjects are part of a population inheriting a "summum" of diabetogenic genes, able to trigger the decompensation of blood glucose regulation. This appears in those patients whose β cells are already functioning at their upper limit of insulin secretion capacity. An extra insulin demand could stimulate the speed of processes involved in the production of secretory vesicles: increased rate of transcription and translation of pre-proinsulin and pre-proamylin genes, increased processing of these molecules in the endoplasmic reticulum / Golgi apparatus and, finally, increased output of nascent secretory vesicles which need a long (hours) process of maturation. An increase in speed of these processes implies a great risk that some secretory vesicle won't attain their final maturation. A part of proinsulin and proamylin will remain incompletely processed, resulting in increased levels inside the pancreatic β cells but also in the peripheral circulation. These events have been well documented in our previous papers[26–32], showing that the main common pathogenetic mechanism associated with the various phenotypes of diabetes could be a defect in the complex posttranslational processing of the two secretory molecules for the β cells: pre-proinsulin and pre-proamylin.

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