Clinical and pathophysiological examination of gestational diabetes

PhD Thesis

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Introduction

Gestational diabetes is one of the most common disease of the pregnancy. The prepregnant overweight, the pregnancy in old age undertaken elevate the frequency of gestational diabetes worldwide. Its multifactorial pathophysiology refers to the autoimmune origin in about 10 % of all GDM cases. The novel classification of diabetes mellitus rests on etiological aspects. The newest recommendation sets the functional and organic impairment of the pancreatic β-cells in the middle of the classification. The genetical, and environmental factors, the insulinresistance, the inflammation, and the dysfunction of the immunsystem have got affect on the function, and mass of β-cells. The fat tissue, the low-grade inflammation, and the oxidative stress are factors on the evaluation of the hyperglycemia. The pathways leading to the final outcome, namely to the hyperglycemia are in elderly maternal age soon in activated state.

The embryonal pancreas begins to produce insulin at the begin of the 11^{th} gestational week. The insulin concentration of the amniotic fluid is elevating until the term of the pregnancy. The insulin molecule due to its size and weight does not across the placenta, therefore the insulin level of the amniotic fluid depends alone on the secretional capacity of the embryonal pancreas. There is a close correlation between the fetal hyperinulinemia, the maternal pregestational weight, and the neonatal macrosomia. Probably before the 24-28^{th} gestational weeks standing maternal subclinical hyperglycemia is one of the causes of the fetal pancreatic insulin overproduction.

The overproduction of the insulin as an adaptive answer to the hormonal change of the pregnancy makes the pancreatic β-cells vulnerable. The damaged protection against the oxidative stress, and the increased production of the free radicals due to the decreasing intracellular concentration of the glutathione, and NADPH levels. The oxidative damage is resulted because of the imbalance of the prooxidant, and antioxidant factors. The free radicals damage the unsaturated fatty acids, the membrane proteins, and lipoproteins. Due to the hyperglycemia the phylogenetically conserved intracellular heat shock proteins' protection system, and gene transcription will be damaged, the cell's protein defence fallen. The presence of prediabetes and diabetes should be taken in account after years of gestational diabetes.

Selenium is an essential trace element and a component of various enzymes with antioxidant functions. Its role in the human organism is ambiguous, because the elevated selenium concentration adapted to the gender, the age, and the BMI has got a positive, but not linear association with the occurence of the type 2 diabetes mellitus.

The C-reactive protein (CRP) is an acute phase protein, producing by the liver, and fat cells, as a response to the cytokines, especially the Il-6. High-sensitive C-reactive protein (hsCRP) is an early assay, where the CRP is measured in a small concentration, early indicating the subclinical, and chronic inflammations. The CRP has got an important role in the pathomechanism of the insulin resistance, and the type 2 diabetes.

The selenium-dependent glutathione peroxidase enzyme (GPX) is pivotal in the inactivation of reactive oxygen species, its activity depends on the state of the oxidative stress, and the selenium concentration of the organism. The biochemical function of GPX is the transmission of lipid hydroxyperoxydes, and free hydrogen-peroxyd to alcohol, and water.

Heat shock proteins are ubiquitous and phylogenetically conserved molecules, which indicate their functional importance. They are usually considered to be intracellular proteins with molecular chaperone and cytoprotective functions. However, 70 kDa heat shock protein (Hsp70) is present in the peripheral circulation of healthy pregnant, and non-pregnant individuals. Previously reported that serum Hsp70 levels are significantly lower in healthy pregnant than in healthy non-pregnant women. The serum Hsp70 concentrations are in positive association with the gestational age. Extracellular Hsp70 through its intensified innate and adaptive proinflammatory Th1-like respons damage the immuntolerance of the pregnancy, causing spontaneous abortion, or premature labour. Recent data indicate that serum Hsp70 levels are also increased in pre-eclampsia, HELLP-syndrome, and diabetes mellitus.

Objectives

1. We investigated the influence of maternal age, pregestational BMI, parity, familial carbohydrate disorder on the occurrence of gestational diabetes in the Hungarian pregnant population.

2. Recent studies revealed that fetal insulin production is under complex regulation. Beside the maternal serum glucose augmentative effect on the fetal insulin production, also has been raised the fetal hyperinsulinemia as an influencing factor on the maternal carbohydrate metabolism. The purpose of our study was to define the amniotic fluid insulin levels of the mid-pregnancy indicating the fetal hyperinsulinism. We assessed whether the arisen amniotic fluid insulin level at the begin of the second trimester is an early marker of maternal subclinical hyperglycemia during the first trimester. The association of the amniotic fluid insulin concentration, the maternal age, and the pregastational BMI were investigated.

3. Based on our study we intented to answer, whether similar to the pregestational diabetes the low state inflammation as a causing, and consequence factor of the carbohydrate disorder is it present at the time of the diagnosis of gestational diabetes? We also tried to detect the imbalance of the pro-, and antioxidant factors during the normal pregnancy. We investigated at the first time the selenium concentrations of the pregnant population in Hungary. The association between the serum selenium concentration, playing essential role as part of selenium-dependent antioxidant compounds, and the prooxidant hsCRP concentration was investigated in normal, and gestational diabetic pregnant women in the second trimester. Our aim was to detect the activity of the selenium-dependent, and antioxidant glutathione-peroxidase during the pregnancy, and to reveal the reduction of GPX activity during the early stage of the formation of GDM.

4. Our mission was to investigate the following subclinical inflammation markers indicating the intensified lipid peroxidation in normal, and gestational diabetic pregnant women: the elevated concentration of the lipoproteins reducing the effect of the insulin, and the increased hsCRP level. We studied whether the maternal lipid peroxidation in the second trimester is associatied with the fasting, or the postprandial venous plasma

glucose value of the OGTT, and the extent of the maternal lipid peroxidation does have any relation to the hypeglycemia of the pregnant?

5. Recent data determined that elevated extracellular level of the Heat shock protein 70 associates with the extent of the insulin resistance, and the decrease of the insulin sensitivity in non-pregnant women. We examined at first the circulating free fraction Hsp70 concentration in gestational diabetes. The purpose of the study was to determine the Hsp70 concentrations in pregestational, gestational diabetic, and healthy pregnant women. We looked for a pattern between the parameters of the carbohydrate metabolism, and the level of the Hsp70.

Materials and methods

Study participants

The determination of the risk factors of gestational diabetes

2521 pregnant women were included in the study at the 1st Department of Obstetrics and Gynaecology at the Semmelweis University between 2000-2003. After fasting blood glucose concentration was determined, a standard seventy-five-gram (75 g) two-hour oral glucose tolerance test (OGTT) screening was performed by the WHO recommendation. The universal screening was constructed within 24-28th gestational weeks, in case of known risk factors during the first trimester. The following risk factors were analysed in all pregnant women: maternal prepregnancy weight (BMI>25 kg/m^2), maternal age (≥25 years), positive family history of diabetes mellitus, poor obstetric outcome of a previous pregnancy (intrauterine death or spontaneous abortion). In the case of multiparas, a previous macrosom newborn (birthweight>4000 g) and GDM during a prior pregnancy were considered risk factors. Pregnant women with no known risk factor were analysed in the low risk group (n=383). Classified the pregnant women as a high risk patients in case of presence of one or more known risk factors (n=2138). The datas were collected from the questionnaire, filled out at the time of OGTT. Women who had prior the pregnancy diabetes mellitus, multiple pregnancy, malformation, or had incomplete questionnaire were excluded.

Amniotic fluid insulin concentrations in the second trimester of the pregnancy

The amniotic fluid insulin concentrations were measured between January 5 and March 12, 2004 at the 1st Department of Obstetrics and Gynaecology, Semmelweis University, Medical School in the 274 pregnant women who had amniocentesis performed there between the two periods**.** The amniocentesis was performed between the 16th and the 23rd weeks of gestation in order to perform karyotyping because of a genetic indication. The indication for the amniocentesis was either advanced maternal age and/or several minor anomalies detected at the ultrasound examination or an obstetric history of congenital malformations. During the analysis of the data pregnant women were excluded because of the following reasons: pregnancy with an abnormal/pathologic karyotype, spontaneous abortion for genetic reasons within 4 weeks

after the sample was taken, bloody amniotic fluid sample and congenital (fetal) malformation at birth.

Serum selenium and hsCRP concentrations in the second trimester of the pregnancy determinated in non-pregnant, and pregnant healthy, and gestational diabetic women

The serum selenium concentration, lipid parameters, and hsCRP values of 20 gestational diabetic pregnant women (GD), 20 control pregnant women (CP), and 20 healthy nonpregnant controls (HC) were compared. Blood was taken at the 1st Department of Obstetrics and Gynaecology, Semmelweis University, Medical School between the 24th and the 28th week of pregnancy when the oral glucose tolerance test was performed. Participiants were excluded because of the following reasons: vitamin and/or food supplement consumption minimum 6 weeks before the blood sample, and maternal chronical disease. The study participiants do not have diabetes mellitus, hypertension, and kidney-, or liver diseases. Healthy pregnant women and nonpregnant healthy control women were selected randomly.

Glutathione peroxidase activity defined in the second trimester of the pregnancy

The study participants (31 gestational diabetic, and 20 healthy control pregnant women) were recruited at the First Department of Obstetrics and Gynecology, Semmelweis University, Budapest, Hungary, between 2007-2008. Blood was collected from each individual after 12 h of fasting, and from pregnant women between the 24th and 28th week of gestation when the oral glucose tolerance test was performed with 75 g glucose according to the World Health Organization guidelines. Women who had taken vitamins and/or nutritional supplements 6 weeks before the start of the study and women on medication because of chronic disease were excluded. In our study the total glutathione peroxidase activity in blood plasma, red blood cell, and whole blood 1:9 hemolysate of gestational diabetic, and control pregnant women were measured and compared.

Determination of the serum Heat shock protein 70 (HSPA1A, Hsp70) in the second trimester of the pregnancy

In this case-control study, we measured serum Hsp70 levels in 11 pregnant women with pregestational diabetes, 38 women with gestational diabetes, and 40 healthy pregnant women in the 1st Department of Obstetrics and Gynecology at the Semmelweis University, between January 2004 and January 2006. The healthy pregnants were considered as control group. All women were caucasian and resided in the same geographic area in Hungary. We examined the most inducible form of the Hsp70 superfamily. The women were fasting, none were in active labor, and none had rupture of amniotic membranes. The measurement was made at the time of the 75-gram oral glucose tolerance test (OGTT) using the World Health Organization criteria. Blood samples were taken before and 120 minutes after the glucose load. All women in the healthy pregnant control group had normal carbohydrate tolerance. Women in the pregestational diabetes group had diabetes mellitus before pregnancy and all of them were in insulin treatment.

Laboratory methods

The serum glucose level was determinated using glucose oxydase method (Dialab, Austria), the samples were collected from venous blood taken at fasting and 120 minutes after the glucose load. Gestational diabetes was diagnosed, if the 120 minute glucose result was 7.8 mmol/l or higher.

The amniotic fluid insulin concentrations were determined using a reagent kit according to a Microparticle Enzyme Immunoassay (MEIA) method, on an Abbot IMX instrument.

The serum selenium and hsCRP concentrations were defined after 12 hours of fasting, than approximately 10 ml of blood was collected from cubital vein between the 24th and 28th week of gestation when the oral glucose tolerance test was performed with 75 g glucose according to the World Health Organization guidelines. Blood was taken in metal-free glass tubes free of anticoagulant, using the standard venipuncture technique. Blood samples were allowed to clot, and the samples were centrifuged for 10 min at 2,400 rpm. Serum was separated into metal-free plastic tubes and stored frozen at −80°C until analysis. Routine laboratory parameters were determined on a Hitachi 917 instrument. HsCRP was measured by immunturbidimetry. Sample preparation for selenium determination was carried out in duplicate using the nitric acid–perchloric acid–sulfuric acid digestion procedure. After

digestion, selenium VI was reduced to selenium IV with hydrochloric acid. Selenium concentration was determined by atomic absorption spectrometry after hydride generation (Solaar M5 AA Spectrometer, Thermo Elemental). The analytical method was verified by analysis of a human reference serum (Seronorm Trace Elements Serum L-1, Ref: 201405, LOT: JL4409). The results for the reference sample agreed with the certified acceptable range of selenium concentration.

The total glutathione peroxidase activity in blood plasma, red blood cell, and whole blood 1:9 hemolysate were measured. Blood was taken after 12 hours of fasting from cubital vein. Glutathion peroxidase activity was measured by an endpoint direct assay in the presence of reduced glutathione and cumene-hydroperoxide as co-substrates. The glutathione peroxidase activity is given in units (U) which means oxidation of 1 nmol reduced glutathione between -1 and +25 ^{o}C and expressed per gram protein content, which was determined by biuret method.

Maternal blood samples were obtained from an antecubital vein into native tubes, as well as tubes containing sodium fluoride and disodium EDTA, and centrifuged at room temperature with a relative centrifugal force of 3000 g for 10 minutes. The aliquots of serum were stored at -80 °C until the measurement of Hsp70 levels. For haemoglobin A_{1c} (HbA_{1c}) determination, EDTA-anticoagulated whole blood was used. In the healthy pregnant and GDM groups, blood samples were taken when OGTT was performed (at 0 minutes). The serum 70 kDA heat shock protein levels were measured by using the ELISA Kit (R&D Systems, DYC1663E, Minneapolis, Minnesota, USA).

Statistical analysis

Analysing the datas of the risk factors the χ^2 statistical analysis was carried out, using the EpiInfo 2000 program. For all statistical analyses, $p<0.05$ was considered statistically significant at the confidence intervallum (CI) of 95%.

The amniotic fluid insulin concentration was considered abnormal if the value was higher than the mean+2SD (standard deviation) (3.5 μU/ml). In case of a negative OGTT result, the 90th percentile value of the amniotic fluid insulin concentration was determined. If amniotic fluid insulin concentration was higher, it was also considered abnormal. Statistical analysis was performed using the Mantel-Hanszel χ^2- test and regression analysis.

Evaluation of the serum selenium and hsCRP concentrations was carried out using the Statsoft Statistica 6.1 software, and the ANOVA and Mann-Whitney U-test. The statistical analysis was made by the Wilcoxon-assay and the logistic regression analysis.

Statistical assessment of the glutathione peroxidase activity was performed using the MS Excel 7.0 software package.

Focusing on the Hsp70's determination the normality of continuous variables was assessed using the Shapiro-Wilk's *W*-test. As the continuous variables were not normally distributed, nonparametric statistical methods were used. To compare continuous variables between two groups, the Mann-Whitney *U*-test was applied, whereas to compare them among multiple groups, the Kruskal-Wallis analysis of variance by ranks test was performed. Multiple comparisons of mean ranks for all groups were carried out as post-hoc tests. As serum levels of Hsp70 showed skewed distributions, we performed analyses of covariance (ANCOVA) with logarithmically transformed data. The Spearman rank order correlation was carried out to calculate correlation coefficients. The scatterplot was created, as a nonparametric method, with logarithmically transformed values of the dependent variable. Statistical analyses were carried out using the following software: STATISTICA (version 12; StatSoft, Inc., Tulsa, Oklahoma, USA) and Statistical Package for the Social Sciences (version 22 for Windows; SPSS, Inc., Chicago, Illinois, USA). Datas are reported as median (interquartile range) for continuous variables, the categorical variables were defined in absolute number (%). For all statistical analyses, $p<0.05$ was considered statistically significant.

The study protocol was approved by the Regional and Institutional Committee of Science and Research Ethics of the Semmelweis University, and written informed consent was obtained from each patient. The study was conducted in accordance with the Declaration of Helsinki.

Results

Determination of the prevalence of the predicting risk factors of gestational diabetes mellitus

The incidence of GDM was 9.26% in the whole examined pregnant population (9.78% in the high risk group and 5.21% in the low risk group). Maternal obesity (OR: 1.66, p<0.0003), maternal age is over 25 years (OR: 2.26, p<0.0002), and GDM in a prior pregnancy (OR: 2.16, p<0.0018) had the most profound effect on the incidence of GDM.

In case of the maternal age is younger than 25 years the incidence of GDM is 5.69 % (23/404), but regarding this pregnant group in only one case accompanied risk factor, namely the elevated maternal BMI (>25 kg/m^2). 12% of the pregnants older than 25 years had gestational diabetes (254/2117, 12.0%). In this pregnant group the strongest risk factors to gestational diabetes mellitus were the maternal BMI (OR: 1.79, p<0,000064), gestational diabetes in the previous pregnancy (OR: 2.06, p<0,003), and diabetes mellitus occured in the family (OR: 2.2, p<0,001). The occurrence of GDM was increasing with the progress of the maternal age: the incidence of GDM was 7.9 % in case of the maternal age was younger than 30 years, and 29.16 % in case of the pregnant woman's age was older than 40 years.

The maternal body-mass index was above 25 kg/m^2 the highest odds ratios were the maternal age over 25 years (OR: 13.69, p<0,00083), the diabetes mellitus in the family history (OR: 2.81, p<0,004), and the previous GDM (OR: 2.81, p<0,01). In case of normal maternal weight (BMI < 25 kg/m^2) only the maternal age influenced significantly the occurrence of the GDM (OR: 1.69, p<0,02).

The amniotic fluid insulin concentration of the second trimester of the pregnancy and the gestational diabetes

The amniotic fluid insulin concentration was considered abnormal if the value was higher than the mean+2SD (standard deviation) (3.5 μU/ml). Abnormal amniotic fluid insulin level was diagnosed in 4.56 % (12/263) of the examined pregnants. Hyperinsulinemia of the amniotic fluid was associated with gestational diabetes mellitus diagnosed during the second trimester in 41.6 % of total cases. Abnormal OGTT indicating GDM was accompanied in only 8 % (20/251) of pregnant women having normal amniotic fluid insulin concentration. The

difference was statistically significant (p<0,001, RR: 5.23, 95 % CI 2.37-11.53). The occurrence of maternal obesity (BMI > 30 kg/m^2) was 16.6 % (2/12) in case of pathological amniotic fluid insulin level, and 9.2 % (23/251) in case of normal amniotic fluid insulin value.

Two-thirds of the pregnant women with abnormal amniotic fluid insulin (8/12, 66.6%) were older than 35 years, while less than half of the women with amniotic fluid insulin in the normal range (117/251, 46.6%) were older than 35 years. The abnormality limit of the amniotic fluid insulin concentration (3.5 μU/ml) predicted gestational diabetes with a 20% sensitivity, 97% specificity, 42% positive predictive value and 92% negative predictive value. There was a significant linear correlation between amniotic fluid insulin and the 2-hour glucose results of the OGTT, but the value of the correlation coefficient was low (p<0.002, r=0.189).

Two or more macrosome newborns in the examined pregnant previous history associated with fetal hyperinsulinism in 50 % of cases. Elevated amniotic fluid insulin concentration adjusted to the gestational age increased the frequence of macrosome newborn.

Serum selenium and hsCRP concentrations in the second trimester of the pregnancy determinated in non-pregnant, and pregnant healthy, and gestational diabetic women

The gestational age at the birth, and the newborn's weight did not showed significant difference between the healthy and gestational diabetic pregnants adjusted them to the maternal age and the BMI. The non-pregnant healthy women's serum selenium concentration (mean±SD 77.4±14.82 μg/l, p<0.001) differed significantly from the mean selenium level of the healthy pregnants (40.5±8.03 μg/l), and the gestational diabetic women (51.7±11.62 μg/l). Gestational diabetic women's serum selenium level was significant elevated to the control pregnants' selenium value (p<0,001). The serum selenium concentration had shown a positive significant correlation with the occurrence of gestational diabetes mellitus.

In the gestational diabetes group between the serum selenium level and the fasting plasma glucose concentration revealed an inverse significant correlation (p=0,802).

The hsCRP level in both diabetes group was significantly higher (p<0.01) than in non-pregnant healthy women indicating increased lipid peroxidation during the pregnancy. The difference of the hsCRP values between the gestational diabetic and healthy women was not significant, but in case of gestational diabetes the frequency of elevated hsCRP level was higher.

In our study the hsCRP level and the fasting plasma triglyceride level (p<0,01), and the serum total cholesterol level (p<0,05) have shown a positive and significant correlation. The

hsCRP concentration correlated also significant with the 2 hours serum glucose value of the OGTT ($p<0,01$).

The serum selenium concentration associated negatively ($p<0.001$) with the serum total cholesterol, the low-density lipoprotein cholesterol, and the triglyceride concentrations. We diagnosed an inverse significant association between the serum selenium level, and the hsCRP concentration indicating a low state inflammation, and an increased lipid peroxidation. (regression coefficient: -0.49, $p<0.001$).

Glutathione peroxidase activity in healthy and gestational diabetic pregnant women defined in the second trimester of the pregnancy

In our study the total glutathione peroxidase activity in blood plasma, and red blood cell of gestational diabetic, and control pregnant women adjusted to maternal age and BMI did not shown significant difference (3.30±0.95 vs. 2.84±0.60 U/g protein and 6.78±2.23 vs. 5.92±0.67 U/g protein, $p=0.035$). The glutathione peroxidase activity of the whole blood 1:9 hemolysate was significantly lower in gestational diabetic pregnants than in control pregnant women (2,03±0,61 U/g protein vs. 2,77±0,70 U/g protein, $p=0.035$). The plasma GPX activity did not differ significantly between gestational diabetic and control pregnant women, despite that at the presence of the GDM measured more frequently higher GPX activity. The association between the serum selenium level and the selenium dependent glutathione peroxidase activity of the plasma was significant in the healthy pregnants compared to the gestational diabetic women ($p=0.005$), where the correlation was a slightly positive, but not significant ($p=0,92$).

Determination of the Heat shock protein 70 (HSPA1A, Hsp70) in the second trimester of the pregnancy

Serum Hsp70 concentrations were significantly higher in women with pregestational (0.39 (0.33-0.45) ng/ml, $p<0.001$) and gestational diabetes mellitus (0.28 (0.24-0.35) ng/ml, $p<0.05$) than in healthy pregnant women (0.26 (0.20-0.30) ng/ml). Furthermore, pregestational diabetic women had significantly higher Hsp70 levels than those with gestational diabetes ($p<0.05$). The differences in serum Hsp70 levels between the study groups remained significant even after adjustment for maternal age, pre-pregnancy BMI and gestational age at blood draw in analysis of covariance (adjusted mean ± standard error of log (serum Hsp70 concentration

(ng/ml)): -0.63 ± 0.03 in normal pregnancy, -0.51 ± 0.03 in GDM and -0.42 ± 0.05 in pregestational diabetes; $p<0.05$). In the group of women with gestational diabetes mellitus, serum Hsp70 levels showed a significant positive correlation with HbA_{1c} values (Spearman $R=0.37$, $p<0.05$.)

Conclusions

1. The strongest risk factors predicting the evolution of gestational diabetes mellitus are the overweight, the previous gestational diabetes, and the diabetes mellitus of firstdegree relatives in pregnants older than 25 years.

2. In 5.2 % of the pregnant women younger than 25 years and having less than 25 kg/m^2 body-mass index developed GDM, supposing else pathomechanism of the gestational diabetes.

3. The maternal age is over 40 years the prevalence of GDM is 29.16 %, wich is approximately six-fold frequent compare to pregnants younger than 25 years.

4. Regardless to the pre-gestational maternal BMI the primary predicting risk factor to gestational diabetes is the maternal age.

5. Abnormal amniotic fluid insulin concentration during the second trimester proved in 4.56 % of all pregnant women. The occurrence of the gestational diabetes mellitus is 8 % in case of normal, and 42 % in case of abnormal amniotic fluid insulin level. In the normoglycemic group of pregnants with pathological amniotic fluid insulin concentration further investigations should be carried out to detect the responsible maternal and/or fetal factors maintaining the maternal normoglycemia. One responsible factor could be the increased fetal glucose uptake.

6. The serum selenium concentration in healthy and gestational diabetic pregnants is significantly lower compared to healthy non-pregnant women, suggesting an increased consumption.

7. The serum selenium level in gestational diabetes is significantly higher to normal pregnants' values.

8. The hsCRP correlates negatively with the serum selenium concentration.

9. The serum hsCRP value of the pregnant healthy and gestational diabetic women during the second trimester is significantly higher to the normal non-pregnant women. The hsCRP level does not differ significant between the normal and gestational diabetic pregnants.

10. The serum selenium concentration at the end of the second trimester associated with negative significant correlation to the total cholesterol, the low-density lipoprotein cholesterol, the triglyceride, and the hsCRP levels.

11. There is a positive correlation between the fasting total cholesterol, the triglyceride, and the serum hsCRP level.

12. To our opinion the decreased serum selenium concentration diagnosed during the pregnancy refers to the increased lipid peroxidation.

13. The 2 hours value of the OGTT at the end of the second trimester shows a significant positive association with the maternal serum hsCRP concentration.

14. The glutathione peroxidase activity of the whole blood hemolysate at the end of the second trimester in gestational diabetes is significant lower compared to the healthy pregnants referring to the increased oxidative stress characteristic the carbohydrate disorder during the pregnancy.

15. The Hsp70 level in gestation diabetic women at the end of the second trimester is significantly increased to the healthy pregnants.

16. In the group of women with gestational diabetes mellitus, serum Hsp70 levels showed a significant positive correlation with HbA_{1c} values, referring to the increased oxidative stress and the systemic inflammation caused by the chronic maternal hyperglycemia. In gestational diabetes the essential factor is the careful maintenance of the carbohydrate metabolism preventing from lesions raised by the oxidative stress.
17. Pregestational diabetic women had significantly higher Hsp70 levels than those with gestational diabetes. The late recognized pregestational diabetes means an enhanced oxidative stress and inflammation to the embryo during the organogenesis.

List of the candidate's publications

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