Mariana Alvarez Arantes

EXPRESSÃO E IMUNIDADE DAS *HEAT SHOCK PROTEINS* (HSP) EM GESTANTES PORTADORAS DE DIABETE E HIPERGLICEMIA GESTACIONAL LEVE

Profa. Titular Dra. Marilza Vieira Cunha Rudge

Orientadora

Prof. Dr. Steven Sol Witkin

Co-orientador

Mestrado

FACULDADE DE MEDICINA DE BOTUCATU
Universidade Estadual Paulista "Julio de Mesquita Filho"
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Dissertação apresentada à Faculdade de Medicina de Botucatu – Unesp, Programa de Pós-Graduação em Ginecologia, Obstetrícia e Mastologia. Área de concentração: Tocoginecologia, para obtenção do título de Mestre.

Orientadora: Profa. Titular Dra. Marilza Vieira Cunha Rudge

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"Que os vossos esforços desafiem as impossibilidades, lembrai-vos de que as grandes coisas do homem foram conquistadas do que parecia impossível."

Charles Chaplin

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Sumário

Capítulo 1

Alterations in circulating heat shock proteins in pregnancies complicated by mild gestational hyperglycemia, gestational diabetes and preexisting diabetes

Mariana A. Arantes [1b], Debora Damasceno [1a], Iracema M.P. Calderon [1a], José C. Peraçoli [1a], Joice Vernini [1b], Glilciane Morceli [1a], Rafael B. Gelaleti [1b], Fernanda Piculo [1b], Steven S. Witkin [2a], Marilza V. C. Rudge [1a]

[a]PhD and [b]Master degree

[1] Laboratory of Experimental Research on Gynecology and Obstetrics, Department of Gynecology and Obstetrics, Unesp – Univ Estadual Paulista, Botucatu Medical School, Sao Paulo, Brazil

[2] Division of Immunology and Infectious Diseases, Department of Obstetrics and Gynecology, Weill Cornell Medical College, Ney York, New York, USA

*_Correspondence to_: Profa. Marilza Vieira Cunha Rudge

Department of Gynecology and Obstetrics

Botucatu Medical School

São Paulo State University, UNESP

Botucatu – São Paulo, BRASIL

Tel./Fax: (+55 14) 3811-6181

RESUMO

O diabete gestacional (DMG) e a hiperglicemia gestacional leve têm consequências importantes para a mãe e o recém-nascido. Gestantes portadoras de hiperglicemia gestacional leve (HGL) não atingem os critérios diagnósticos para DMG, porém, os recém-nascidos apresentam macrossomia mediada pela glicemia, mesma mortalidade perinatal e resultados perinatais adversos semelhantes ao das DMG. Estudos relatam que as atividades das proteínas de choque térmico (*Heat Shock Proteins*– hsp) são relevantes para relacionar com as doenças maternas. A família das hsp consiste de diversas proteínas, altamente conservadas durante a evolução.

O objetivo do presente estudo foi estudar os níveis de hsp 60, hsp70 e seus respectivos anticorpos no soro materno entre 24-28 semanas e 34-38 semanas de gestação e 6 semanas pós parto em 208 mulheres normoglicêmicas (ND) , 121 mulheres com hiperglicemia gestacional leve , 55 mulheres com diabete gestacional e 178 mulheres com overt diabetes. Níveis de hsp60, hsp70, anti-HSP60 e anti-HSP70 foram quantificados através da técnica de ELISA. hsp60 e hsp70 estão diminuídos nos três grupos hiperglicêmicos em cada momento estudado quando comparados com as concentrações do grupo controle. No grupo ND, mas não nos grupos hiperglicêmicos, níveis de hsp60 aumentaram, e níveis de hsp70 diminuíram. Níveis de anti-HSP60 foram similares entre os grupos enquanto níveis de anti-HSP70 foram menores nos três grupos hiperglicêmicos em comparação ao grupo ND durante a gestação, mas não no pós parto. Como conclusão, concentrações circulantes de hsp60 e hsp70 estão diminuídas em mulheres hiperglicêmicas durante a gestação, independente do grau de hiperglicemia,

quando comparado com mulheres normoglicêmicas. Outros estudos serão necessários para entender os mecanismos responsáveis por estas alterações nos níveis de hsp60 e hsp70 na hiperglicemia e as consequências na gestação.

PALAVRAS-CHAVE: heat shock proteins (hsp), diabete gestacional, hiperglicemia gestacional leve

ABSTRACT

Objective

The aim of the present study was to compare serum levels of heat shock proteins (hsp) 60, hsp70 and their respective antibodies in maternal serum.

Research design and methods

In maternal serum at 24-28 and 34-38 weeks gestation and at 6 weeks postpartum from 208 normoglycemic (ND) women and 121 women with mild gestational hyperglycemia (MGH), 55 with gestational diabetes (GDM) and 178 with overt diabetes. hsp60, hsp70, anti-HSP60 and anti-HSP70 levels were quantitated by enzyme-linked immunosorbent assay (ELISA).

Results

Hsp60 and hsp70 levels were decreased in all three hyperglycemic groups at each time period compared to concentrations in the ND controls. In the ND group, but not in the hyperglycemic mothers, hsp60 levels increased, and hsp70 levels decreased with each successive sample. Anti-HSP60 levels were similar between groups while anti-HSP70 levels were lower in the three hyperglycemic groups than in the ND controls during pregnancy, but not postpartum.

Conclusions

Circulating hsp60 and hsp70 concentrations are decreased in hyperglycemic women during pregnancy, regardless of the degree of hyperglycemia, compared to ND women. Further studies are warranted to

explore the mechanism(s) responsible for these alterations in hsp60 and hsp70 levels in hyperglycemia and consequences for pregnancy outcome.

KEYWORDS: Heat-shock protein (hsp); gestational diabetes; mild gestational hyperglycemia; pregnancy

INTRODUCTION

The Hyperglycemia and Adverse Pregnancy Outcome (HAPO) Study demonstrated that levels of maternal hyperglycemia that were below values diagnostic for diabetes were, nevertheless, still associated with elevations in perinatal risks (1). This confirmed previous findings that even mild glycemia increased susceptibility to adverse perinatal outcome (2). These observations identified a group of hyperglycemic women who were previously classified as normal according to the 4th International Workshop Conference criteria (3) but who, nevertheless, had metabolic characteristics and pregnancy outcomes resembling that of women positive for gestational diabetes mellitus (GDM) by previous criteria.

We have combined two parallel diagnostic tests, the 100g Oral Glucose Tolerance Test (OGTT) and Glycemic Profile (GP), to characterize mild gestational hyperglycemia (MGH) in pregnant women, i.e., women with a negative diagnosis for GDM but with hyperglycemia detected in the GP (4). MGH was identified in 13.8% of pregnant women screened for GDM and, added to the 7.0% of pregnancies complicated by diabetes, increased the occurrence of hyperglycemic disorders in pregnancy to over 20% (5). These women were at high risk for hypertension, obesity and hyperglycemia, and seemed to reproduce the model of metabolic syndrome (MS) in pregnancy, with hyperinsulinemia and insulin resistance, that continued six weeks postpartum (6). After 10 to 12 years of the index-pregnancy, type 2 diabetes mellitus (DM) was confirmed in 16.7% of women who had MGH during pregnancy (7). Exploratory analyses of newborn outcomes indicated that 53.8% had macrosomia, a similar proportion (51.9%) to that described for overt diabetes

and for GDM (8). A high perinatal mortality rate (41‰) was also similar to that present in diabetic women and was 10 times greater than that observed in normoglycemic pregnant women (9). Some of these newborns also exhibited hypoglycemic crises, hyperbilirubinemia, prematurity and congenital anomalies (2). Therefore, despite previously being identified as low-risk, MGH in pregnancy characterizes a group of women at high risk for adverse maternal and perinatal outcomes.

Cells respond to environmental stresses by up-regulating expression of heat-shock proteins (hsp) (10, 11). Intracellular hsps, especially the 60kDa (hsp60) and 70 kDa (hsp70) function as molecular chaperones, promoting accurate assembly of polypeptides, protecting proteins from denaturation and marking terminally altered proteins for removal from the cell. They also prevent programmed cell death (apoptosis). hsp60 and hsp70 may be released into the circulation under conditions of stress or as a consequence of cell necrosis. These extracellular hsps can bind to receptors on immune and non-immune system cells activate the innate immune response and, thereby, alert the organism to the presence of an environmental stress. The binding of hsp60 and HSP70 to altered proteins that are released into the circulation can result in induction of antibodies to these hsps (11,12).

Circulating hsp70 levels are decreased in pregnant women compared to the non-pregnant state (13). In type 2 diabetes, intracellular hsp70 concentrations are decreased compared to normoglycemic individuals while extracellular hsp levels may be increased or remain unchanged (14), Associations between hsp concentration and glycemic status in pregnancy have not been evaluated. The aim of the present study was to compare serum levels

of hsp60, hsp70 and their respective antibodies in maternal serum at two stages of pregnancy (24-28 weeks, 34-38 weeks) and at 6 weeks postpartum from women who were normoglycemic (ND), MGH, GDM or who had overt diabetes. We sought to evaluate whether determination of these protein levels would vary with a woman's glycemic status and if they would be of value in the prediction of hyperglycemia-related pregnancy outcome.

RESEARCH DESIGN AND METHODS

This case control study was conducted in the setting of an ongoing prospective observational analysis in which a cohort of women recruited at the time of antepartum GDM screening were undergoing a longitudinal metabolic characterization in pregnancy and postpartum. At our institution eligible pregnant women underwent screening for GDM, including a fasting glycemia level ≥ 90 mg/dL and risk factors for GDM in late second trimester, according to the Brazilian Health Ministry recommendation, followed by referral for a diagnostic oral standard 75- g glucose tolerance test (OGTT) and a glycemic profile (GP) between 24-28 weeks gestation. GDM was confirmed when an abnormal screening test was followed by one or more elevated values on a standard 75-g OGTT using criteria of Carpenter & Coustan (1982). MGH was diagnosed when an abnormal screening was followed by normal values on the OGTT and an altered glycemic profile characterized by fasting glycemia >90 mg/dl and/or postprandial glycemia > 130 mg/dl (2,16). Pregnant women with type DM came to the service with a prior diagnosis. Gestational age was calculated from the date of the last menstrual period. The study protocol was approved by the UNESP - Botucatu Medical School Board for Human Investigation, and all participants provided written informed consent. The study was conducted in accordance with the Declaration of Helsinki. The present investigation was undertaken at the Diabetes and Pregnancy Tertiary Center, at the Botucatu Medical School, UNESP- Brazil. This analysis was performed on the first 524 women who completed the oral GTT and glycemic profile during two stages of pregnancy and at the 6-week postpartum visit. Recruitment was

over a two year time interval. Exclusion criteria were type 1 diabetes, multifetal gestation, maternal or fetal infection and fetal congenital anomaly.

Participant assessments

On the morning of the antepartum diabetes diagnosis, data regarding medical, obstetrical, and family history were collected by an interviewer – administered questionnaire. The results of the oral GTT plus glycemic profile stratified pregnant women into four glucose tolerance groups: IA – control NT group (normal OGTT and glycemic profile (n = 280); IB –MGH (normal OGTT and abnormal glycemic profile) (n = 121); IIA – GDM (abnormal OGTT and normal glycemic profile) (n = 55); and IIB – GDM or overt diabetes (abnormal OGTT and glycemic profile) (n = 178). At 6 weeks postpartum, participants underwent a 2-h 75g OGTT. Samples of maternal blood (7 ml) were collected at between 24- 28 weeks and 34 -38 weeks of gestation and at 6 weeks postpartum.

Figure 1 shows flowchart of women included in the study.

The samples were centrifuged at 2700 g for 10 min at 4°C to separate serum from particulate matter. The aliquots of serum were stored at -80°C until the analyses were performed. MGH and GDM women were treated with dietary counseling, exercise and insulin, if necessary. To obtain glycemic control. Maternal blood glucose levels were monitored weekly and reviewed at our maternal fetal unit once a week. Maternal blood glycosylated hemoglobin (HbA_1C) was measured and the goal of the treatment was to maintain fasting glycemia lower than 90 mg/dl and post prandial glucose lower than 120 mg/dl (17,18). The control group was managed as nondiabetic patients.

Anthropometrical and biochemical analyses

The body mass index (BMI) was calculated as body weight divided by the square of height; plasma glucose was measured using the glucose oxidase method (Glucose - Analyzer II Beckman, Fullerton, California, USA); glycosylated hemoglobin (HbA1c) was analyzed by HPLC (high-performance liquid chromatography – D10™ Hemoglobin Testing System, BIO RAD® laboratories, Hercules, CA, USA). The glycemic mean was calculated by the arithmetic mean of plasma glucose measured in all glycemic profiles performed at diagnosis. Large for gestational age (LGA) was defined as neonatal birth weight **≥** 90th percentile for gestational age and sex, and was determined according to our standard protocol.

Paired samples were assayed for hsp60 (EKS-600 *Assay Designs*), hsp70 (EKS-715 *Assay Designs*), IgG anti-HSP60 (EKS-650 *Assay Designs*) and IgG anti-HSP70 (EKS- 750 *Assay Designs*) by commercial enzyme-linked immunosorbent assay (ELISA) kits (ENZO *Life Science*). The lower level of sensitivities were 3.125 ng/ml for hsp60, 0.099 ng/ml for hsp70, 2.88 ng/ml for IgG anti-HSP60 and 6.79 ng/ml for IgG anti-HSP70.

Statistical analysis

We estimated the need to evaluate at least 27 pregnant women per group to provide sufficient statistical power (>80% at a type I error rate of 0.05) to detect differences in serum hsp60, hsp70, anti-HSP60 and anti-HSP70 levels between hyperglycemic and normoglycemic women.

Analysis of variance (ANOVA with simple measures) was used to evaluated maternal, placental and newborn infants' (NB) characteristics and ANOVA with

repeated measures and followed by the Tukey's test with adjustment was used to evaluate hsp60 and 70 and their respective antibodies. Pearson's test was used to correlation coefficient to assess the relationship between variables. Statistical significance was considered as a p value < 0.05.

RESULTS

Characteristics of the mothers, newborns and placenta are summarized in Table 1.The fasting glycemia level did not differentiate the groups, although HbA_1C in women with overt diabetes (6.08%) was higher compared to that in normoglycemic (5.31%) and MGH (5.28%) women (p= 0.007). BMI was elevated only in women with overt diabetes (31.25 km/m^2) as compared to the other three groups (p =0.02). Newborn infants from the MGH group had a higher birthweight (3483.97 g) and length (49.34 cm) compared to babies in the ND group (3134.57 g, 48.39 cm) (p = 0.001). LGA newborns were also more frequent in the MGH group (29.41 %) and the neonatal ponderal index was higher in the overt diabetes group (2.94) than in ND women (p = 0.04). Placental weight and placental index were similar between groups.

The concentrations of hsp60, hsp70 and their respective antibodies in the four groups at each of the three time periods are summarized in Table 2.

Each of the three hyperglycemic groups had lower hsp60 levels at all times analyzed compared to ND mothers. At 24-28 weeks, women with GDM had the lowest serum concentration of hsp60, which differed from all other groups (p = 0.002). At 34-38 weeks gestation women with MGH or GDM had the lowest concentrations of hsp60 (p<0.0001). In the postpartum period, women with overt diabetes or GDM had the lowest concentration of hsp60 that differed from levels present in the ND and MGH groups (p = 0.003). The ND women showed a progressive increase in hsp60 level from 24 weeks to postpartum. Hsp60 in the MGH women decreased at 34-38 weeks and then increased postpartum. Hsp60 in women with overt diabetes decreased

postpartum in relation to levels at 34-38 weeks gestation. In women with GDM the hsp60 levels did not vary significantly over the study time interval.

Anti-HSP60 levels were similar between groups. The only significant difference noted was a lower level in the ND group at 24-28 weeks compared to levels at 34-38 weeks and postpartum (p = 0.02).

The three hyperglycemic groups had lower serum hsp70 levels during pregnancy compared to the normoglycemic mothers. At 24-28 weeks gestation the hsp70 level only in the MGH reached statistical significance as compared to the ND group (p = 0.001). In the postpartum period, ND women and women with MGH had levels of hsp70 that were much higher than in women with GDM or overt diabetes (p = 0.006). The ND group showed a progressive decrease in hsp70 concentration from 24 weeks gestation to the postpartum period. In contrast, there was no significant difference in the hsp70 level over time in the three hyperglycemic groups.

Antibodies to hsp70 were lower in women with MGH and overt diabetes compared to levels detected in the ND and GDM groups ($p < 0.0001$). At 34-38 weeks gestation, all three hyperglycemic groups had lower anti-HSP70 levels compared to the ND mothers (p= 0.003). There was no difference between the groups in the postpartum period. The concentration of anti hsp70 in ND women, but not in the three hyperglycemic groups, was significantly lowered postpartum in relation to the levels during pregnancy ($p < 0.0001$). Table 3 shows the correlations between hsp60 and hsp70 and anti-HSP60 and anti-HSP70 concentrations as well as maternal, neonatal and placental data. Significant

correlations were found only between hsp60 and hsp70 levels ($p < 0.0001$) and between, hsp60 and anti-HSP60 ($p < 0.0001$).

Table 3 shows the correlations between hsp60 and hsp70 and anti-HSP60 and anti-HSP70 concentrations as well as maternal, neonatal and placental data. Significant correlations were found only between hsp60 and hsp70 levels ($p < 0.0001$) and between, hsp60 and anti-HSP60 ($p < 0.0001$).

Significant negative correlations were found between IgG antibodies to hsp70 and maternal BMI, and between antibody to hsp60 and newborn weight, placental weight and ponderal index (Table 4).

DISCUSSION

Our results confirm prior investigations (9,19). That plasma glucose concentrations that are below levels that are diagnostic of diabetes are nevertheless associated with adverse perinatal outcomes. The present study also demonstrates that, regardless of the extent of maternal hyperglycemia, elevated glucose concentrations are associated with reduced circulating levels of hsp60 and hsp70 during gestation and at 6 weeks postpartum. Whether this altered hsp expression contributes to the pregnancy-related pathology seen in hyperglycemic mothers, and identity of potential mechanisms, remain to be determined.

Previous studies on the relation between heat shock proteins and diabetes have yielded divergent results. In monkeys who were hyperglycemic due to spontaneous or streptozotocin-induced type 2 diabetes, hsp70 levels in plasma and in liver and muscle were reduced compared to control animals (20). In rats rendered diabetic by streptozotocin, hsp70 production was inhibited (21,22). Similarly, in humans with type 2 diabetes hsp70 expressions in skeletal muscles were lower than in normoglycemic subjects (23).

Other studies have reported that extracellular hsp concentrations are elevated in individuals with type 1 (24) or type 2 (14) diabetes. However, the increase in hsp70 in type 2 diabetics was confined to individuals who were obese. Associations between hsp70 levels and hyperglycemia in pregnancy and between hsp60 and hyperglycemia in pregnant and non-pregnant women have received scant research attention.

Our observations in the present study suggest that, similar to the decrease in intracellular hsp70 levels that occurs in diabetic animals and humans, the induction of hsp60 and hsp70 production in pregnant and early postpartum women is inhibited by elevated glucose levels. The observed diminution of extracellular hsp70 under these conditions differs from the rise in hsp70 levels seen in the circulation of non-pregnant individuals with type 1 or type 2 diabetes. Possibly, differences in the degree of oxidative stress and protein glycation that occurs in pregnant and non-pregnant hyperglycemic individuals and between men and women contributes to this difference. It is known that the extracellular hsp70 concentration is reduced in pregnant women compared to non-pregnant women (13). This suggests that hsp70 induction is normally down-regulated during gestation and that, whatever may be the involved mechanism, this is enhanced by hyperglycemia. The decreased levels of hsp60 and hsp70 in sera of hyperglycemic pregnant women may be a manifestation of reduced gene activation. It appears that hyperglycemia results in a reduction in hsp70 levels by inhibiting activation of heat shock factor-1 (HSF-1), the factor that induces heat shock protein gene transcription (14,20,22).

Interestingly, protocols that elevated hsp70 levels have been shown to reduce insulin resistance. Mice that were genetically engineered to overexpress hsp70 in skeletal muscle were protected against development of insulin resistance (25). hsp70 induction by heating protected rats fed a high fat diet from becoming insulin resistant (26). Induction of HSF-1 reversed development of hyperglycemia in diabetic mice (27). Administration of an adenovirus that carried a constitutively active form of HSF-1 was shown to improve glucose

levels in diabetic rats (28). Administration of the hsp70-inducing drug, geranylgeranylacetone increased hsp70 levels in diabetic monkeys (29). Protocols to increase hsp70 production during pregnancy and their possible effect on maternal and neonatal outcome remain to be evaluated.

Antibodies to heat shock proteins have been identified in the circulation of both pregnant and non-pregnant women and concentrations were similar in women with preeclampsia (30). In the present study antibodies to hsp70 were present at lower levels in women in the three hyperglycemic groups compared to the ND women. This parallels the findings with hsp70 antigen, suggesting that formation of antibodies varies with the concentration of the involved protein and that an excessive immune response to hsp70 does not occur in women who are hyperglycemic during their pregnancy.

CONCLUSION

The principal limitation of the current study is the lack of follow up, since not the same patient was evaluated in all moments. These patients are overweight and control pregnant group without obesity must be considered in next studies.

In conclusion, we have provided evidence of progressively decreased from 24-28 weeks through 6 weeks post-partum of hsp60 and hsp70 in the peripheral circulation of pregnant women with MGH, GDM and overt diabetes. The observation of anti-HSP60 and anti-HSP70 in four groups is less evident in hyperglycemic patients.

The increase of hsp60 and hsp70, anti-HSP60 and anti-HSP70 antibodies in normoglycemic patients from pregnancy to postpartum period seems to be an important strategy for protection of proteins against harmful changes and conservation of their native molecule structures. These increases may serve to protect the structure of proteins, leading to beneficial effects. All these protective effects were losing in all hyperglycemic patients independently of the diagnostic test used to identify these women. GDM, overt diabetes and MGH represent groups of hyperglycemic patients with adverse maternal and perinatal outcome. Even well treated in a tertiary referral center for diabetes, with good general metabolic state, maternal BMI and HbA1C, newborn weight and length, ponderal index and LGA were significantly increased and could be associated with the pathological decrease in heat shock proteins and respective antibodies. The hyperglycemia seems to be an evidence of mitochondrial

dysfunction and may also take part into the pathogenesis of adverse maternal and perinatal outcomes.

The reduced levels of hsp60 and hsp70 suggest that hyperglycemic pregnant women may be less capable of dealing with the normal endogenous stresses associated with rapid cell differentiation and development during gestation as well as additional exogenous stresses that may be encountered by any specific individual. The cells would be less able to survive transient alterations in cellular homeostasis and would be more susceptible to apoptosis. In addition, decreased extracellular concentrations of these hsp would result in a decreased ability to activate the innate immune system and, subsequently, render the mother and fetus more susceptible to infection.

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TABLES AND FIGURES

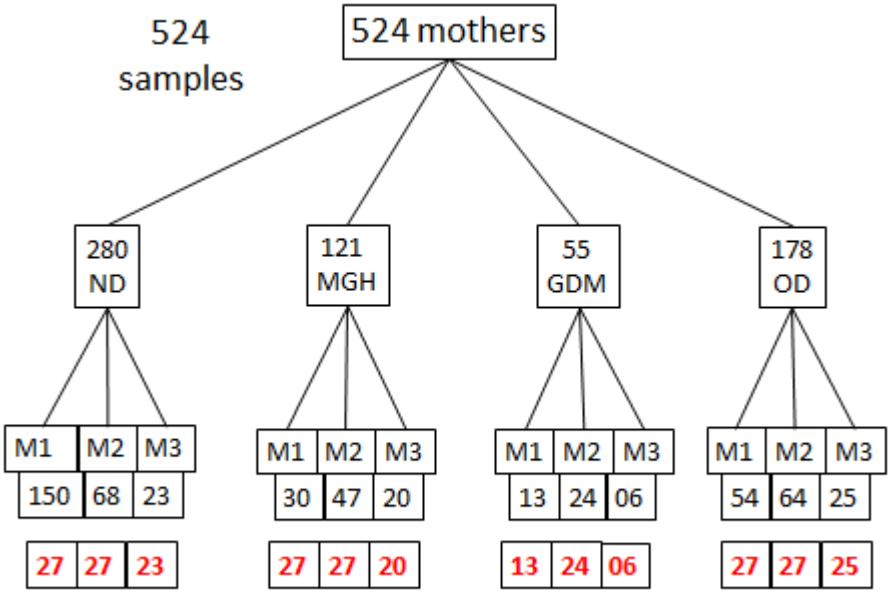

Fig. 1 Flowchart of women included in the study

M1: 24-28 weeks gestational age

M2: 34-38 weeks gestational age

M3: 6 weeks postpartum

Table 1. Mean (±SD) of maternal, newborn infants (NB) and placental characteristics of the study population.

	ND	MGH	GDM	Overt diabetes
Age	28,45 ± 2,5	28,25 ± 2,01	31,66 ± 2,83	32,66 ± 2,81
BMI (kg/m^2)	28,70 ± 6,5	28,92 ± 6,86	29,22 ± 6,92	31,25 ± 7,01*
Glycemia (mg/dl)	86,06 ± 21,82	95,29 ± 12,26	100,44 ± 15,48	133,36 ± 34,87
HbA_1C (%)	5,31 ± 0,48	5,28 ± 0,57	5,34 ± 0,51	6,08 ± 0,63*$^{\#}$
Smoking (%)	6,06	25,71	16,66	33,33
Caucasian (%)	90,90	62,85	75	54,76
Married (%)	67,82	69,72	76,90	79,02
Fetal Weight (g)	3134,57 ± 571,51	3483,97 ± 774,62*	3162,31 ± 464,68	3283,80 ± 570,26
Fetal Length (cm)	48,39 ± 2,74	49,34 ± 3,05*	48,45 ± 1,60	48,05 ± 2,36
Apgar	7,45 ± 2,56	7,72 ± 1,46	8,00 ± 1,71	7,63 ± 1,45
Apgar <7 (%)	21,05	18,75	11,11	16
Hb NB	15,92 ± 2,39	16,18 ± 1,94	18,80 ± 4,09	15,93 ± 1,47
SGA	0	17,65	22,22	12,50
AGA	91,67	52,94	77,78	79,19
LGA	8,33	29,41*	0	8,33
Placental weight (g)	533,57 ± 131,25	588,04 ± 172,25	550,00 ± 106,71	601,48 ± 163,92
Ponderal index	2,74 ± 0,23	2,86 ± 0,33	2,78 ± 0,26	2,94 ± 0,23*
Placental index	0,16 ± 0,06	0,16 ± 0,05	0,18 ± 0,04	0,18 ± 0,06

*p<0,05 – statistically significant difference compared to the ND (ANOVA with simple measures).
$^{\#}$p<0,05 – statistically significant difference compared to the MGH (ANOVA with simple measures).

Table 2. Mean (± SD) HSP60, HSP70 and respective antibodies from different groups in maternal blood.

	HSP60			Anti HSP60			HSP70			Anti HSP70		
	24-28 weeks	34-38 weeks	Postpartum	24-28 weeks	34-38 weeks	Postpartum	24-28 weeks	34-38 weeks	Postpartum	24-28 weeks	34-38 weeks	Postpartum
ND	24,02 ±45,23	36,78 ±1,73	49,03 ±19,7[a]	28,17 ±25,08	58,93 ±48,5[a]	48,63±17,31[a]	0,49 ±0,42	0,35 ±0,52	0,25 ±0,23[ab]	179,85 ±103,95	215,78 ±96,53	103,82 ±20,25[ab]
MGH	11,66 ±9,42	5,61 ±5,15*[a]	26,16±20,74[ab]	45,61 ±32,59	37,68 ±36,28	46,68 ±10,93	0,21 ±0,13*	0,27 ±0,25	0,32 ±0,32	107,77 ±33,04*	139,29 ±93,33*[a]	93,22 ±16,02[ab]
GDM	4,41 ±1,77*[#]	6,53 ±7,74*	7,56 ±4,82	30,54 ±25,36	61,86 ±38,81	53,87 ±34	0,21 ±0,14	0,17 ±0,17	0,19 ±0,11	173,36 ±28,57[#]	130,28 ±98,49*	162,89 ±48,85
Overt diabetes	12,16 ±10,33[$]	16,87 ±15,07[#]	7,76 ±6,15*[#b]	32,76 ±22,85	39,43 ±27,93	41,95 ±22,49	0,21 ±0,18	0,17 ±0,23	0,15 ±0,08*[#]	107,15 ±64,58*[$]	140,94 ±84,84*[a]	113,55 ±53,78[ab]

*p<0,05 – statistically significant difference compared to the ND (ANOVA with repeated measures).
[#]p<0,05 – statistically significant difference compared to the MGH (ANOVA with repeated measures).
[$]p<0,05 – statistically significant difference compared to the GDM (ANOVA with repeated measures).
[a]p<0,05 – statistically significant difference compared to the 24-30 weeks (ANOVA with repeated measures).
[b]p<0,05 – statistically significant difference compared to the 34-38 weeks (ANOVA with repeated measures).

Table 3. Relationship between heat shock proteins and maternal,neonatal and placental data.

	HSP60		HSP70	
	R	*P*	*R*	*P*
HSP60	-	-	**0,58**	**<0,0001**
HSP70	**0,58**	**<0,0001**	-	-
Anti-HSP60	**0,38**	**<0,0001**	0,15	0,112
Anti-HSP70	**0,06**	**0,53**	0,04	0,67
Glucose	0,08	0,47	-0,06	0,56
HbA_1C	0,20	0,04	0,12	0,22
BMI	0,31	0,18	0,08	0,42
Fetal weight	-0,13	0,22	-0,04	0,66
Fetal Length	-0,17	0,10	-0,14	0,42
Apgar	-0,007	0,94	0,10	0,35
Placental weight	-0,01	0,87	-0,04	0,69
Ponderal index	0,01	0,87	0,14	0,19
Placental index	-0,08	0,45	0,04	0,70

Table 4. Relationship between antibodies to heat shock proteins and maternal, neonatal and placental data.

	Anti-HSP60		Anti-HSP70	
	r	*p*	*r*	*p*
Anti-HSP60	-	-	-0,02	0,80
Anti-HSP70	0,02	0,82	-	-
Glucose	**0,23**	**0,04**	-0,12	0,31
HbA_1C	-0,09	0,33	0,0001	0,99
BMI	-0,09	0,34	**-0,23**	**0,01**
newborn weight	**-0,28**	**0,04**	-0,11	0,30
Newborn length	0,03	0,77	-0,02	0,84
Apgar	0,07	0,51	-0,16	0,14
Placental weight	**-0,30**	**0,04**	-0,20	0,06
Ponderal index	**-0,45**	**0,001**	-0,17	0,11
Placental index	0,04	0,66	-0,13	0,22
Fetal hemoglobin	**-0,34**	**0,050**	-	-

Anexos

unesp **Universidade Estadual Paulista**
Faculdade de Medicina de Botucatu

Distrito Rubião Junior, s/nº - Botucatu – S.P.
CEP: 18.618-970
Fone/Fax: (0xx14) 3811-6143
e-mail secretaria: capellup@fmb.unesp.br
e-mail coordenadoria: tsarden@fmb.unesp.br

Registrado no Ministério da Saúde
em 30 de abril de 1997

Botucatu, 14 de julho de 2011. Of. 307/11-CEP

Ilustríssima Senhora
Profª Titular Marilza Vieira Cunha Rudge
Departamento de Ginceologia e Obstetrícia da
Faculdade de Medicina de Botucatu.

Cara Drª Marilza,

De ordem do Senhor Coordenador deste CEP, e com referência ao Projeto de Pesquisa **"Diabete e Hiperglicemia diária na gravidez-Pesquisa Clinica e Experimental para validação do Grupo IB de Rudge"**, Coordenado por Vossa Senhoria, com a colaboração de Iracema de Mattos Paranhos Calderon, Daisy Maria Fávero Salvadori, Débora Cristina Damasceno, já aprovado por este CEP em 08/11/2004, informo que nesta data **(14/07/2011)** foi autorizado a inclusão de 02 momentos a mais no projeto a saber:

- Antes da 24ª semana de gestação e 6 semanas após o parto para colheita de amostras de sangue (10ml) para determinação de HSP (proteínas de choque térmico).
- O TCLE ora apresentado cumpre as normas da Resolução 196/96, portanto deverá ser aplicado.

Na mesma data **(14/07/2011)**, foi autorizada a inclusão dos alunos **Karina Zanotel de Oliveira e Felipe Hiroshi Saito**, com objetivo de pós graduação, em nível de Mestrado e Doutorado.

Atenciosamente,

Alberto Santos Capelluppi
Secretário do CEP.

unesp UNIVERSIDADE ESTADUAL PAULISTA
"JÚLIO DE MESQUITA FILHO"
Campus de Botucatu

FMB FACULDADE DE MEDICINA BOTUCATU

Botucatu, 28 de janeiro de 2014.

Ao ilustríssimo senhor

Alberto Santos Capelluppi

Venho por meio deste solicitar a inclusão da aluna de Pós-Graduação Mariana Alvarez Arantes, como nova participante do projeto "DIABETE E HIPERGLICEMIA DIÁRIA NA GRAVIDEZ-PESQUISA CLÍNICA E EXPERIMENTAL PARA VALIDAÇÃO DO GRUPO IB DE RUDGE" , aprovado em 08/11/2004 e inclusão de novos momentos em 14/07/2011, sob minha orientação e colaboração das professoras Iracema de Mattos Paranhos Calderon, Daysi Maria Fávero Salvadori, Débora Cristina Damasceno.

Atenciosamente,

Profa. Titular Marilza Vieira Cunha Rudge

PG-GOM Faculdade de Medicina de Botucatu – Programa de PG em Ginecologia, Obstetrícia e Mastologia
Distrito de Rubião Júnior, s/n CEP 18618-970 / Botucatu São Paulo / Brasil
Tel /Fax 55 14 3811 6227 / e-mail: pggo@fmb.unesp.br

TERMO DE CONSENTIMENTO LIVRE E ESCLARECIDO

I. IDENTIFICAÇÃO DO PACIENTE (VOLUNTÁRIO)

Nome:		
RG:		**Código (não preencher):**
Endereço:		
Cidade:		**Bairro:**
CEP:	**Estado:**	**Telefone:**

II. TÍTULO DA PESQUISA

DIABETE E HIPERGLICEMIA LEVE DIÁRIA NA GRAVIDEZ – PESQUISA CLÍNICA E EXPERIMENTAL PARA VALIDAÇÃO DO GRUPO IB DE RUDGE

Pesquisador – Responsável: Profª Drª Marilza Vieira Cunha Rudge, Departamento de Ginecologia e Obstetrícia – Faculdade de Medicina de Botucatu, UNESP, Botucatu – SP. (140 3811-6227); mrudge@fmb.unesp.br

III. EXPLICAÇÕES DO PESQUISADOR AO PACIENTE

O objetivo deste estudo é identificar marcadores que possam caracterizar a hiperglicemia gestacional diária como uma forma de diabete e, assim, contribuir para o tratamento. Além disso, o estudo visa avaliar possíveis alterações em órgãos e células que possam causar efeitos adversos no futuro. Desta forma, solicitamos sua autorização para coleta de uma amostra de sangue antes de 24ª semana de gestação (máximo de 10ml) para determinação de HSP (proteínas de choque térmico), outra amostra no final da gestação para realização de exames bioquímicos e extração de material genético (DNA) das células para avaliação de características genéticas (genes) que são responsáveis pelas respostas do seu organismo à exposição a algumas substâncias. Solicitamos, também, sua autorização para que sejam coletadas amostras de placenta e amostra de sangue do cordão umbilical do nenê para realização dos mesmos exames e 6 semanas após o parto para colheita de amostras de sangue (10ml) para determinação de HSP (proteínas de choque térmico) e autorização para que parte das amostras de sangue e placenta possam ser armazenadas, sob responsabilidade dessa Instituição, para a realização de estudos semelhantes no futuro. Esclarecemos que, a não ser o pequeno desconforto no momento da picada da agulha, a coleta do sangue não tem risco, pois será feita por profissional qualificado e utilizando material descartável. A senhora pode, a qualquer momento, se recusar em contribuir com o estudo sem ser prejudicada no seu tratamento e acompanhamento médico, ou pode, também, ter acesso aos resultados. Sua

identidade não será revelada e será mantido o caráter confidencial de todas as informações obtidas.

Os resultados deste estudo serão divulgados em congressos científicos e publicados em revistas especializadas, preservando sua identidade. Os resultados do estudo não trarão benefícios imediatos a sua pessoa, mas poderão contribuir, no futuro, para redução dos efeitos adversos causados por essa patologia.

Os pesquisadores responsáveis por este estudo, sempre que solicitados, estaraão à sua disposição para o esclarecimento de qualquer questão relacionada à pesquisa.

Ressaltamos que nem os pesquisadores e nem o paciente receberá qualquer remuneração financeira por participar desta pesquisa.

IV. CONSENTIMENTO PÓS-INFORMADO

Eu, _____ abaixo

assinado, declaro que fui esclarecido sobre o objetivo do presente estudo e sobre os eventuais desconfortos que poderei sofrer, assim como os benefícios do estudo.

Concordo, portanto, em participar, na qualidade de voluntário, do referido Projeto de Pesquisa, sob livre e espontânea vontade.

Botucatu, _____ de_____ de _____

_____

Paciente

_____

Pesquisadoras: Profa. Marilza Vieira Cunha Rudge

e/ou Profa. Iracema de Mattos Paranhos Calderon

telefone contato: (14) 38116227

FICHA PARA COLETA DE DADOS INDIVIDUAIS

Projeto: ***"Avaliação da genotoxicidade de gestantes com diagnóstico de hiperglicemia leve" e "Avaliação gênica e de proteínas do sangue e da placenta de gestantes diabéticas e com hiperglicemia leve"***

Responsáveis: Profa. Dra. Marilza Vieira Cunha Rudge

Profa. Dra. Iracema de Mattos Paranhos Calderon

Profa. Dra. Paula Helena Ortiz Lima

DADOS DA PACIENTE

Nome:……Paciente nº ……

Data:**……/……/……** **Registro FMB.....**

Data de nascimento:**……/……/……**

RG:.....

Qual o seu endereço (rua, nº , cidade, CEP):

_____

Telefone para contato _____

Qual é sua nacionalidade?_____

Cor: Branca ( ) Parda ( ) Negra ( ) Amarela ( )

Estado civil

(1)casada (2)divorciada (3)solteira

(4)amasiada (5)viúva

Hábito de fumar: Nunca fumou ( ) Parou de fumar ( ) há quanto tempo?.....

Por quanto tempo fumou?.....

Fuma ( ) Nº de cigarros/dia……...

Tipo: ( )cachimbo ( ) charuto ( ) palha ( ) papel com filtro ( ) outros

Convive com fumante? Sim ( ) Não ( )

Local: ( )casa ( )trabalho ( )outros

No. de pessoas?.....Quantas horas/dias?.....

Consume bebida alcoólica? Sim ( ) Não ( )

Até 7 doses/sem. ( ) Mais de 7 doses/sem ( )

Usa drogas? Sim ( ) Não ( )

Tipo: ( )maconha ( )cocaína ( )craque ( )outros

Vias de administração: ( )inalatória ( )endovenosa ( ) outras

Durante quanto tempo?.....

Já usou? Sim ( ) Não ( ) Quanto tempo?.....

Parou há quanto tempo?.....

Contato com substâncias tóxicas? Sim ( ) Não ( )

Quais?.....

Por quanto tempo (meses)?.....

Período sem contato com a (s) substância (s) (meses):.....

Possui algum tipo de doença? Sim ( ) Não ( ) Qual(is)?

Diabetes mellitus ( )

Já teve infarto? Sim ( ) Não ( ) Há quanto tempo?……..

É hipertensa? Sim ( ) Não ( )

É obeso? Sim ( ) Não ( )

Peso (Kg)…… Altura (cm)…….. IMC:…….

Toma medicamento? Sim ( ) Não ( )

Qual(is)? Em que dosagem?……..

...……..……..……

Profissão:…… Há exigência de esforço físico? Sim ( ) Não ( )

Pratica exercício físico? Sim ( ) Não ( ) Com que freqüência?……

HISTÓRICO FAMILIAR

Possui algum parente com:

Diabetes Mellitus ( ) Obesidade ( ) Hipertensão ( )

Doença cardiovascular ( ) Hipercolesterolemia ( ) ( ) outros

Quem?……

Não tem ( ) Não sabe ( )

HISTÓRIA GESTACIONAL

G:.....P:.....A:.....C:.....

DUM:.....

Pré-natal: Sim ( ) Não ( ) No. de consultas?.....

Intercorrências durante a gravidez: (..)náuseas ( )vômitos ( ) dor abdominal

( )dor para urinar ( )corrimentos ( ) sangramento ( )outros

Doenças durante a gestação: Sim ( ) Não ( ) Tipo?.....

Tratamento: Sim ( ) Não ( ) Qual?.....

Fatores de risco para HIV? Sim ( ) Não ( )

AVALIAÇÕES

	Inicial	Final
Peso (kg) (verificar no cartão de pré-natal ou na folha rosa do prontuário)		
Pressão Arterial (mmHg) (verificar no cartão de pré-natal ou na folha rosa do prontuário)		

DADOS DO RECÉM-NASCIDO (RN)

Tipo de parto: ( )normal ( )cesária ( )fórceps ( )outros

Sinais de sofrimento fetal? Sim ( ) Não ( )

Qual?.....

Mecônio: Sim ( ) Não ( )

Tipo: ( )fluido ( ) moderado ( ) espesso

Apgar: 1' .....5'.....10'.....

New Ballard (NB):.....Sexo:.....Peso:.....Estatura:.....

PC:.....PT:.....PA:.....

Classificação do RN: ( ) PIG ( ) AIG ( ) GIG

Necessidade de reanimação: ( ) máscara ( )ventilação sob pressão ( ) intubação

( ) drogas Quais drogas?.....

Óbito RN: Sim ( ) Não ( )

Vasos do cordão: ( ) 2 artérias e 1 veia ( ) 1 artéria e 1 veia ( ) outros

Anomalias ou malformações: Sim ( ) Não ( )
Quais?.....

Tipo de placenta: ( ) normal ( ) calcificada ( ) outras