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Análise da proteína quimiotática de monócitos-3 (CCL7) em gestantes hiperglicêmicas com incontinência urinária: coorte prospectiva da gestação ao primeiro ano pós-parto

Tese apresentada à Faculdade de Medicina, Universidade Estadual Paulista "Júlio de Mesquita Filho", Câmpus de Botucatu, para obtenção do título de Doutor em Ginecologia, Obstetrícia e Mastologia.

Orientadora: Profa. Dra. Marilza Vieira Cunha Rudge
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Palavras-chave: CCL7; Diabetes gestacional; Gestação; Incontinência urinária.

Dedicatória

"Nenhuma invenção do ser humano, por mais completa e evoluída que seja, equivale à magia que é o encontro de duas células num ambiente propício a formar um novo ser".

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"Porque cada um, independente das habilitações que tenha, ao menos uma vez na vida fez ou disse coisas muito acima da sua natureza e condição, e se a essas pessoas pudéssemos retirar do quotidiano pardo em que vão perdendo os contornos, ou elas a si próprias se retirassem de malhas e prisões, quantas mais maravilhas seriam capazes de obrar, que pedaços de conhecimento profundo poderiam comunicar, porque cada um de nós sabe infinitamente mais do que julga e cada um dos outros infinitamente mais do que neles aceitamos reconhecer."

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Sumário

Contextualização

Grupo de Pesquisa "Diabete e Gravidez - Clínico e Experimental"

O Grupo de Pesquisa Acadêmico "Diabete e Gravidez - Clínico e Experimental" é liderado por Rudge, M.V.C. desde 1980 e vinculado ao Programa de Pós-Graduação em Ginecologia, Obstetrícia e Mastologia da Faculdade de Medicina de Botucatu-UNESP. Em sua tese de livre-docência (1984) investigou o diagnóstico do *Diabetes mellitus* gestacional (DMG) por meio do teste oral de tolerância à glicose (TTG) e perfil glicêmico (PG).

O uso dos dois testes, aplicados em paralelo, identificou quatro grupos de gestantes com características diferentes das apontadas pela literatura, nomeados grupos de Rudge: IA - gestantes não diabéticas com os 2 testes normais; Grupo IB - portadoras de hiperglicemia gestacional leve com apenas o PG alterado; Grupo IIA - portadoras de DMG com apenas o TTG alterado e o grupo IIB- portadoras de DMG e/ou DM2 com os 2 testes alterados (1). Usando a macrossomia fetal como padrão ouro do diagnóstico, Rudge *et al.* identificaram que as gestantes com perfil glicêmico alterado, independente do resultado do TTG, apresentaram maior prevalência de macrossomia fetal. As gestantes que apresentaram alteração apenas no PG foram denominadas portadoras de Hiperglicemia gestacional leve (HGL) e caracterizam um grupo diferenciado de gestantes que vem sendo estudado em diferentes aspectos maternos e perinatais (1). Este estudo deu origem à pesquisas clínicas e experimentais de forma translacional para elucidação das repercussões da hiperglicemia gestacional em diferentes níveis glicêmicos (2).

Dentre os temas de interesse do grupo, a associação entre hiperglicemia gestacional e incontinência urinária (IU) chama atenção pela escassez de estudos na literatura. Pesquisas com associação entre DMG e alteração muscular do assoalho pélvico iniciaram-se a partir de investigações clínicas sobre IU gestacional em mulheres com histórico de DMG. A tese de doutorado de Barbosa em 2006 "Prevalência e fator de risco para incontinência urinária e disfunção do assoalho pélvico dois anos após *Diabetes mellitus* gestacional" gerou resultados

importantes (3) para a fundamentação de uma nova linha de pesquisa, credenciada junto ao grupo de pesquisa no CNPq. Esta linha de pesquisa, denominada "Tríade hiperglicemia gestacional, incontinência urinária gestacional e perfil da miopatia hiperglicêmica gestacional" tem como objetivo identificar biomarcadores morfológicos, bioquímicos, moleculares e ômicos da miopatia diabética gestacional na predição de incontinência urinária pós-parto.

O estudo clínico de Barbosa *et al.* mostrou que a incidência da IU gestacional em mulheres com histórico de DMG foi maior (50,8%) em comparação ao grupo normoglicêmico (31,6%). Dois anos após parto cesárea a IU nas mulheres com histórico DMG prevaleceu 44,8% vs. 18,4% nas normoglicêmicas. Na análise multivariada, o DMG foi considerado fator de risco para IU (OR 2,26; IC 95%: 1,116-4,579). Além disso, a IU pós-parto relacionou-se com a baixa pressão de contração dos músculos do assoalho pélvico (MAP) (OR 20,416; IC 95%: 3,548;117,479) (3).

Estes achados demonstraram déficit funcional dos MAP, possivelmente advindos do processo patológico do DMG, e deram origem à primeira etapa do estudo translacional em relação à interação DMG-MAP ("*bedside to bench*") (2) que teve finalidade de investigar, por meio de estudos experimentais, a musculatura estriada uretral de ratas prenhes diabéticas, análogos aos MAP de mulheres. Esta fase foi conduzida em parceria com pesquisadores da *Case Western Reserve University* (CWRU), Cleveland Ohio/USA. No mestrado de Marini, 2010 (4) (FAPESP 2008/00989-4) e Piculo, 2013 (5) (FAPESP 2010/13303-3) foram desenvolvidos estudos experimentais em ratas prenhes com diabete grave e moderado que demonstraram achados semelhantes em relação à morfologia das fibras musculares do músculo estriado uretral de ratas submetidas à cesárea, como atrofia, adelgaçamento, desorganização e rompimento associado à perda de localização anatômica normal e alteração na proporção das fibras rápidas e lentas. Além dos achados musculares foi evidenciada

mudança significativa na matriz extracelular com presença de fibrose e na ultraestrutura como o acúmulo de mitocôndrias, gotas de lipídios e grânulos de glicogênio. Aspectos relacionados aos danos e exposição temporal a níveis glicêmicos moderados, relacionados à metodologia empregada, levantaram questionamentos que foram elucidados com o desenvolvimento de dois estudos experimentais desenvolvidos em teses de mestrado e doutorado (5, 6). O efeito da indução do diabete de intensidade e tempo de exposição diferentes foi verificado no doutorado de Marini, 2014 (FAPESP 2010/10740-3). Diferentemente do esperado, o diabete moderado presente a longo prazo foi mais agressivo para integridade do tecido muscular comparado ao diabete grave a curto prazo, confirmando que o tempo de exposição ao ambiente hiperglicêmico foi mais determinante para lesão muscular do que a intensidade glicêmica (6). Estes resultados experimentais demonstraram que a associação diabete e prenhez danifica gravemente o músculo estriado uretral, corroborando os resultados clínicos anteriores de que a IU em diabéticas gestacionais está associada à disfunção muscular do assoalho pélvico (3).

A etapa "*bed to bench*" da pesquisa translacional realizada nos trabalhos publicados pelo nosso grupo consolidaram a base fisiopatológica da IU no DMG e na HGL (2). A etapa "*bench to bed*" deveria ser a intervenção na gestação de diabéticas. Entretanto, o contato e as discussões das proponentes deste projeto com o serviço de Urologia da *Case Western Reserve University–Cleveland (USA)* supervisionado pelo Dr. Adonis Hijaz, indicaram que antes da intervenção é importante o desenvolvimento de mais pesquisa para investigar os mecanismos de recuperação envolvidos nos distúrbios do assoalho pélvico feminino em mulheres com DMG e HGL e que resultarão no melhor atendimento às pacientes. Frente a isso, nosso grupo iniciou a investigação de marcadores envolvidos na mobilização de células-tronco mesenquimais como mecanismo natural de reparo dos MAP envolvidos na IU em mulheres com hiperglicemia gestacional durante a gestação e após o parto, que é tema da presente tese.

Papel da CCL7 na recuperação da IU

A proteína quimiotática de monócitos-3 (MCP-3), atualmente conhecida como CCL7, é uma citocina *homing* de células-tronco mesenquimais, significativamente super-expressa após a distenção vaginal em ratas, principalmente na uretra (7). *Homing* é definido como a captura das células-tronco mesenquimais pela vasculatura de um tecido, seguido de sua transmigração através do endotélio (8). Embora esse processo ainda não esteja totalmente esclarecido, muitos estudos têm sido realizados a respeito da mobilização de células-tronco mesenquimais nativas e do *homing* das células-tronco mesenquimais exógenas infundidas por diversas vias em resposta a um insulto isquêmico/inflamatório.

Em investigação experimental recente de causas de incontinência urinária induzida por trauma de parto e mecanismos de recuperação, foi verificada a relação entre a duração da distenção vaginal e subsequente aumento da expressão de CCL7 e um dos seus receptores associados, CCR-1, na uretra imediatamente após distenção vaginal (9). A infusão intravenosa de célula-tronco mesenquimal em ratas esteve associada a uma recuperação funcional acelerada de perda da resistência uretral (10), sugerindo que a promoção da mobilização de células-tronco pode ter um efeito positivo sobre a recuperação da IU. A importância sobre o papel da CCL7 e sua associação com a mobilização de células-tronco na recuperação da lesão de um tecido segue observação semelhante ao que ocorre no coração após uma lesão isquêmica. As células-tronco são funcionalmente responsáveis pelo desenvolvimento e regeneração dos tecidos e órgãos (11, 12). No adulto, as células-tronco quiescentes normalmente tornam-se ativadas após um episódio de dano tecidual, durante a qual o corpo gera sinais químicos específicos, que servem para direcionar a migração e diferenciação celular (32). Este método tem o potencial de facilitar a recuperação da função sem as dificuldades inerentes no uso de células transplantadas.

Dados atuais sugerem que o parto humano simulado por distensão vaginal em ratas

virgens é conhecido por aumentar a expressão de quimiocinas e receptores envolvidos na migração de células-tronco e reparação de tecidos. Apesar de ser bem caracterizado e ter uso validado, o conhecimento dos efeitos da distenção vaginal na migração de células-tronco e reparação tecidual no cenário da gravidez e do parto ainda vem sendo melhor investigado. Lenis *et al.* (2013) investigaram a hipótese de que a gravidez e o parto em ratas facilitam o reparo da distenção vaginal, aumentando a expressão de quimiocinas e de seus receptores, facilitando a migração de células-tronco endógenas e a reparação tecidual. Os dados mostraram que a distenção vaginal regula a expressão de CCL7 imediatamente após a lesão em ratas virgens e pós-parto (13), o que validou estudos anteriores. A distenção vaginal regulou a expressão de CCL7 uretral em aproximadamente oito vezes comparado aos controles, independentemente da paridade, sendo consistente com os resultados de Wood *et al.* (2008), apesar do uso de uma linhagem diferente de rato (9). Por outro lado, Woo *et al.* relataram aumento aproximado de vinte vezes na expressão uretral de CCL7 em ratas após distenção vaginal. Variações na técnica de dissecção podem explicar estas diferenças entre as investigações (7, 14).

Devido a necessidade de realizar um estudo clínico para mensurar o CCL7 em mulheres com incontinência urinária no período gestacional e pós-parto e a impossibilidade de fazê-lo em tecido específico (uretra), Hijaz *et al.*, 2013 (15) investigou a expressão de CCL7 no soro de modelos murinos de incontinência urinária induzida por trauma de parto. Os resultados mostraram superexpressão de CCL7 no soro de camundongos após a distenção vaginal, indicando que a expressão local de CCL7 está associada a uma resposta sistêmica após lesão, além de sugerir um potencial translacional (15).

Diante disso, o estudo proposto estabelece uma ligação translacional, mensuração de CCL7 em mulheres com hiperglicemia gestacional, e ainda proporciona oportunidades para a exploração futura do papel mecanicista do fator de recuperação na IU feminina.

A presente tese está dividida em 3 partes: Contextualização, Capítulo 1 e Capítulo 2.

Os capítulos estão formatados de acordo com as normas das revistas a que serão submetidos.

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Capítulo 1

PREGNANCY-SPECIFIC URINARY INCONTINENCE IN GESTATIONAL HYPERGLYCEMIA WORSE THE OCCURRENCE AND SEVERITY OF URINARY INCONTINENCE AND QUALITY OF LIFE OVER THE FIRST YEAR POSTPARTUM

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ABSTRACT

Background: The lack of epidemiologic data of long-term repercussion of pregnancy-specific urinary incontinence (PS-UI) in gestational hyperglycemia over the first year postpartum led us to conduct this cross-sectional questionnaire-based study in Brazil. **Objective:** To determine the occurrence and severity of PS-UI in gestational hyperglycemic women and its impact on quality of life (QoL) over the first year postpartum. **Design, Setting, and Participants:** 388 pregnant women with PS-UI distributed into two study groups (normoglycemic and hyperglycemic) were analysed at five time-points during pregnancy and the first year postpartum. Gestational hyperglycemia was established according to ADA's criteria, followed by glucose profile. **Outcome Measurements and Statistical Analysis:** Relationships with outcome analyzed using Chi-square test for categorical variables and Student-t test for quantitative variables. **Results and Limitation:** PS-UI prevalence was 54.1% being 43.3% in normoglycemic and 56.7% in hyperglycemic Brazilian pregnant women. Gestational hyperglycemia increased the amount ($p < 0.0027$), frequency ($p < 0.0014$), impact of PS-UI on the QoL ($p < 0.0001$), severity ($p = 0.0003$) and total scores of ICIQ-SF and ISI questionnaires ($p < 0.0001$), at the two time-points of pregnancy. In the postpartum period, the PS-UI in gestational hyperglycemic women increased the amount ($p = 0.0079$), frequency ($p = 0.0382$), impact of UI on QoL ($p < 0.0001$), severity ($p = 0.0053$), and questionnaires scores ($p < 0.0001$ for ICIQ-SF and $p = 0.003$ for ISI) over the first year postpartum. **Conclusions:** The PS-UI in hyperglycemic women worse the occurrence and severity of UI and affected their QoL proportionally to the severity of symptoms over the first year postpartum. The results emphasize the interaction between PS-UI, gestational hyperglycemia and long-term maternal outcome. **Patient Summary:** PS-UI were found to be severe and very severe in hyperglycemic women with negative impact on QoL during pregnancy over the first year postpartum.

Keywords: Gestational diabetes; pregnancy; quality of life; urinary incontinence.

INTRODUCTION

Urinary incontinence (UI) over the first year postpartum is a highly prevalent disorder and has an negative effect on quality of life (QoL) (1-3). The question of how to prevent postpartum UI has led to increasing interest in the impact of several potential risk factors for this disorder. Pregnancy itself is identified as a risk factor for postpartum UI and is independent of the mode of delivery (4, 5). With the presence of UI in the first time during pregnancy, named as pregnancy-specific UI (PS-UI) (6), the woman had an excessive tendency to show a similar reaction later in life (6, 7). Gestational diabetes mellitus (GDM) and mild gestational hyperglycemia (MGH) are hyperglycemic status resulting from inadequate insulin sensitivity, first recognized or developed during pregnancy (8), which increase UI up to two years postpartum (5, 9). Diabetes increases the risk of UI 2.5 fold (10, 11), and is associated with an impairment of muscle strength and physical function (12). For type 2 DM, there are changes in muscle strength and functional capacity, excessive loss of skeletal muscle mass, abnormal lipid deposition and high collagen levels in diabetic tissues (12).

The inter-relationship between diabetes mellitus (DM) and UI, and between pregnancy and UI are well established. Despite these associations, the inter-relationships among DM, pregnancy, and UI have rarely been investigated (9). Currently, few studies reveal positive correlation between GDM and UI (5, 9, 13). The long-term association of PS-UI in hyperglycemic pregnant women over a continuous period since the pregnancy until the first year postpartum are unclear. In the light of the complex inter-relationship among GDM and MGH, and PS-UI and postpartum UI, we hypothesize that gestational hyperglycemia (GH) associated with PS-UI will increase the occurrence and severity of UI having a negative impact on the QoL during pregnancy and up to 12 months postpartum. We conducted a cross-sectional questionnaire-based study to investigate two complementary aims at five time-points

during pregnancy and postpartum, i.e., (1) to determine the impact of GH on the occurrence and severity of PS-UI and its impact on the QoL at two time-points of pregnancy, and (2) to examine the impact of the new association of PS-UI and GH on the occurrence, severity, and impact of UI on the QoL over three time-points in the first year postpartum.

MATERIALS AND METHODS

Research design and subjects

This study was part of a follow-up of hyperglycemic pregnant women's health with PS-UI, and was conducted between 2013 and 2015 at the University Hospital (Perinatal Diabetes Research Center), São Paulo State University (UNESP), Brazil. The pregnant women attending Prenatal Care Unit were recruited at the time of ante partum screening for GDM. This study counted with the approval from the Research Ethics Committee of institution (CAAE: 20639813.0.0000.5411). Written informed consent was obtained from all subjects and all the procedures used in the study were in accordance with the guidelines of the Helsinki Declaration on human experimentation.

We used a cross-sectional design, five time-points during pregnancy until 1-year postpartum to determine the occurrence, severity, and impact of PS-UI on the QoL of two groups of pregnant women: normoglycemic and hyperglycemic. The diagnosis of gestational hyperglycemia (GDM or MGH) was established between 24^{th} and 28^{th} gestational weeks, by 75 g-OGTT test according to American Diabetes Association (ADA) criteria (14) followed by the glucose profile (GP) test according to Rudge (15, 16). MGH and GDM were considered as gestational hyperglycemia and reflect the full spectrum of glucose tolerance in pregnancy since normal to mildly abnormal to GDM (17).

UI was defined as any urinary leakage new onset during pregnancy (6). UI was classified according to the International Continence Society (ICS) guidelines as stress urinary

incontinence (SUI) (involuntary leakage on effort or exertion, or on sneezing, or coughing), urge urinary incontinence (UUI) (involuntary leakage accompanied by or immediately preceded by urgency), and mixed urinary incontinence (MUI) (involuntary leakage associated with urgency and with exertion, effort, sneezing, or coughing) (18).

A total of 717 pregnant women were subjected to the 75-g-OGTT and GP determinations, and inquiry for UI. Based on the collected information women were distributed into two study groups: normoglycemic incontinent (NI, with normal 75g-OGTT and GP, n = 168) and hyperglycemic incontinent (HI, with abnormal 75g- OGTT and/or abnormal GP, n = 220).

The current analysis was restricted to women with singleton pregnancies, who underwent their OGTT and GP between 24 and 28 weeks of pregnancy with PS-UI. Pre-pregnancy UI, known type 1 or type 2 DM, preterm delivery (<37 weeks of gestation), multiple pregnancies, known fetal anomaly, or any clinical condition may have jeopardised the health status of the woman, were excluded from this study.

Data collection

Individuals who satisfied the inclusion criteria were invited to participate in the study. Baseline information (maternal characteristics, demographics, anthropometrics, previous obstetric history) and symptoms of UI were evaluated at five time-points during pregnancy and the postpartum period: at 24 and 28 weeks gestational age (visit 1), 34 and 38 weeks gestational age (visit 2), within 24 and 48 hours postpartum (visit 3), 6 weeks postpartum (visit 4), and 6-12 months postpartum (visit 5). A form completed immediately after the birth was used to record the labour process, the mode of delivery, and the neonatal birth profile.

Next, pregnant women were asked to complete the Brazilian version of the International Consultation on Incontinence Questionnaire-Urinary Incontinence-Short Form

(ICIQ-SF) (19). The ICIQ-SF comprises three scored items and one non-scored item, and is assessable for prevalence, severity, interference in daily life, and type of UI (19). The ICIQ-SF score represents a combined severity and bother-score, with range 0–21 (20). Scores on the interference in QoL represent are set from '0' as not at all to '10' as a great deal (2). The one non-scored item of the ICIQ-SF includes eight answers and is a self-diagnostic item to understand the participant's perception of the cause and type of leakage.

The participants were also asked about the frequency and quantity of urinary leakage in order to assess the validated Incontinence Severity Index (ISI). According to ISI questionnaire, the severity of incontinence was classified as slight (score 1-2), moderate (score 3-6), severe (score 8-9) and very severe (score 12) based on composite scores (21). From visit 1 to visit 5, all the participants were followed by face-to-face interview by the researcher to assess incontinence status.

Statistical analysis

Data from pregnant women of the normoglycemic and hyperglycemic urinary incontinent groups were compared using the Chi-square test for categorical variables and Student-t test for quantitative variables. All analyses were performed using SAS software for Windows (v.9.3, SAS Institute Inc., Cary, NC, USA). Statistical significance was set up at the 5% level ($p<0.05$).

RESULTS

Of the 717 participants initially screened for GH and interviewed for UI, 388 were eligible for inclusion in the final analysis and successfully included in this study (Figure 1). Of the PS-UI participants screened and interviewed (n = 388), 220 women were in the hyperglycemic group, and 168 women were in the normoglycemic group. The prevalence of

PS-UI was 54.1% (388/717). Women allocated at five time-points were 107 at visit 1 and 130 at visit 2 during pregnancy, 72 at visit 3, 25 at visit 4, and 54 at visit 5 over the first year postpartum (Figure 1).

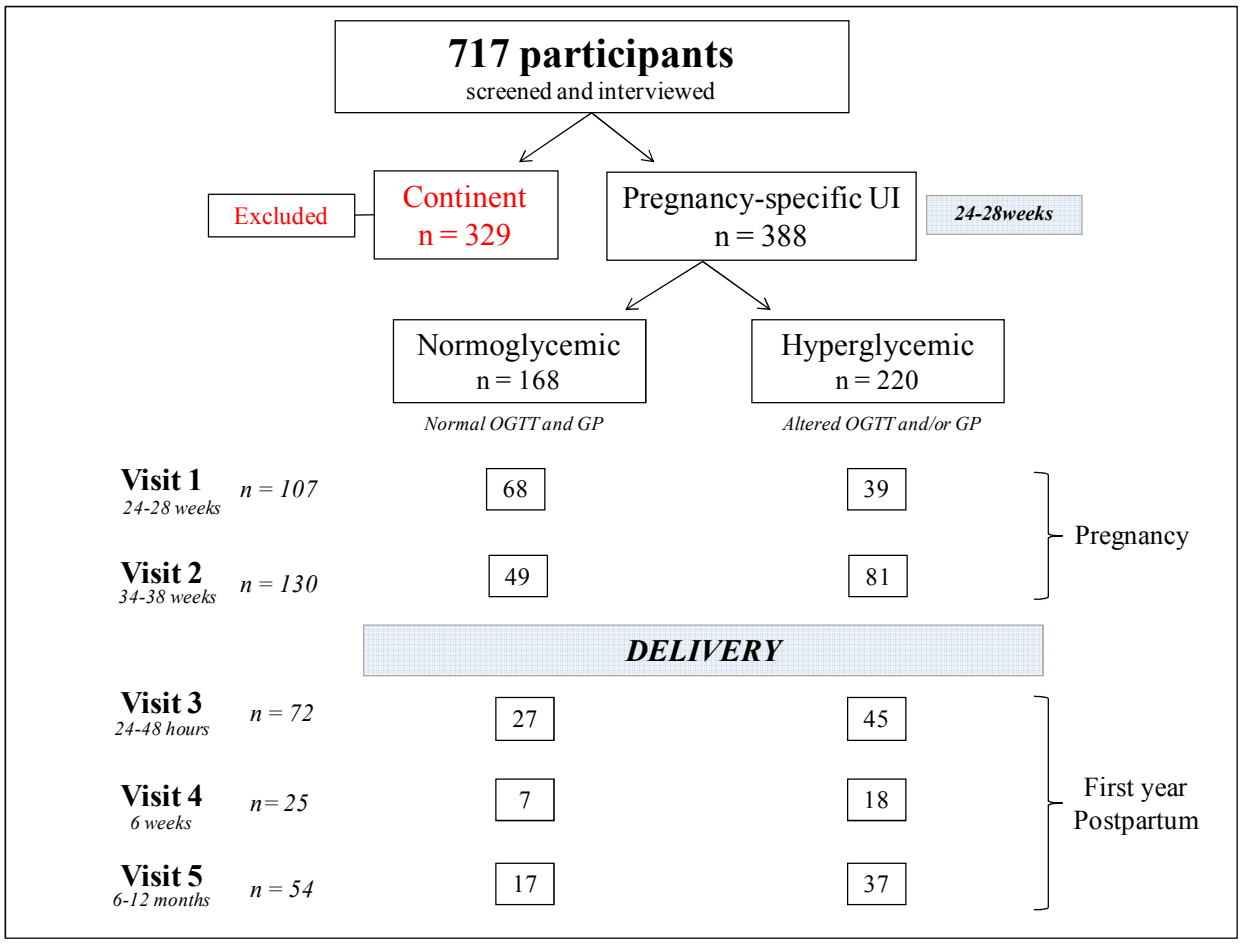

Figure 1. Flow chart indicating the distributions of study participants at each scheduled time-point. UI, urinary incontinence; OGTT, oral-glucose tolerance test; GP, glucose profile test.

Table 1 presents the baseline characteristics of the 388 participants within the Normoglycemic incontinent and Hyperglycemic incontinent groups. The PS-UI hyperglycemic women were older, with higher pre-pregnancy and final body mass index (BMI), lower total gestational weight gain, and higher percentage of hypertension, C-section, and smoking habits. The glucose level on OGTT (fasting, 1 hour, 2 hours), the glycaemic

average from the results of the GP, and glycated HbA_{1C} showed that HI group presented higher values in the three tests compared to the NI group.

Table 1. Demographic and clinical parameters of study subjects (total n = 388)

	NI (n=168)		HI (n=220)		*p*-value
	n	%	n	%	
Age (years)					
Mean (SD)	25.8 (6.2)		31.2 (6.1)		< 0.0001
<25	78	46.4	38	17.3	
25-29	44	26.2	48	21.8	
30-34	30	17.9	56	25.5	
35-39	16	9.5	56	25.5	
≥40	0	0.0	22	10.0	
Pre-pregnancy BMI (kg/m²)					
Mean (SD)	27.8 (6.1)		32.4 (8.2)		< 0.0001
<18.5	6	3.6	5	2.3	
18.5-24.9	51	30.4	24	10.9	
25-29.9	61	36.3	66	30.0	
30-34.9	25	14.9	63	28.6	
≥35	25	14.9	62	28.2	
Final BMI (kg/m²) **					
Mean (SD)	32.3 (5.9)		35.8 (7.5)		0.0025
<18.5	0	0.0	0	0.0	
18.5-24.9	15	8.9	12	5.5	
25-29.9	42	25.0	31	14.1	
30-34.9	43	25.6	78	35.5	
≥35	39	23.2	96	43.6	
Total maternal weight gain (kg) **					
Mean (SD)	10.2 (5.7)		8.0 (6.3)		0.0014
≤10	82	48.8	133	60.5	
>10	57	33.9	84	38.2	
Parity					
Primiparous	61	36.3	40	18.2	0.0794
Multiparous	107	63.7	180	81.8	
Ethnicity					
White	135	80.4	137	62.3	0.0003
Other	33	19.6	83	37.7	
Civil status					
Single	30	17.9	18	8.2	0.0033
Not single	138	82.1	202	91.8	
Smoking status					
Previous/current smoker	7	4.2	28	12.7	0.0035
Never smoke	161	95.8	192	87.3	
Alcohol status					
Yes	3	1.8	2	0.9	0.4481
No	165	98.2	218	99.1	
Hypertension					
Yes	16	9.5	68	30.9	< 0.0001

No	152	90.5	152	69.1	
Mode of delivery **					
Vaginal delivery	41	24.4	55	25.0	0.0215
Caesarean delivery	49	29.2	121	55.0	
Birthweight (g) **					
<4000	72	42.9	151	68.6	0.3497
≥4000	10	6.0	21	9.5	
Birthweight classification **					
SGA	3	1.8	15	6.8	0.3393
AGA	69	41.1	137	62.3	
LGA	10	6.0	20	9.1	
OGTT (Mean (SD))					
Fasting (mg/dL)	72.4 (7.8)		87.5 (13.9)		< 0.0001
1 hour (mg/dL)	104.1 (24.4)		160.2 (37.7)		< 0.0001
2 hours (mg/dL)	91.9 (18.2)		133.9 (32.5)		< 0.0001
Glycemic profile					
Glycemic mean (SD)	83.3 (8.4)		95.9 (11.3)		0.0001
HbA_{1C} (%)					
Mean (SD)	5.0 (0.5)		5.5 (0.5)		< 0.0001

NI, normoglycemic incontinent group; HI, hyperglycemic incontinent group; BMI, body mass index (weight (kg)/height $(m)^2$); SD, standard deviation. *$p < 0.05$ significant difference between two groups. **Incomplete N due to incomplete follow-up, delivery outside of service.

The characteristics of PS-UI in normoglycemic and hyperglycemic groups are shown in Table 2. Analysis of the type of UI revealed that the most frequent type was SUI during pregnancy until the first year postpartum. This difference is not statistically significant when evaluated in each group per separate. The percentages of the different types of PS-UI according to the groups and visits are shown in Table 2.

The responses to the ICIQ-SF questions showed that GH increased the amount (moderate/large) of urine loss (p<0.0027) and the frequency (several times a day) of PS-UI (p<0.0014) at the two-time-points of pregnancy (Table 2). For QoL, the impact of PS-UI showed higher average score (p<0.0001) in the hyperglycemic group at two pregnancy-visits (Table 2). Supplementary Table 3 shows the breakdown of values of QoL score of the ICIQ-SF question.

Compared to normoglycemic women, the total score (0-21) of ICIQ-SF in gestational hyperglycemic women exhibited higher mean values during pregnancy, reflecting a worse combined severity and bother-impact of PS-UI. No significant difference in the leakage

situations/causes was found between the groups at any visit (Table 2). The specific evaluation of severity of PS-UI by ISI questionnaire showed higher average score in hyperglycemic women during two visits of pregnancy, with higher proportion of severe/very severe leakage. Likewise, the UI impact on QoL was predominantly severe and very severe (Table 2, Figure 2).

Table 2. Pregnancy-specific UI data and questionnaire responses at two-time-points of pregnancy and three-time-points of first year postpartum in hyperglycemic and normoglycemic women.

	PREGNANCY						FIRST YEAR POSTPARTUM								
	24-28 weeks			34-38 weeks			24-48 hours			6 weeks			6-12 months		
	NI (n=68)	HI (n=39)	*p*-value	NI (n=49)	HI (n=81)	*p*-value	NI (n=27)	HI (n=45)	*p*-value	NI (n=7)	HI (n=18)	*p*-value	NI (n=17)	HI (n=37)	*p*-value
Type of urinary incontinence															
Stress	55.9%	64.1%	**0.4794**	51.0%	60.5%	**0.3901**	51.9%	53.3%	**0.5628**	57.1%	61.1%	**0.8557**	76.5%	56.8%	**0.1635**
Urge	7.3%	10.3%		4.1%	1.23%		7.4%	2.2%		0.0%	0.0%		0.0%	0.0%	
Mixed	36.8%	25.6%		44.9%	38.3%		40.7%	44.4%		42.9%	38.9%		23.5%	43.2%	
Amount of urine lost (ICIQ-SF)															
A small amount	77.9%	48.7%	**0.0022**	67.4%	33.3%	**0.0005**	63.0%	22.2%	**0.0017**	57.1%	44.4%	**0.6219**	70.6%	27.0%	**0.0079**
A moderate amount	22.1%	43.6%		22.5%	54.3%		33.3%	57.8%		42.9%	44.4%		29.4%	62.2%	
A large amount	0.0%	7.7%		10.2%	12.4%		3.7%	20.0%		0.0%	11.1%		0.0%	10.8%	
Frequency of urine lost (ICIQ-SF)															
About once a week or less often	38.2%	15.4%	**0.0090**	38.8%	9.9%	**0.0004**	29.6%	4.4%	**<0.0001**	28.6%	27.8%	**0.1277**	41.2%	13.5%	**0.0382**
Two or three times a week	45.6%	41.0%		20.4%	25.9%		48.2%	13.3%		57.1%	16.7%		29.4%	24.3%	
About once a day	8.8%	23.1%		20.4%	18.5%		7.4%	33.3%		14.3%	16.7%		23.5%	24.3%	
Several times a day	7.4%	20.5%		20.4%	45.7%		14.8%	48.9%		0.0%	38.9%		5.9%	37.8%	
All the time	0.0%	0.0%		0.0%	0.0%		0.0%	0.0%		0.0%	0.0%		0.0%	0.0%	
Qol (ICIQ-SF) (0-10)	4.8	7.2	**<0.0001**	5.4	7.6	**<0.0001**	5.4	8.5	**<0.0001**	6.3	7.9	**0.1105**	5.8	8.3	**<0.0001**
ICIQ-SF mean score (0-21)	9.0	12.8	**<0.0001**	10.5	14.2	**<0.0001**	10.3	15.8	**<0.0001**	11	13.9	**0.0194**	10.3	14.8	**<0.0001**
When does urine leak?															
Leaks before you can get to the toilet	42.7%	41.0%	**0.8701**	46.9%	40.7%	**0.4892**	44.4%	46.7%	**0.8546**	42.9%	44.4%	**0.9428**	17.7%	40.5%	**0.0974**
Leaks when you cough or sneeze	76.5%	84.6%	**0.3157**	87.8%	85.2%	**0.6810**	85.2%	93.3%	**0.2586**	85.7%	88.9%	**0.8264**	94.1%	91.9%	**0.7718**
Leaks when you are asleep	27.9%	12.8%	**0.0711**	30.6%	28.4%	**0.7877**	25.9%	26.7%	**0.9450**	14.3%	27.8%	**0.4782**	41.2%	24.3%	**0.2078**
Leaks when you are physically active/exercising	25.0%	28.2%	**0.7166**	30.6%	28.4%	**0.7877**	14.8%	33.3%	**0.0843**	28.6%	33.3%	**0.8187**	17.6%	40.5%	**0.0974**
Leaks when you have finished urinating and are dressed	48.5%	35.9%	**0.2051**	40.8%	53.1%	**0.1749**	40.7%	53.3%	**0.3007**	42.9%	38.9%	**0.8557**	23.5%	29.7%	**0.6366**
Leaks for no obvious reason	32.4%	23.1%	**0.3087**	26.5%	23.5%	**0.6934**	25.9%	13.3%	**0.1787**	0.0%	5.6%	**0.5245**	23.5%	24.3%	**0.9494**
Leaks all the time	1.5%	0.0%	**0.4467**	2.0%	4.9%	**0.4051**	0.0%	4.4%	**0.2666**	0.0%	0.0%		5.9%	0.0%	**0.1365**
ISI score (1-12)	3.4	5.8	**0.0056**	4.7	7.0	**<0.0001**	4.0	7.7	**<0.0001**	3.6	6.1	**0.0339**	3.6	6.7	**0.0003**
Severity (ISI)															
Slight	29.4%	2.6%	**<0.0001**	28.6%	6.2%	**0.0003**	25.9%	2.2%	**<0.0001**	14.3%	11.1%	**0.2797**	47.1%	8.1%	**0.0053**
Moderate	64.7%	64.1%		44.9%	35.8%		59.3%	28.9%		85.7%	50.0%		35.3%	37.8%	
Severe	5.9%	28.2%		16.3%	44.4%		7.4%	48.9%		0.0%	33.3%		17.7%	43.2%	
Very Severe	0.0%	5.1%		10.2%	13.6%		7.4%	20.0%		0.0%	5.6%		0.0%	10.8%	

NI, normoglycemic incontinent group; HI, hyperglycemic incontinent group.

Table 3. Quality of life affection in normoglycemic and hyperglycemic groups in different periods of pregnancy and postpartum (Supplementary data).

QoL (ICIQ-SF)	PREGNANCY				FIRST YEAR POSTPARTUM					
	Visit 1 (24-28weeks)		Visit 2 (34-38weeks)		Visit 3 (24-48hours)		Visit 4 (6 weeks)		Visit 5 (6-12 months)	
	NI (n= 68)	**HI** (n= 39)	**NI** (n=49)	**HI** (n=81)	**NI** (n=27)	**HI** (n=45)	**NI** (n=7)	**HI** (n=18)	**NI** (n=17)	**HI** (n=37)
0	4.4%	0.0%	4.1%	1.2%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
1	4.4%	2.6%	0.0%	3.7%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
2	5.9%	0.0%	6.1%	1.2%	14.8%	0.0%	0.0%	0.0%	0.0%	0.0%
3	10.3%	0.0%	16.3%	1.2%	11.1%	0.0%	0.0%	0.0%	5.9%	0.0%
4	19.1%	5.1%	12.2%	3.7%	7.4%	4.4%	14.3%	0.0%	29.4%	0.0%
5	32.4%	17.9%	24.5%	6.2%	25.9%	2.2%	28.6%	11.1%	23.5%	8.1%
6	5.9%	7.7%	4.1%	4.9%	14.8%	2.2%	14.3%	5.6%	11.8%	0.0%
7	2.9%	15.4%	8.2%	11.1%	7.4%	8.9%	14.3%	11.1%	11.8%	10.8%
8	5.9%	23.1%	4.1%	18.5%	0.0%	22.2%	14.3%	33.3%	0.0%	40.5%
9	1.45%	12.8%	6.1%	28.4%	7.4%	31.1%	14.3%	27.8%	5.9%	18.9%
10	7.4%	15.4%	14.3%	19.8%	11.1%	28.9%	0.0%	11.1%	11.8%	21.6%

NI, normoglycemic incontinent group; HI, hyperglycemic incontinent group.

Regarding the postpartum period, the PS-UI associated with GH increased the amount (p=0.0079), frequency (p=0.0382), impact of UI on QoL (p<0.0001) and severity (p=0.0053), even as the questionnaires score (p<0.0001 for ICIQ-SF and p=0.003 for ISI) over the first year postpartum. Within this period, some differences at three time-points were observed: at 24-48 hours postpartum, this binomial maintained the same characteristics of amount (p=0.0017), frequency (p<0.0001), impact on QoL (p<0.0001) and severity of UI (p<0.0001) verified during pregnancy. Unexpectedly, at 6 weeks postpartum, the influence of the binomial "PS-UI plus GH" on urinary conditions seem to regress in both groups, as only the ICIQ-SF mean score (p=0.0194) and ISI score (p=0.0339) was increased in hyperglycemic group. Lastly, at 6-12 months postpartum, the influence of PS-UI plus GH on the persistence of UI conditions was strongly observed in previous hyperglycemic pregnant women (Table 2).

In addition, compared to normoglycemic group, the women with GH had a noticeable reversal of the severity and impact of UI on QoL, with the predominance of severe and very severe classification during pregnancy until the end of the follow-up period (Figure 2A, B).

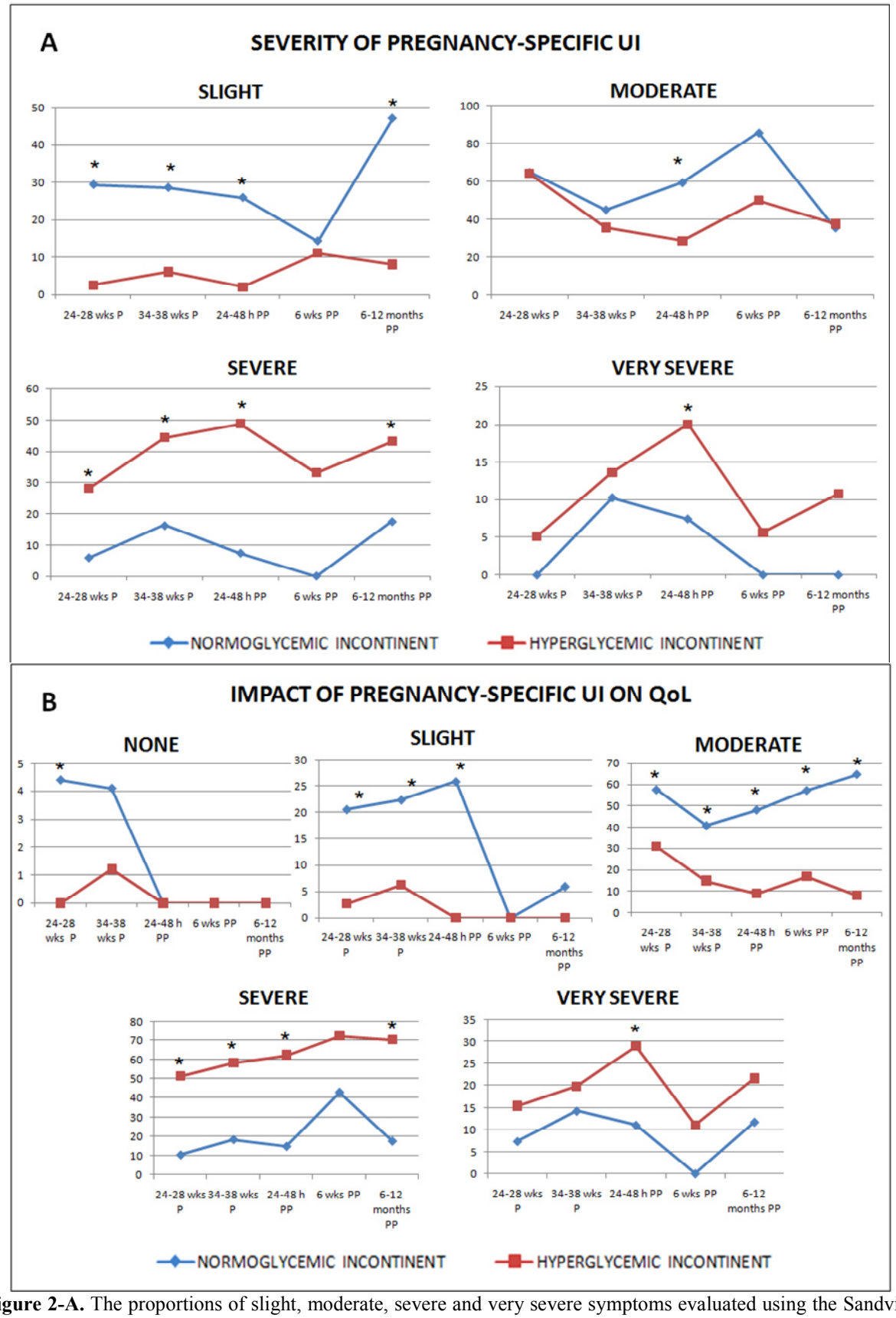

Figure 2-A. The proportions of slight, moderate, severe and very severe symptoms evaluated using the Sandvik Incontinence Severity Index among pregnancy-specific UI women with gestational hyperglycemia and normoglycemia. **B**. The proportions of none, slight, moderate, severe and very severe impact of pregnancy-specific UI on QoL evaluated with question of the ICIQ-SF questionnaire among women with gestational hyperglycemia and normoglycemia. Wks, weeks; h, hours; P, pregnancy; PP, postpartum.

DISCUSSION

The epidemiological value of the current research highlights the relationship between GH and self-reported UI. Through the use of validated specific questionnaires, tools with grade A recommendation (22), our results show higher PS-UI occurrence in gestational hyperglycemic women with more severe UI and negative impact on QoL not only in pregnancy but also over the first year postpartum. Additionally, this study has brought to light a clear profile of the natural history of PS-UI in women with GH, including types, amount, frequency, severity, and interference on daily life during pregnancy and its persistence up to first year postpartum.

The overall prevalence of PS-UI in this study was 54.1%, being 43.3% in normoglycemic and 56.7% in hyperglycemic women. The occurrence of PS-UI is variable in the literature, ranging from 17 to 54% (23), which is consistent with our results. Our previous clinical findings (5) showed that the overall prevalence of PS-UI and UI two-year postpartum in primiparous women delivered by caesarean-section (C-section) was significantly higher (p<0.01) among GDM (50.8 and 44.4%, respectively) compared to normoglycemic pregnant women (31.6 and 18.4%, respectively). SUI was the most prevalent of all types of UI either in NUI or HUI over the first year postpartum, which is consistent with previous studies (1, 24).

To the best of our knowledge, this is the first study to evaluate the association of PS-UI and postpartum UI with severity and QoL in women with GH in several follow-up periods with validated specific questionnaires. Importantly, at the end of the follow-up period, there were still higher proportions of severe symptoms of UI likewise severe impact of PS-UI on daily life in previous gestational hyperglycemic women. These findings suggest that the impact of binomial GH and PS-UI on postpartum genitourinary function was relatively prolonged in these women, despite the higher rates of C-section in this specific population.

The potential mechanisms by which GH cause PS-UI cannot be elucidated from the results of this study. However, we previously showed that UI prevalence and pelvic floor muscle dysfunction (PFMD) after C-section were high among women with prior GDM (5). Thus, UI may also be an indirect long-term consequence of GDM. This framework confirms an association between GDM and subsequent PFMD postpartum (5). Additional results demonstrated that short-term severe and long-term mild diabetes causes detrimental effects on pregnant rat urethral muscle (32, 33). These alterations may be involved in the failure to maintain healthy skeletal muscle thus contributing to the PFMD. These results may have implications for the monitoring and prevention strategies to improve the QoL and PFMD in previous GDM women (25).

The long-term associations of delivery mode with UI and changes in UI over a continuous period remain controversial (26), so it is questionable whether C-section can prevent PFMD (3, 27). In this study, women with GH had higher rates of C-section, indicating that this procedure was not a protective factor for postpartum UI. This finding agrees with our previous results showing that C-sections do not protect against UI after childbirth in Brazil (28). A systematic review and meta-analysis demonstrated an increase in the risk of developing long-term SUI, an increase of ~8% in moderate or severe SUI when comparing vaginal delivery with C-section (29). These findings suggest that C-section offers substantial protection against pelvic floor trauma. In hyperglycemic pregnant women with PS-UI, the C-section has no benefits.

Along the first year postpartum, differences at the three-time points were observed. At the early postpartum, the amount, frequency, impact on QoL and severity of UI was maintained. At 6 weeks postpartum, the binomial PS-UI and GH decreased the UI repercussions. Additionally, the ICIQ-SF and ISI scores could be explained because the muscle strength of the pelvic floor returns to the ante partum value 6–10 weeks postpartum in

most women (30). At 6-12 months postpartum, the influence of the binomial was observed and ICIQ-SF and ISI scores confirm the worse of occurrence, severity, and negative impact on QoL. These results suggest that incomplete long-term postpartum evaluation may demonstrate wrong conclusions. PS-UI in gestational hyperglycemic women caused distress in a severe and very severe form as well as had severe and very severe impact on QoL. The most severely affected women with UI symptoms seek help since most of them suffer silently and have probably not been properly diagnosed (31).

Several limitations of the present study should be considered. First, even if the study sample is representative of pregnant women assisted in the High Risk Prenatal Care Unit-University Hospital, an over representation of hyperglycemic pregnant women was observed. These conditions occur since this is a reference center for clinical obstetric and neonatal for GDM treatment and were not controlled in the current study. Second, the prevalence of UI may vary considerably since the monocentric design of our study consider not only GDM but also mild gestational hyperglycemia as hyperglycemic pregnant women. Consequently, our results are likely to underestimate the true prevalence of pregnancy-specific UI in GDM. Third, it was a cross-sectional study although with a control group, but the relatively short follow-up of participants may have limited the results. Larger or cohort studies with longer follow-up may help better characterize the natural history of pregnancy-specific UI and postpartum UI in gestational hyperglycemic women. Fourth, the blood sugar levels were not monitored in the postpartum period, leading to a postpartum missed diagnosis of type 2 DM. Weight gain or loss, which is an important factor for UI, was recorded just before and during pregnancy, but was not in the postpartum period. It would also been interesting to compare demographic characteristics between women with and without pregnancy-specific UI, but we have not demonstrated this information in this study. After all, the underlying biological pathway was not investigated in the current research, and merits further in-depth study.

The PS-UI verified in gestational hyperglycemic women predicts the future occurrence, severity, and negative impact on QoL, which not only contradicts the old concept that the effects of GDM vanish soon after delivery (9), but also reinforce the interaction between pregnancy, GH and long-term maternal outcome (32).

CONCLUSIONS

The PS-UI in gestational hyperglycemic women worse the occurrence and severity of UI and affected their QoL proportionally to the severity of symptoms and impairment over the first year postpartum. The results emphasize the need for policy development for UI prevention and intervention planning for hyperglycemic women.

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COMPETING INTERESTS

The authors declare that there are no competing interests.

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Capítulo 2

INTERACTION BETWEEN GESTATIONAL HYPERGLYCEMIA, PREGNANCY-SPECIFIC URINARY INCONTINENCE, AND CCL7 SERUM PROFILE ON LONG-TERM MATERNAL OUTCOME

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ABSTRACT

Purpose: Little published data exist on the impact of gestational hyperglycemia, pregnancy-specific urinary incontinence and CCL7 overexpression on long-term maternal urinary outcome. We investigated the serum CCL7 profile in hyperglycemic women during pregnancy, delivery and postpartum period up the 12 months and correlate the level of CCL7 with continence status.

Materials and Methods: A total of 102 pregnant women who underwent the hyperglycemia diagnosis with any urinary leakage new-onset during pregnancy were allocated into 4 groups with and without gestational hyperglycemia and/or pregnancy-specific urinary incontinence. The CCL7 serum concentration was measured by ELISA at 6 time-points of the cohort follow-up study. Univariate and multivariate analysis looked for predictors of longitudinal effects of maternal glucose levels, pregnancy-specific urinary incontinence and CCL7 serum concentration with UI 12 months postpartum.

Results: The CCL7 serum concentration was similar in early pregnancy in 4 groups and was lower in hyperglycemic groups continent and incontinent during all the analyzed period**.** At the gestational hyperglycemia diagnostic period, the serum CCL7 profile in hyperglycemic incontinent women showed lower concentration and stable levels at all time-points of the follow-up study, without a peak soon after birth.

Conclusions: The CCL7 gradient demonstrated the inter-relationship among hyperglycemic status during pregnancy, pregnancy-specific urinary incontinence and CCL7 serum concentration, peak and progression in predict long-term maternal urinary incontinence.

Keywords: CCL7, cytokines, gestational diabetes; pregnancy; urinary incontinence.

INTRODUCTION

Hyperglycemia is one of the most common medical conditions that affect women during pregnancy (1). Gestational diabetes is considered one of the risk factors for the development of persistent urinary incontinence (2). Women with previous gestational diabetes mellitus (GDM) associated with pregnancy-specific urinary incontinence (PS-UI) were significantly more likely to have urinary incontinence (UI) and pelvic floor muscle dysfunction two years after cesarean section (3). The pathophysiological mechanisms involved in the development of UI in GDM are not fully elucidated. In addition to the proposed role of diabetic myopathy (4), recent preclinical research demonstrated a negative impact of diabetes on recovery mechanism from the vaginal injury trauma (5).

The pathophysiology of urinary incontinence is complicated with multiple predisposing, inciting and decompensating factors contributing to the eventual occurrence of the symptoms (6). Yet vaginal delivery is considered a pivotal event in women's lifespan and in the occurrence on urinary incontinence. Epidemiological and preclinical data suggest that there is an inherent reparative mechanism that contribute to the recovery of continence following injury.

Based on the aforementioned efforts, preclinical research has identified chemokine (C-C motif) ligand 7 (CCL7) as a chemokine that is overexpressed in vaginal distention injury in rats (7). Overexpression of CCL7 has been shown in rat serum, urethral and vaginal tissues immediately following induction of stress urinary incontinence (SUI) in a rat model simulating birth trauma (8, 9). Moreover, the overexpression levels were impacted by age and diabetes status in preclinical studies (5). Significant to the recovery mechanisms, CCL7 is considered a potent stem cell chemoattractant protein (9). CCL7 was first identified in a model of myocardial infarction and shown to facilitate functional recovery and have since been identified in additional injury models (10). Further evidence, in clinical scenarios,

indicates CCL7 stimulates stem cell homing for regenerative repair (11). The resultant cytokine gradient recruits hematopoietic stem cells and mesenchymal stem cells to the injured area, where they engraft, produce growth factors, facilitate healing from injury, and improve functional recovery (10). CCL7 overexpression has shown potency for stimulating targeted stem cell migration and provide a translational link (clinical measurement) which further provide opportunities for treatment (10).

In separate but parallel research, a new cellular–based approach for regenerative repair of genitourinary tissue involves the injection of mesenchymal stem cells (MSCs), either systematically or locally, and has shown early promise in animal models of SUI (12-14). Despite the success of early MSCs treatment in SUI, outcomes are inconsistent, and evidence shows cell migration away from the therapeutic site (15, 16). One of the challenges with MSCs therapy is homing and retention of the injected MSC to the site of injury (17). In SUI, homing of the MSCs depends on the presence of chemoattractant gradients in the vicinity of the urethra (18). Thus it is likely that one reason some women fail to recover continence years after birth trauma is due to a lack of sufficient chemokine concentration gradient at the time of injury to stimulate MSCs homing for induction of repair and tissue regeneration.

These results denote the need for exploration the mechanistic role of a recovery factor in gestational hyperglycemic women with PS-UI. The last several years basic science and clinical research have focused on muscle or adipose derived stem cells for the treatment of SUI (19). The next step in analyzing the underlying mechanism through which the body begins the natural reparative process at the molecular signalling level of stem cell homing needs to take place.

Based on the above, we hypothesized that CCL7 response to childbirth injury will be likely absent or compromised in the natural repair process of hyperglycemic pregnant women plus PS-UI. This could negatively impacts inherent reparative mechanisms and delay/prevent

the migration of MSCs to the site of injury caused by childbirth, with consequences in the postpartum period. We designed the current prospective study to evaluate the serum CCL7 profile in hyperglycemic women during pregnancy, delivery and postpartum period up the 12 months compared to normoglycemic controls, and correlate the level of CCL7 with continence status.

MATERIALS AND METHODS

Research design and subjects

This is a single-center prospective cohort study. Pregnant women were recruited at 12-18 weeks gestation and followed up to 12 months after delivery. The study was conducted at the Perinatal Diabetes Research Center of Botucatu Medical School/UNESP/Brazil, between 2013 and 2015, and was approved by the Research Ethics Committee of the Institution (CAAE: 20639813.0.0000.5411). The study protocol was explained to each participant before admission. Voluntary participation was emphasized and privacy was assured. The participants were informed that they could terminate the follow-up at any time. Written informed consent was obtained from all subjects and all procedures used in the study were in accordance with the guidelines of the Helsinki Declaration on human experimentation.

Women who satisfied the inclusion criteria were invited to participate in the study. Baseline information (maternal characteristics, demographics, anthropometrics, previous obstetric history), symptoms of UI, and blood samples (10 ml) for measurement of CCL7, were obtained at six time-points during pregnancy and the postpartum period: at 12 and 18 weeks gestational age (visit 1), 24 and 28 weeks gestational age (visit 2), 34 and 38 weeks gestational age (visit 3), within 24 and 48 hours postpartum (visit 4), 6 weeks postpartum (visit 5), and 12 months postpartum (visit 6). A form completed immediately after the birth was used to record the labour process, the mode of delivery, and the neonatal birth profile.

Inclusion criteria: pregnant women (>18 yrs of age) with singleton pregnancy and no history of urinary incontinence prior to current pregnancy, who underwent the diagnostic tests for gestational hyperglycemia between 24 and 28 weeks of pregnancy, delivery at University maternity, with follow-up from visit 1 to visit 6 were approached for inclusion in the study.

Exclusion criteria: Pre-pregnancy UI, Pre-gestational BMI> 35 kg/m^2, high risk pregnancy, known type 1 or type 2 DM, preterm delivery (<37 weeks of pregnancy), multiple pregnancies, pre-eclampsia, eclampsia, known fetal anomaly, maternal age <18 years old, urogenital cancer, multiple sclerosis, asthma, chronic sinusitis, ulcerative colitis, biliary cirrhosis or any clinical condition may have jeopardised the health status of the woman, were excluded from this study.

Important definitions:

Gestational hyperglycemia: The Perinatal Diabetes Research Center—Botucatu Medical School—UNESP diagnoses gestational hyperglycemia (gestational diabetes mellitus-GDM and mild gestational hyperglycemia- MGH) using screening, with fasting blood glucose ≥90 mg/dL and risk factors (personal, obstetric and family). Positive screening women is followed by the diagnostic phase with 75 g-OGTT, combined with the glycemic profile (GP) between 24th and 28th gestational weeks. These strategies classifying the pregnant women in four groups identified by Rudge (20), including pregnant women with GDM and MGH. Both two groups are nominated as gestational hyperglycemia and reflect the full spectrum of glucose tolerance in pregnancy since normal to mildly abnormal to GDM (21). Patients with positive screening and negative diagnosis of gestational hyperglycemia (normal OGTT and glycemic profile) were classified as normoglycemic pregnant women. We categorized gestational hyperglycemia (GDM and Mild Hyperglycemia) according to cutoffs recommended by ADA (American Diabetes Association) (22) and Rudge *et al.*, 1990 (20).

Pregnant women with MGH have a normal 75 g-OGTT, but an abnormal GP with mildly elevated glucose levels— fasting plasma glucose levels are ≥90 mg/dL and/or postprandial plasma glucose is ≥130 mg/dL (20). Diagnosis of GDM was confirmed by fasting glucose level ≥ 92 mg/dL, 180 mg/dL after 1-hour, or 153 mg/dL after 2-hours glucose load. One changed value is sufficient for the diagnosis of GDM (22).

Urinary incontinence was defined according to the International Continence Society definition of urinary incontinence (23). Pregnancy Specific Urinary Incontinence (PS-UI) was defined as any urinary leakage new onset during pregnancy (24) ascertained by self-reported. Participants were asked whether before pregnancy they had leaked or lost control of even a small amount of urine with activity such as coughing, lifting or exercise, or urge or pressure to urinate and could not get to the toilet fast enough for urge UI. Pregnant women with SUI and/or urge urinary incontinence before pregnancy were excluded as noted in the exclusion criteria.

Based on the above, we identified 4 groups of patients that we categorized as normoglycemic continent (NC), normoglycemic incontinent (NI), hyperglycemic continent (HC) and hyperglycemic incontinent (HI). The groups classification was established between 24-28 weeks of pregnancy, the corresponding period to the diagnosis of gestational hyperglycemia.

ELISA Quantification of Blood Serum CCL7

Serum analysis was performed using the Human CCL7 Quantikine ELISA Kit (R&D Systems, Catalog Number DCC700, Minneapolis, USA). Whole blood samples (10 mL) were collected with serum separator tubes and centrifuged at 2000g for 15 minutes at 4°C. Serum layer was extracted and stored at -80°C. Before analysis, serum samples were slowly warmed to room temperature. One hundred microliters of Assay Diluent was added to all sample

wells, as well as standard wells (to produce a concentration gradient of 1000, 500, 250, 125, 62.5, 31.3, and 15.6 pg/mL) and blank wells. Fifty microliters of serum were added to sample wells with all samples run in duplicate. The 96-well plate was incubated at room temperature for 3 hours followed by 4 exchanges with wash buffer. Afterward, 200µL of Human MCP-3 Conjugate was added to each well and the plate was incubated at room temperature for 1 hour. The aspiration and 4 washes were repeated. Thereafter, 100µL of substrate solution was added to all wells, and the plate was incubated at room temperature for 30 minutes. Stop solution (50 µL) was added to all wells once the highest standard reached a dark blue color. The plate was read on a Power Wave XS with Gen5™ Software (1.05) - *Biotek* (Winooski, Vermont, USA) at 450 nm, and the wavelength correction was made to 570nm for correct optical imperfections in the plate.

Statistical analysis

The sample was restricted to women who completed all follow-up period from 12 weeks of pregnancy to 12 months postpartum. Continuous data are presented as medians with 25th to 75th percentiles and categorical data as frequencies and percentages.

Prevalence of PS-UI and UI 12 months postpartum were estimated as the ratio of the number of women who answered "yes" to the screening question to the total number of women included in the study.

The Kruskal-Wallis test, followed by Dunn's multiple comparisons and chi-square test were performed to assess the CCL7 serum profile in four groups at each time-point of pregnancy and postpartum. CCL7 serum profile among each time-point from the 4 groups (normoglycemic continent, normoglycemic incontinent, hyperglycemic continent and hyperglycemic incontinent) were then compared by Friedman, followed by Dunn's multiple comparisons.

The relative risk of gestational hyperglycemia plus PS-UI on UI 12 months postpartum were estimated by Cox regression.

Associations between independent variables and UI 12 months postpartum are shown as the OR and 95% CI. Logistic regression models were used to determine risk factors related to UI 12 months postpartum. Variables that were significantly related to UI 12 months postpartum in the univariate model were included in the multivariate model. Multivariable linear regression analyses were used to estimate longitudinal effect of the associations among maternal glucose concentrations, PS-UI, CCL7 serum concentration with UI 12 months postpartum.

Association of mode of delivery with CCL7 serum concentration at each pregnancy and postpartum time-points was analyzed by Mann-Whitney test.

All statistical analyses were carried out using SPSS software (Version 21.0 Armonk, NY: IBM Corp.), and an open source statistical software R (www.r-project.org), version 3.1.2. Figures are generated in Graph Pad, version 5. All of the statistical tests were two sided, and P value less than 0.05 was considered significant.

RESULTS

A total of 4506 consecutive patients enrolled for recruitment in the Prenatal Care Unit, 3629 were excluded as not eligible due to confirmed UI prior to current pregnancy and/or they did not meet the inclusion criteria. A total of 877 pregnant women consented to participate at 12-18 weeks, who constituted the study register. Of these, 316 women had higher final BMI or preterm delivery and 459 women failed to complete six visits and/or declined to participate during the follow-up period. Thus, 102 women completed the clinical data, evaluations of PS-UI and postpartum UI and CCL7 analysis over six visits and were successfully included in this study. Of the included participants (n = 102), 56 women were in the normoglycemic

group and 46 women in the hyperglycemic group (GDM and MGH). Of the 56 normoglycemic women, 34 were continent and 22 incontinent; of the 46 hyperglycemic women, 11 were continent and 35 incontinent (Figure 1).

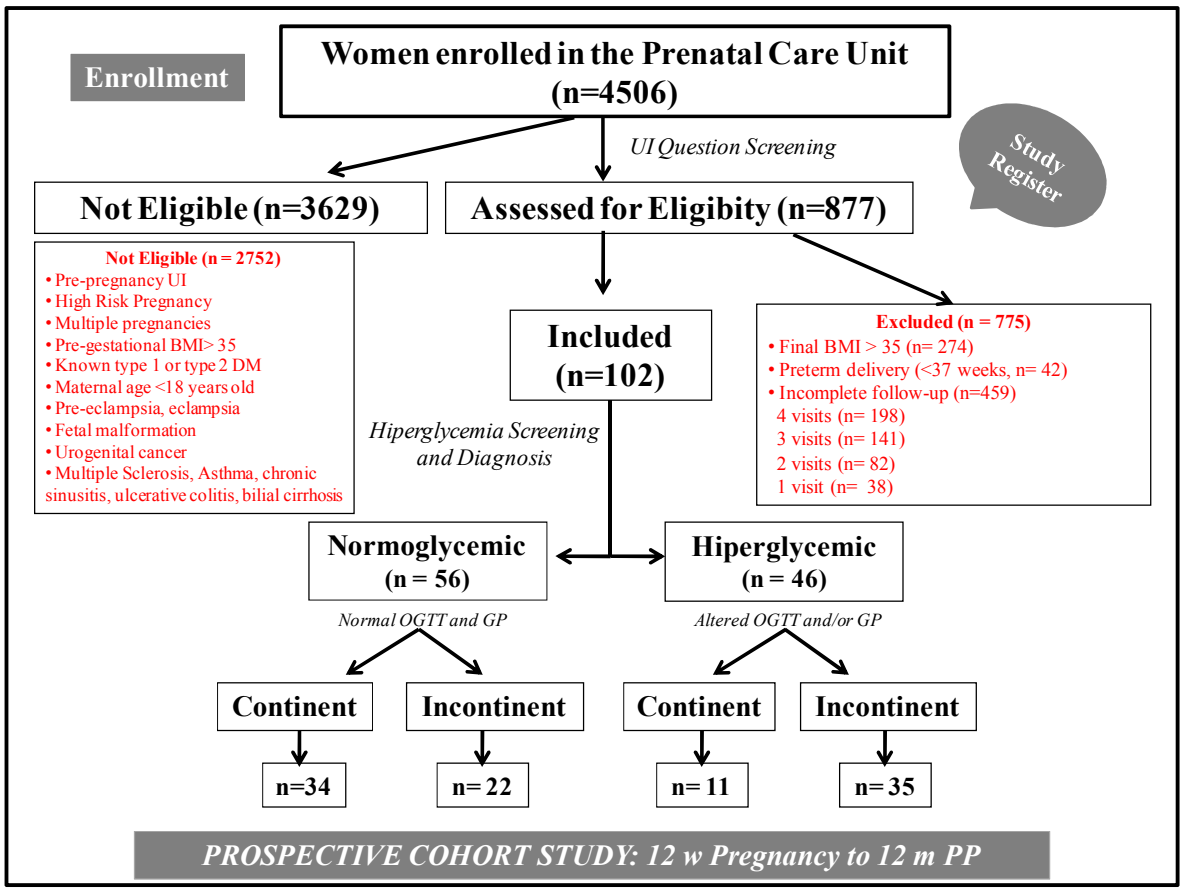

Figure 1. Flow diagram of pregnant women's screening, enrollment and follow-up in the analysis. OGTT, oral-glucose tolerance test; GP, glucose profile test; W: weeks, M: months, PP: postpartum

Table 1 summarizes the baseline characteristics of the 102 pregnant women included in the study at the time of OGTT and GP. Several variables differed between the eligible groups. The HI group were older, had lower total gestational weight gain, and higher percentage of hypertension, C-section, and smoking habits. As expected, the hyperglycemic groups presented higher values on OGTT (fasting, 1 hour, 2 hours), glycemic average of glucose profile, and glycated HbA_{1C}. The prevalence of UI 12 months postpartum was higher among PS-UI women either normoglycemic or hyperglycemic.

Table 1. Baseline characteristics of study participants between groups (total n = 102).

	NC (n=34)			NI (n=22)			HC (n=11)			HI (n=35)				
	Med	**Min**	**Max**	**Med**	**Min**	**Max**	**Med**	**Min**	**Max**	**Med**	**Min**	**Max**	***p***	
Age (years)	29.0	16.0	39.0	27.5	17.0	39.0	31.0	22.0	41.0	32.0	16.0	41.0	*0.009*	#
Parity	2.0	1.0	4.0	2.0	1.0	9.0	2.0	1.0	4.0	2.0	1.0	5.0	*0.849*	
Pre-pregnancy BMI (kg/m^2)	26.1	17.9	36.7	26.4	18.4	35.6	26.4	18.6	31.2	27.9	18.1	34.9	*0.205*	
Final BMI (Kg/m^2)	31.7	23.0	38.8	21.4	23.6	38.2	31.0	19.6	37.7	33.0	22.2	48.6	*0.303*	
Total maternal weight gain (kg)	12.1	-7.0	29.4	9.7	0.8	22.9	11.5	3.0	19.0	8.0	-5.0	18.0	*0.011*	$
OGTT fasting (mg/dL)	75.0	64.0	89.0	73.0	57.0	81.0	86.0	81.0	102.0	86.0	61.0	139.0	*0.000*	&
OGTT 1hour (mg/dL)	117.5	79.0	185.0	106.5	57.0	134.0	170.0	125.0	213.0	153.0	83.0	226.0	*0.000*	&
OGTT 2hours (mg/dL)	107.0	74.0	153.0	88.0	68.0	129.0	140.0	87.0	1471.0	120.0	71.0	211.0	*0.000*	*#
Glycemic mean	83.0	67.5	111.5	83.7	69.8	100.2	97.2	75.2	113.3	96.8	63.3	123.8	*0.000*	&
HbA_{1C} (%)	4.9	3.6	6.1	5.1	4.2	6.3	5.4	4.8	5.8	5.3	4.7	6.4	*0.001*	+
Birthweight (g)	3233.5	1365.0	4125.0	3105.0	2595.0	4065.0	3195.0	2590.0	3605.0	3340.0	2185.0	4505.0	*0.080*	
	n	**%**		**n**	**%**		**n**	**%**		**n**	**%**		***p***	
White	8	23.5		6	27.3		4	36.4		12	34.3		*0.736*	
Stable union/Married	24	70.6		20	90.9		10	90.9		34	97.1		*0.010*	$
Hypertension	6	17.6		0	0.0		2	18.2		9	25.7		*0.045*	#
Smoking	8	23.5		0	0.0		1	9.1		3	8.6		*0.041*	##
Alcoholism	2	5.9		0	0.0			0.0		0	0.0		*0.358*	
Cesarean delivery	17	47.1		9	40.9		5	45.5		28	80		*0.006*	#
Birthweight classification														
AGA	24	70.6		20	90.9		10	90.9		23	65.7			
SGA	6	17.6		1	4.5		1	9.1		3	8.6		*0.138*	
LGA	4	11.8		1	4.5		0	0.0		9	25.7			
UI 12months postpartum	5	14.7		14	63.6		5	45.5		29	82.9		*<0.001*	$$ ##

Kruskal-Wallis test, followed by Dunn's multiple comparisons and chi-square test. NC: Normoglycemic continent group; NI: Normoglycemic incontinent group; HC: Hyperglycemic continent group; HI: Hyperglycemic incontinent group

# $p<0.05$- NI ≠ HI
$ $p<0.05$- NC ≠ HI
& $p<0.05$- NC and NI ≠ HC and HI
* $p<0.05$- NC and NI ≠ HC
+ $p<0.05$- NC ≠ NI and HC
$p<0.05$- NC ≠ NI and HC
$$ $p<0.05$- HI ≠ NC and HC

The CCL7 levels in normoglycemic and hyperglycemic groups were compared in Table 2 and Figure 1. Analysis of the CCL7 serum concentration revealed homogeneous groups in early pregnancy (12-18 weeks). Compared to normoglycemic groups, mainly continent, serum content of CCL7 was lower in hyperglycemic groups either continent or incontinent, from 24-28 weeks of pregnancy over the first year postpartum.

Table 2. Biochemical measures of CCL7 serum levels at each pregnancy and postpartum time-points in the four cohort study groups.

		NC			NI			HC			HI				
		Med	**Min**	**Max**	**Med**	**Min**	**Max**	**Med**	**Min**	**Max**	**Med**	**Min**	**Max**	***p***	**Comparisons**
PREGNANCY	CCL7 **(12-18 weeks)**	27.0	25.0	29.7	26.2	24.0	30.0	27.7	26.6	29.6	25.3	23.1	30.0	*0.041*	NC=NI=HC=HI
	CCL7 **(24-28 weeks)**	27.2	24.0	30.9	25.9	22.1	30.0	24.0	21.4	27.9	24.7	20.1	26.8	*0.000*	NC > HC, HI
	CCL7 **(34-38 weeks)**	26.6	21.4	31.3	25.9	22.1	29.1	23.7	20.8	26.9	24.2	21.7	35.1	*0.000*	NC > HC, HI
POSTPARTUM	CCL7 **(24-48 hours)**	29.8	27.2	32.4	28.2	26.1	29.7	25.9	22.0	28.8	25.2	22.4	28.5	*0.000*	NC, NI > HC, HI
	CCL7 **(6 weeks)**	26.9	25.1	30.4	26.5	23.7	42.6	24.6	20.8	27.8	25.2	22.0	28.7	*0.000*	NC, NI > HC, HI
	CCL7 **(12 months)**	27.2	24.7	30.9	26.6	24.0	31.7	24.3	22.7	26.9	25.0	22.4	27.5	*0.000*	NC > HC, HI

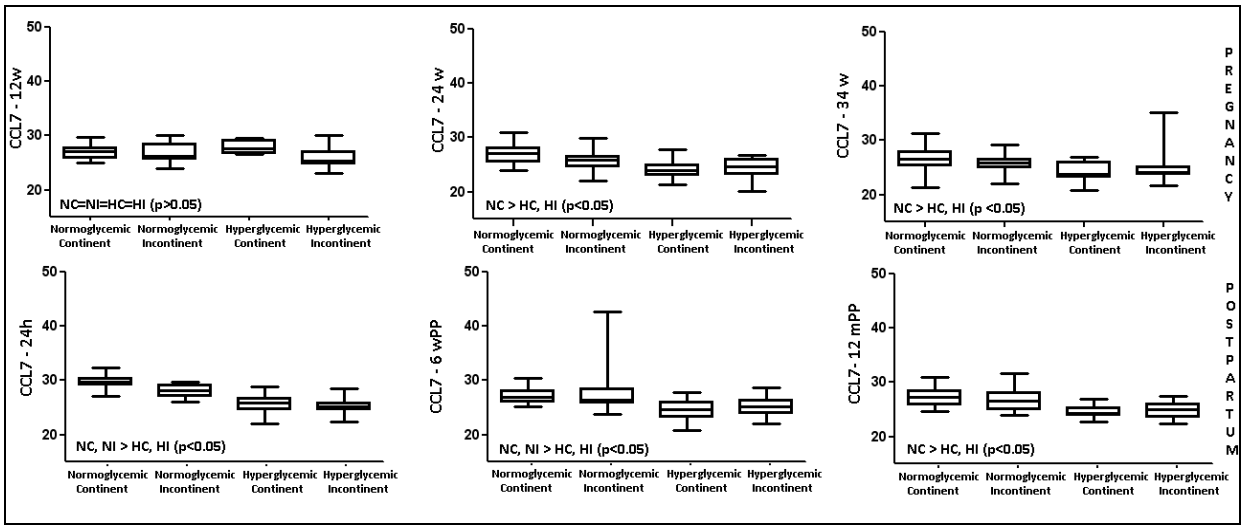

Figure 2. Median ± 25th - 75th percentiles of CCL7 serum concentration in patients who developed, or did not develop, gestational hyperglycemia and PS-UI at 6-time-points from pregnancy (12-18; 24-28; 34-38 weeks) to postpartum (24-48 hours; 6 weeks and 12 months).

The Table 3 and Figure 2 presents the progression of CCL7 serum concentration over the study period from 12 weeks of pregnancy till 12 months postpartum in each cohort study groups.

In NC group, CCL7 serum concentration revealed the highest values, which remains stable during pregnancy, increases significantly at 24-48 hours after delivery and returns to 12th weeks baseline value at 12th month postpartum. Trends of peak increase in CCL7 serum concentration at 24-48 hours postpartum were found in this group compared to all time-points

of pregnancy and postpartum.

In NI group, the CCL7 serum concentration was similar to NC group. However, the peak of CCL7 soon after birth (visit 4) reached statistical significance only compared to late pregnancy (34-38 weeks).

Conversely, the hyperglycemic groups did not demonstrate the same profile of CCL7 during the 6 time-points: the concentration was lower, the progression of CCL7 was unequal and the peak at 24-48 hours postpartum was absent.

In HC group, at 12-18 weeks, the highest CCL7 serum concentration measured was similar to normoglycemic continent group (visit 1). After this, CCL7 serum concentration decreased progressively and reached the lower value at 34-38 weeks of pregnancy and did not return to initial baseline value at 12 months postpartum.

By 6-time-points, CCL7 serum concentrations in HI group demonstrated the same CCL7 chemokine concentration at all follow-up visits.

Table 3. Progression of CCL7 serum concentration in the four groups at cohort study: from 12 weeks of pregnancy until the first year postpartum.

	PREGNANCY			POSTPARTUM				
	CCL7 (12-18weeks)	**CCL7 (24-28weeks)**	**CCL7 (34-38weeks)**	**CCL7 (24-48hours)**	**CCL7 (6 weeks)**	**CCL7 (6-12 months)**	**p**	**Comparisons**
NC	27.7 (26.1-29.7)	27.4 (24.8-28.4)	27.8 (25.1-29.4)	30.2 (29.4-32.4)	27.8 (25.1-29.5)	27.4 (24.7-29.8)	0.001	12w, 24w, 34w, 12mpp < 24h
NI	26.1 (24.6-29.3)	25.4 (22.7-27.5)	25.6 (22.1-26.1)	28.4 (26.6-29.5)	25.9 (23.7-28.5)	24.9 (24.0-27.4)	0.026	34w < 24h
HC	27.7 (26.6-29.6)	24.6 (22.7-27.9)	23.3 (21.4-26.0)	26.3 (25.3-26.7)	24.6 (24.0-26.0)	24.0 (22.7-25.3)	0.003	12w > 34w, 12mpp
HI	25.3 (24.5-28.7)	24.4 (22.0-26.1)	24.3 (23.1-25.8)	24.5 (23.1-26.4)	24.7 (22.1-25.3)	25.1 (23.4-26.1)	0.378	12=24=34=24=6=12

NC: Normoglycemic continent group; NI: Normoglycemic incontinent group; HC: Hyperglycemic continent group; HI: Hyperglycemic incontinent group

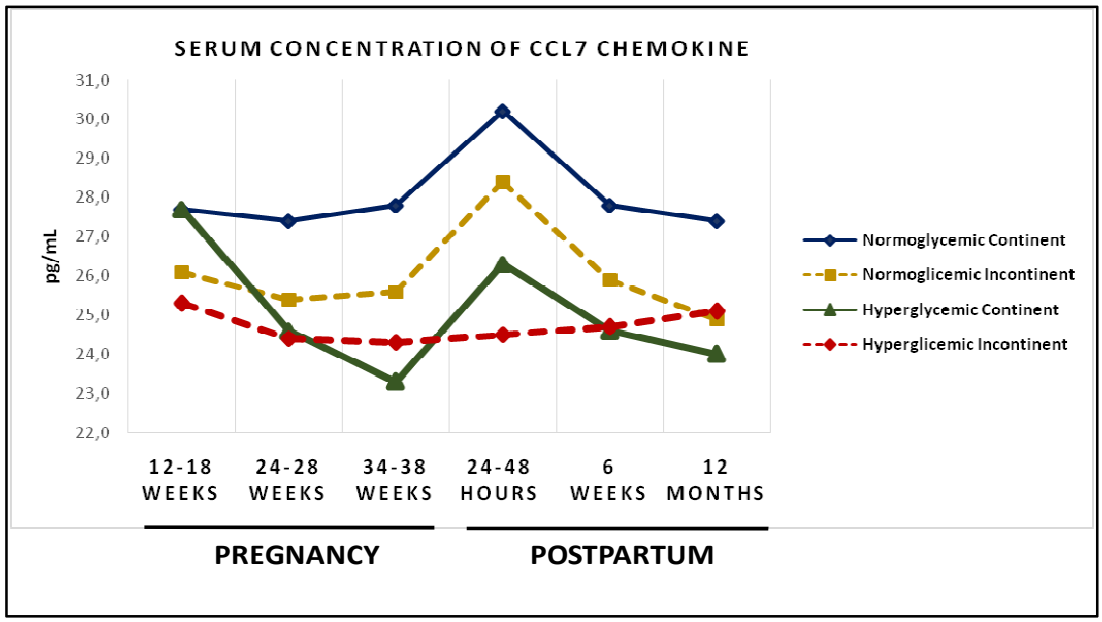

Figure 2. Progression of CCL7 serum concentration in the four groups at cohort study.

Cox regression was applied to test the associations between gestational hyperglycemia plus PS-UI on UI 12 months postpartum. In the presence of PS-UI either in hyperglycemic (RR, 5.6; 95% confidence interval (CI), 2.2 - 14.6) or normoglycemic group (RR, 4.3; 95% confidence interval (CI), 1.6 - 12.0), pregnant women significantly increased the relative risk to present UI 12 months postpartum (Table 4).

Table 4. Relative risk for UI 12 months postpartum in the groups.

Group	UI 12 MPP	RR (CI95%)
Normoglycemic Continent	5/34 (14.7%)	1.0
Normoglycemic Incontinent	14/22 (63.6%)	4.3 (1.6 - 12.0)
Hyperglycemic Continent	5/11 (45.5%)	3.1 (0.9 - 10.6)
Hyperglycemic Incontinent	29/35 (82.9%)	5.6 (2.2 - 14.6)

MPP: months postpartum

Multiple logistic regression analysis was performed to assess the association between clinic features and UI 12 months postpartum in the four groups (Table 5). The results showed that NI (OR, 13.57; 95% confidence interval (CI), 1.96-93,86; p=0.008) and HI groups (OR, 58.89; 95% confidence interval (CI), 6.09-569.17; p=0.000) are more likely to have UI 12

months postpartum. Birth(s) by cesarean section conferred some protection with respect to urinary incontinence at 12 months postpartum (OR, 0.12; 95% confidence interval (CI), 0.02-0.63; p=0.012).

Table 5. Multivariable regression model of gestational hyperglycemia, PS-UI, clinical features and UI at 12 months postpartum.

Variable	UI 12 MPP			
	OR	CI 95%		*p*
Normoglycemic Continent	1.00			*0.003*
Normoglycemic Incontinent	13.57	1.96	93.86	*0.008*
Hyperglycemic Continent	3.38	0.30	37.85	*0.323*
Hyperglycemic Incontinent	58.89	6.09	569.17	*0.000*
Age (years)	0.99	0.88	1.10	*0.816*
Stable union/Married	0.64	0.09	4.35	*0.644*
Hypertension	1.42	0.12	17.03	*0.784*
Smoking	0.21	0.01	3.30	*0.268*
Pre-pregnancy BMI (kg/m^2)	1.01	0.92	1.17	*0.732*
Final BMI	1.01	0.92	1.10	*0.817*
Total maternal weight gain (kg)	1.00	0.90	1.12	*0.973*
OGTT fasting (mg/dL)	1.02	0.95	1.08	*0.609*
OGTT 1hour (mg/dL)	1.01	0.99	1.04	*0.364*
OGTT 2hours (mg/dL)	1.00	0.98	1.01	*0.715*
Glycemic mean	0.99	0.91	1.08	*0.819*
HbA_{1C} (%)	1.29	0.23	7.12	*0.773*
Cesarean delivery	0.12	0.02	0.63	*0.012*

MPP: months postpartum
BMI: Body mass index
OGTT: oral glucose tolerance test
HbA_{1C}: Glycated hemoglobin

The univariate analysis showed significant association between normoglycemic incontinent, hyperglycemic continent and hyperglycemic incontinent groups and CCL7 values on UI 12 months postpartum. No correlation was identified between CCL7 serum concentrations during pregnancy and UI 12 months postpartum. By contrast, CCL7 serum concentration since 24-48 hours after delivery to 12 months postpartum correlated with better prognosis over the first year postpartum (Table 6).

Table 6. Univariate analysis of gestational hyperglycemia, PS-UI, CCL7 serum concentration and UI at 12 months postpartum.

	UI 12 MPP			
	OR	**CI95%**		***p***
Normoglycemic Continent	1.00			
Normoglycemic Incontinent	10.15	2.80	36.75	*0.000*
Hyperglycemic Continent	4.83	1.06	22.09	*0.042*
Hyperglycemic Incontinent	28.03	7.69	102.21	*0.000*
CCL7 (12-18 weeks P)	0.82	0.61	1.11	*0.199*
CCL7 (24-28 weeks P)	0.87	0.71	1.07	*0.182*
CCL7 (34-38 weeks P)	0.88	0.73	1.06	*0.181*
CCL7 (24-48 hours PP)	0.60	0.47	0.78	*0.000*
CCL7 (6 weeks PP)	0.74	0.57	0.95	*0.018*
CCL7 (12 months PP)	0.63	0.46	0.85	*0.002*

MPP: months postpartum ; P: pregnancy; PP: postpartum

Table 7 presents that no association was found between mode of delivery with CCL7 serum concentration at all cohort time-point periods ($p>0.05$).

Table 7. Association of mode of delivery with CCL7 serum concentration at each pregnancy and postpartum time-points.

	Vaginal Delivery				**Cesarean Delivery**				
	n	Med	Min	Max	n	Med	Min	Max	*p*
CCL7 (12-18 weeks P)	33	26.63	24.63	29.63	40	26.99	23.14	30.00	0.838
CCL7 (24-28 weeks P)	41	25.99	21.40	30.90	51	25.54	20.12	30.27	0.245
CCL7 (34-38 weeks P)	42	25.27	20.76	31.32	51	25.79	21.65	35.10	0.482
CCL7 (24-48h PP)	27	27.07	22.04	32.38	49	26.45	22.41	31.10	0.587
CCL7 (6 weeks PP)	34	26.00	20.76	42.61	46	26.14	22.04	29.45	0.869
CCL7 (12 months PP)	27	25.54	22.43	31.69	48	25.85	22.70	29.79	0.711

Mann-Whitney test. P: pregnancy; PP: postpartum

DISCUSSION

To the best of our knowledge, this is the first population-based cohort study to evaluate the interaction between gestational hyperglycemia, PS-UI, and serum CCL7 profile during pregnancy and postpartum on UI over time. Only a few prospective studies have examined the persistence, resolution or recurrence of UI symptoms beyond 12 months postpartum (25-27) in either normoglycemic or hyperglycemic pregnant women (3, 28). There

are no data published regarding clinical measurement of CCL7 in pregnant women long-term after delivery, mainly in special groups likewise maternal diabetes.

The prevalence and relative risk of UI 12 months postpartum in pregnant women with PS-UI was higher in hyperglycemic incontinent group and normoglycemic incontinent group evidencing that the development of UI during pregnancy is an important tool to predict UI 12 months postpartum. It has been published that UI during pregnancy is an independent risk factor for incontinence in the postpartum period (29, 30) and long after delivery (31). Dietz-Itza *et al.* (2010) revealed that the new onset of UI during pregnancy is strongly associated with the presence of this symptom 1 year after delivery (30).

Consistent with previous studies (7-9, 19), the need for exploration a recovery factor for UI allowed us to confirm the translational potential of CCL7 cytokine. The overexpressed CCL7 serum of mice soon after vaginal distension can be used as a surrogate for injury after delivery (9). We demonstrated the impact of gestational hyperglycemia plus PS-UI on CCL7 profile during pregnancy until 12 months postpartum emphasizing variable pattern of the chemokine concentration, peak and progression. The analysis of the CCL7 serum concentration revealed homogeneous groups in early pregnancy (12-18 weeks), although with the development of pregnancy, shortly after birth up to 1 year postpartum, varied according to the group characteristics.

In spite of the main objective of this paper was to analyse the interaction among gestational hyperglycemia, PS-UI and CCL7 serum concentration in the long-term maternal UI, we did not find a standard curve of CCL7 in hyperglycemic incontinent group during pregnancy and postpartum. Alternatively, the CCL7 serum concentration in normoglycemic continent group were significantly higher at all time-points, with distinctive peak at 24-48 hours postpartum and impressive return to initial baseline value at 12 months postpartum. This profile denotes a "standard curve of CCL7 serum concentration" from 12 weeks of

pregnancy to 12 months postpartum and were useful in predicting long-term maternal UI. A potential protective mechanism by which the CCL7 peak at 24-48 after delivery in normoglycemic groups, may represent a biomarker action that promote regenerative tissue repair later in life.

The preliminary data of vaginal distension (VD) in a rat model of SUI showed significantly overexpression of CCL7 in the urethra and anterior vaginal wall immediately following distention, with decreasing values toward the level of control, and sham values at 24 hours after distention (8).These findings provided the basis for continued evaluation of CCL7 in stem cell homing for the future treatment of UI and it is an exciting step in understanding the body's natural reparative process. In this context, Hijaz *et al.* suggested that additional time points and duration of VD were necessary to fully evaluate the effects of chemokines on regenerative tissue repair (9). Although earlier studies support rapid changes in CCL7 levels after VD, recent data from Salcedo *et al.* suggested that 24 hours may not be long enough to detect changes in cytokines after injury (32). Besides, the authors suggest large epidemiological studies with clinical measurement of CCL7, among other chemokines, during, immediately after, and up to 12 months after delivery to determine if the findings in animal models correlate with clinical observations in postpartum women (9).

Hyperglycemic pregnant women with PS-UI revealed lower concentration of CCL7 compared to healthy controls, since gestational hyperglycemia diagnostic period (24-28 weeks) to 12 months postpartum. Otherwise, the pattern of this chemokine profile was surprisingly stable in the hyperglycemic incontinent group at all time-points of the follow-up study, without tendency of peak soon after birth (24-48 hours). These results indicate that CCL7 gradient is likely absent or compromised in the natural repair process of hyperglycemic incontinent pregnant women who continue to suffer from UI long-term after delivery. Our results persuade the clinical measurement of CCL7 since 24-28 weeks of pregnancy and

monitoring the concentration and progression throughout the first year postpartum.

The two intermediate groups, normoglycemic incontinent and hyperglycemic continent, exhibited singular patterns of CCL7 serum concentration showing the influence of altered parameters either gestational hyperglycemia or PS-UI. In normoglycemic incontinent pregnant women, this chemokine concentration demonstrated a distinctive peak at 24-48 hours postpartum but only compared to late pregnancy (34-38weeks). In hyperglycemic continent group, the influence of hyperglycemia was clearly observed, by reduced CCL7 serum concentration from the time of gestational hyperglycemia diagnosis to all monitoring period. In addition, CCL7 concentration did not reach peak soon after birth and did not return to initial baseline value at 12 months postpartum.

Changes in CCL7 serum levels during the follow-up period of pregnancy and postpartum are reflective of a complex inter-relationship between gestational hyperglycemia and PS-UI and the maternal long-term effect on UI 12 months after delivery. The new emphasis in diagnosing gestational hyperglycemia and PS-UI represents a unique window of opportunity for the health-care professional to introduce educate and advising patients on the different approaches to prevent or delay the onset of UI later in life. CCL7 serum concentration, peak at 24-48 hours after delivery and postpartum progression could potentially be used as marker of UI over the first year postpartum.

In this study, we validate previous experimental studies in rats' model of SUI by which CCL7 was significantly overexpressed in urethral and vaginal tissues immediately following vaginal distension (7, 8, 11). Furthermore, our clinical results of raised CCL7 serum concentration in control healthy women correlate well with experimental findings of overexpression of CCL7 in the serum of mice after VD, complying that CCL7 measurement could be used as a surrogate for injury after delivery (9). Although previous experimental studies demonstrated overexpression of CCL7 in urogenital tissues immediately following

vaginal distention, the present study found no association between the mode of delivery with the clinical measurement of CCL7.

The legacy of the maternal hyperglycemic environmental and UI acquired during pregnancy associated with low levels of CCL7 concentration without a peak at 24-48 hours after delivery cannot be ignored. Our results confirmed the widely recognized effects of hyperglycemia environment extend beyond those apparent at birth. Therefore, diabetes prevention is critical for women with gestational diabetes mellitus (GDM), which affects 7–14% of pregnancies (33), as women with GDM are seven times more likely to develop type 2 diabetes than parous women without GDM (34). Yet evidence to support health system adoption of postpartum diabetes prevention programs for women with GDM is lacking.

CCL7 serum concentration evaluated during pregnancy may represent a risk assessment tool and/or lab technique to predict pelvic floor damage after delivery. Even short-term hyperglycemic state during pregnancy with PS-UI, the CCL7 determination may be a potentially indicator of long-term maternal UI. This condition causes embarrassment and can have a personal and economic impact on women (35).

Taken together, these findings of overexpression of CCL7 in the serum of NC group and decreased levels in the HI group, may confirm that diabetes delays the recovery from child birth induced UI (5), and that CCL7 could potentially be used as a serum marker of muscle injury, ex. the development of UI 12 months postpartum. Our results emphasize the need for policy development for UI prevention in previous hyperglycemic pregnant women.

As an alternative to provide this missing CCL7 gradient, as in hyperglycemic women with PS-UI, Rivera-Delgado *et al*. (2016) evaluated the feasibility of locally providing the CCL7 gradient by means of an affinity-based implantable polymer in rats (11). Using chemokines to attract therapeutically injected stem cells has been a goal of the field for some time. Chemokines are a subset of cytokines that bind to G protein coupled receptors. MSCs

migrate in response to gradients of these chemokines depending on the set of receptors expressed in the cell surface and the chemokines present in their direct vicinity (11). This preclinical approach indicated conjugation of heparin to a polymer backbone (using either bovine serum albumin or poly (ethylene glycol) as the base polymer) can be used as a delivery system capable of providing sustained concentrations of CCL7 in a therapeutically useful range up to a month *in vitro*. With this approach it was possible to detect, after polymer implantation, significant increase in CCL7 in the urethral tissue directly surrounding the polymer implants with only trace amounts of human CCL7 present in the blood of the animals (11). Thus, affinity delivery of bioactive molecules was an effective long-term drug delivery strategy (11).

The strengths of our study included a population-based, prospective cohort study design with several time-points and a first comprehensive clinical measurement of the CCL7 as an effective bioactive molecule to predict UI long-term after delivery, mainly in hyperglycemic women. This study was characteristically translational since the findings in animal models could correlate with clinical observations in postpartum women.Weakness of this study included the small sample size and the short-tem follow-up (12 months postpartum). Larger studies and long observational periods are required to investigate the link between hyperglycemia during pregnancy, PS-UI, CCL7 concentration, peak at 24-48hours postpartum and the development of UI 12-48 months postpartum. Beyond that, it has been shown that the assessment of single biomarker is not accurate enough to be used as diagnostics tool due to their not-specific nature (36), while the measurement of selected inflammatory proteins in combination with classical clinical markers may improve the predictive value of UI postpartum. Therefore, our findings need to be confirmed in future studies performed on larger population of hyperglycemic patients with PS-UI.

In conclusion, the inter-relationship among hyperglycemic status during pregnancy,

PS-UI and CCL7 serum concentration, peak and progression can better predict UI 12 months postpartum. This association increase the accuracy of the fate in UI 1 year postpartum and allow us to prevent and promote better management in pelvic floor muscle dysfunction, insuring positive outcomes.

CONCLUSION

Our study demonstrates that CCL7 profile in hyperglycemic pregnant women with PS-UI was characterized by low concentration after 24 weeks of pregnancy, stable levels at all time-points of the follow-up study, without a peak soon after birth.

Therefore, the complex inter-relationship among gestational hyperglycemia, PS-UI and CCL7 profile increased the prevalence of UI 12 months postpartum. These findings point the need for further investigation of therapeutically useful for hyperglycemic control plus pelvic floor muscle training, associated with local or systemic release of CCL7 to stimulate stem cell homing for regenerative tissue repair, in order to prevent future development of UI.

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COMPETING INTERESTS

The authors declare that there are no competing interests.

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Anexos

ANEXO 1 – APROVAÇÃO DO COMITÊ DE ÉTICA

FACULDADE DE MEDICINA DE BOTUCATU -UNESP

PARECER CONSUBSTANCIADO DO CEP

DADOS DO PROJETO DE PESQUISA

Título da Pesquisa: ANÁLISE DA PROTEÍNA QUIMIOTÁTICA DE MONÓCITOS-3 (CCL7) EM GESTANTES HIPERGLICÊMICAS COM INCONTINÊNCIA URINÁRIA: Coorte prospectiva da gestação até seis meses pós-parto

Pesquisador: Fernanda Piculo

Área Temática:

Versão: 2

CAAE: 20639813.0.0000.5411

Instituição Proponente: Departamento de Ginecologia e Obstetrícia

Patrocinador Principal: FUNDACAO DE AMPARO A PESQUISA DO ESTADO DE SAO PAULO

DADOS DO PARECER

Número do Parecer: 401.058

Data da Relatoria: 19/09/2013

Apresentação do Projeto:

A hiperglicemia gestacional leve (HGL) e o Diabetes mellitus gestacional (DMG) têm consequências importantes para a mãe, o feto e o recém-nascido. A associação entre hiperglicemia gestacional e incontinência urinária (IU) é escassa na literatura, havendo necessidade de mais pesquisas para ampliar a compreensão dos fenômenos que resultarão no melhor atendimento às pacientes. Considerando que a proteína quimiotática de monócitos-3 (CCL7) é um importante fator para o mecanismo de recuperação da incontinência urinaria, a hipótese é que as pacientes portadoras de DMG ou de HGL apresentem alteração nos níveis de CCL7 e isto retarde/impeça a migração de células tronco mesenquimais para o local de lesão causada por IU, com consequências no período pósparto.

Será realizado estudo de coorte prospectivo para análise da proteína quimiotática de monócitos-3 (CCL7) em gestantes hiperglicêmicas com incontinência urinária (IU) desde o início da gestação até seis meses pós-parto.

A coleta de dados ocorrerá em aproximadamente 36 meses consecutivos no Centro de Investigação do Diabete Perinatal da FMB/UNESP (financiamento FAPESP no Infra IV)e a análise do material será realizada no Laboratório de Pesquisa Experimental em Ginecologia, Obstetrícia e Mastologia da FMB/Unesp. Serão selecionadas todas as gestantes entre 12 e 18

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ANEXO 2

TERMO DE CONSENTIMENTO LIVRE E ESCLARECIDO
(TERMINOLOGIA OBRIGATÓRIA EM ATENDIMENTO A RESOLUÇÃO 466/12-CNS-MS)

A Sra. está sendo convidada a participar de uma pesquisa chamada "**ANÁLISE DA PROTEÍNA QUIMIOTÁTICA DE MONÓCITOS-3 (CCL7) EM GESTANTES HIPERGLICÊMICAS COM INCONTINÊNCIA URINÁRIA: Coorte prospectiva da gestação ao primeiro ano pós-parto"**, que pretende estudar uma proteína conhecida como CCL7 em gestantes com diabete que apresentam perda de urina desde o início da gestação até doze meses pós-parto.

A Sra. foi selecionada a participar dessa pesquisa por estar entre as gestantes que realizam assistência pré-natal no Centro de Investigação do Diabete Perinatal da Faculdade de Medicina de Botucatu, UNESP, e estar entre a 12ª e 18ª semana gestacional.

A pesquisa consta de algumas perguntas sobre dados pessoais e clínicos, uso de medicamentos, hábitos de vida e questionários relacionados à perda de urina que serão aplicados pelo responsável da pesquisa. A entrevista terá duração de cerca de 20 minutos e será realizada nos dias de consulta de pré-natal, visitas de acompanhamento ou agendamento, em sala reservada. A entrevista não será gravada. O pesquisador irá tomar os devidos cuidados para proteger a privacidade do indivíduo e dos dados pertencentes à sua participação neste estudo de pesquisa. Registros médicos e pessoais da participante serão mantidos em armários trancados e o acesso à informação do estudo será dado apenas à equipe de investigação.

A pesquisa consta de coleta de pequena quantidade de sangue para análise através de teste laboratorial. Esclarecemos que, a não ser o pequeno desconforto no momento da picada da agulha, a coleta do sangue não terá riscos, pois será feita por profissional qualificado e utilizando material descartável. O procedimento tem por finalidade estudar uma proteína que pode interferir na perda urinária feminina.

O conhecimento dessas características possibilitará estabelecer intervenção para o tratamento da perda de urina, bem como uma rápida recuperação às gestantes que possam vir a perder urina, proporcionando menor desconforto pessoal e social e melhor qualidade de vida.

Caso você não queira participar da pesquisa, é seu direito e isso não vai interferir no seu acompanhamento médico ou preferência de agendamento médico durante e após a gestação. Você poderá retirar seu consentimento, em qualquer fase da pesquisa sem nenhum prejuízo.

É garantido total sigilo do seu nome e resultados de exame, em relação aos dados relatados nesta pesquisa. Você receberá uma via deste termo, e outra via será mantida em arquivo pelo pesquisador por cinco anos.

Qualquer dúvida adicional, você poderá entrar em contato com o Comitê de Ética em Pesquisa, através do fone: (14) 3880-1608 /1609.

CONCORDO EM PARTICIPAR DA PESQUISA

Nome:_____

Assinatura:_____

Fernanda Piculo, Data: ____/____/____Assinatura:_____

Orientadora: Marilza Vieira Cunha Rudge, Departamento de Ginecologia e Obstetrícia- Faculdade de Medicina de Botucatu, UNESP, Botucatu – SP. Fone: (14) 3882-6227. E-mail: marilzarudge@gmail.com

Pesquisadora: Fernanda Piculo, Rua Visconde do Rio Branco, nº. 891, Botucatu-SP Fone: (14)99117-1959. E-mail: fer_piculo@yahoo.com.br

ANEXO 3
FICHA DE AVALIAÇÃO CLÍNICA

Data da avaliação:_____/_____/_____ Número da amostra:_____
Pré Natal em: _____

1. Identificação

Nome:_____
RG (H.C.):_____ RG:_____
Endereço: _____ nº _____ CEP: _____
Bairro: _____ Cidade: _____ Estado: _____
Tel.: ( )_____ Cel.: ( ) _____ Profissão:_____
Data de Nascimento:_____/_____/_____ ( ) anos Raça:_____
Idade Gestacional:_____ sem ____ d Estado Civil:_____
Peso inicial:_____ Altura:_____ IMC (gestacional) inicial:_____
Peso final: _____ IMC (gestacional) final:_____ Classificação: _____

Critérios de inclusão
G____ P____C____A_____

Partos	Peso do Bebê ao nascer
1º	
2º	
3º	

Grupo de Inclusão: Grupo de Rudge IA ( ) IB ( ) II A ( ) II B ( )

Média glicêmica: _____ GTT jejum_____ 1h_____ 2h_____
Hemoglobina glicada: _____

Refere IU ( ) **ou Não refere IU** ( )

Critérios de não-inclusão
( ) DM 1 ( ) DM 2
( ) IU prévia a gestação
( ) Cirurgia prévia para IU
( ) Cirurgia abdominal (exceto cesárea)
( ) Infecção ativa com vírus da hepatite A, B, C e/ou HIV e/ou Sífilis
( ) Gestação gemelar (atual ou prévia)
( ) Esclerose múltipla
( ) Diagnóstico de Miomas
( ) Doença inflamatória pélvica
( ) Neoplasias malignas
( ) Asma, sinusite crônica
( ) Colite ulcerosa, cirrose biliar
( ) Alterações cognitivas
( ) Ceratoconjuntivite vernal
( ) Constipação intestinal crônica
( ) Tosse Crônica

Considerar
( ) Hipertensão Arterial Crônica (HAC)
( ) Etilista
( ) Até 3 doses (destilada) ou garrafas (fermentadas)
( ) De 3 a 7 doses (destilada) ou garrafas (fermentadas)
( ) Acima de 7 doses (destilada) ou garrafas (fermentadas)
( ) Tabagista ( ) quantidade por dia
( ) DMG em partos anteriores

Dados Uroginecológicos

- A Sra. teve qualquer perda de urina em lugares impróprios ou em horas impróprias por duas vezes ou mais em um mês independente da quantidade de perda de urina?Sim ( ) ou Não ( )

- A Sra. já apresentou perda de urina nas gestações anteriores? Sim ( ) ou Não ( )

- A Sra. perde urina quando faz algum esforço? Sim ( ) ou Não ( )

- Quantas vezes a Sra. perde urina por semana? Diariamente ( ) Poucas Vezes ( )

Sobre perda de urina aos esforços:

Situação	**Assinale Sim ou Não**	**Período: 1- antes da gestação, 2- nesta gestação, 3- antes e nesta gestação**
Tosse		
Espirra		
Ri		
Muda de posição		
Carrega peso		
Outros esforços		

ANEXO 4
QUESTIONÁRIO - ÍNDICE DE GRAVIDADE DA INCONTINÊNCIA

Incontinence Severity Index (ISI)

Português

(1) Com qual frequência você apresenta perda de urina?

1. Menos de uma vez ao mês
2. Algumas vezes ao mês
3. Algumas vezes na semana
4. Todos os dias e/ou noites

(2) Qual quantidade de urina você perde cada vez?

1. Gotas
2. Pequeno jato
3. Muita quantidade

Fonte: Pereira *et al*., 2011.

ANEXO 5
QUESTIONÁRIO- ICIQ-SF

International Consultation on Incontinence Questionnaire - Short Form

Nome: _____ Data de hoje ___/___/___

Muitas pessoas perdem urina alguma vez. Estamos tentando descobrir quantas pessoas perdem urina e o quanto isso as aborrece. Ficaríamos agradecidos se você pudesse nos responder às seguintes perguntas, pensando em como você tem passado, em média nas ÚLTIMAS QUATRO SEMANAS:

1. Data de nascimento: _____/_____/_____ (Dia/Mês/Ano)
2. Sexo: □ Feminino □ Masculino

3. Com que freqüência você perde urina? (assinale uma resposta)

Nunca □ 0
Uma vez por semana ou menos □ 1
Duas ou três vezes por semana □ 2
Uma vez ao dia □ 3
Diversas vezes ao dia □ 4
O tempo todo □ 5

4. Gostaríamos de saber a quantidade de urina que você pensa que perde? (assinale uma resposta)

Nenhuma □ 0
Uma pequena quantidade □ 2
Uma moderada quantidade □ 4
Uma grande quantidade □ 6

5. Em geral o quanto que perder urina interfere em sua vida diária? Por favor, circule entre o número 0 (não interfere) e 10 (interfere muito).

0 1 2 3 4 5 6 7 8 9 10

Não interfere Interfere muito

ICIQ- Soma de todos resultados: 3+4+5=

6. Quando você perde urina? (Assinale todas as alternativas que se aplicam a você)

Nunca □
Perco antes de chegar ao banheiro □
Perco quando tusso ou espirro □
Perco quando estou dormindo □
Perco quando estou fazendo atividades físicas □
Perco quando termino de urinar e estou me vestindo □
Perco sem razão óbvia □
Perco o tempo todo □

"Obrigado por ter respondido às questões"

Fonte: Tamanini *et al*., 2004