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Original article

Associations of Spexin and cardiometabolic parameters among women with and without gestational diabetes mellitus

Nasser M. Al-Daghri [a,*], Hazim A. Al-Hazmi [a], Abdulrahman Al-Ajlan [b], Mohammad S. Masoud [a], Abeer Al-Amro [c], Amani Al-Ghamdi [c], Abdullah M. Alnaami [a], Omar S. Al-Attas [a], Majed S. Alokail [a]

[a] Prince Mutaib Chair for Biomarkers of Osteoporosis, Biochemistry Department, College of Science, King Saud University, Riyadh 11451, Saudi Arabia
[b] Department of Clinical Laboratory Sciences, College of Applied Medical Sciences, King Saud University, PO Box 10219, Riyadh 11433, Saudi Arabia
[c] Biochemistry Department,College of Science,King Saud University, Riyadh 11451, Saudi Arabia

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ABSTRACT

Spexin (SPX) is a novel biomarker abundantly expressed in several animal and human tissues implicated in food intake and glucose control, respectively. As new roles for SPX are emerging, the present study explored for the first time, the associations of SPX to several cardiometabolic indices and inflammatory markers in pregnant women, a demographic not yet investigated with respect to SPX. A total of 117 Saudi women subdivided to those with gestational diabetes mellitus (GDM) (N = 63) and those without (N = 54) were included in this cross-sectional study. Anthropometry, glycemic, lipid, vitamin D, adipocytokines and inflammatory markers were measured consecutively at baseline and after the 2nd and 3rd trimesters. Age- and BMI adjusted comparisons revealed that levels of SPX were not significantly different in pregnant women with and without GDM. In all subjects, circulating levels of SPX showed modest associations with glucose ($R = 0.18$; $p = .08$) and HOMA β ($R = -0.19$; $p = .09$) as well as significant positive associations with total cholesterol ($R = 0.25$; $p = .02$), LDL-cholesterol ($R = 0.25$; $p = .02$), 25(OH)D ($R = 0.22$; $p = .04$), albumin ($R = 0.30$; $p < .01$) and IL1β ($R = 0.41$; $p < .01$). Stepwise regression analysis also suggested that IL1β, leptin and albumin were the significant predictors of SPX. In summary, SPX levels modestly affect glucose and insulin sensitivity in pregnant women but is not associated with GDM and obesity. The significant association of SPX to ILβ warrants further investigation as to the role of SPX in immune modulation.

1. Introduction

The novel peptide spexin (SPX) was recently discovered in 2007 (Mirabeau et al., 2007; Sonmez et al., 2009). In humans, SPX is a product of the Ch12orf39 gene consisting of 14 amino acids (116 amino acids as pre-peptides) and widely expressed in endocrine and epithelial tissues, among others (Gu et al., 2015; Mirabeau et al., 2007; Porzionato et al., 2010; Sonmez et al., 2009). The abundant expression of SPX in human tissues suggests its potential involvement in many physiological functions that are yet to be established. Studies performed in animals so far offered clues. In goldfish (*Carassius auratus*), SPX injection inhibited basal and neuropeptide Y (NPY)-induced feeding behavior and consumption (Wong et al., 2013). Consequently, Walewski et al. (2014) demonstrated anti-obesity activity of SPX on mice with diet-induced obesity (DIO), seen as reduced energy intake (~32%) and an inverse correlation between circulating SPX and leptin. Both *in vivo* animal studies hint that SPX modulates satiety. Early investigations performed among children and adolescents seem to support this premise, with SPX observed to be down regulated in obese adolescents, and, similar to animal models, associated inversely with leptin (Kumar et al., 2017). Among the limited studies performed in adult humans so far, Gu et al. (2015) demonstrated that SPX is inversely correlated to glycemic indices and lipids, with reduced SPX levels reported in patients with type 2 diabetes mellitus (T2DM) as compared to non-diabetic subjects, suggesting that SPX may have a role in glucose and lipid metabolism. These

* Corresponding author at: Prince Mutaib Chair for Biomarkers of Osteoporosis, Biochemistry Department, College of Science, King Saud University, PO Box 2455, Riyadh 11451, Saudi Arabia.
E-mail address: ndaghri@ksu.edu.sa (N.M. Al-Daghri).

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preliminary findings however were not replicated in adolescents (Hodges et al., 2017), suggesting possible differences in SPX functions according to age groups. Other populations also remain under investigated, including pregnant women.

Gestational Diabetes Mellitus (GDM) is defined as impaired tolerance of glucose diagnosed at the 24–28th week of pregnancy (Rani and Begum, 2016). Worldwide the prevalence of GDM varies 1.0–14% depending on the definition used (Chen et al., 2016). The absence of universal definition prevalence of GDM is on the rise worldwide according to WHO (4). In Saudi Arabia, the over-all prevalence of GDM was ~37% (Al-Rubeaan et al., 2014) with regional variations: Medina (Western region) at 51% (Alfadhli et al., 2015) and Riyadh (Central region) at 24% (Wahabi et al., 2017). As pregnancy is considered an insulinogenic state (Barbour et al., 2007), GDM as a complication of pregnancy may potentially provide interesting insights about SPX's evolving role in glucose metabolism as this type of investigation has never been carried out in this population. Furthermore, no study has been undertaken ascertaining the associations of SPX to a multitude of biomarkers including adipocytokines and inflammatory markers, aside from glycemic, lipid and anthropometric indices combined in the same population. In this cross-sectional study, we assessed circulating SPX levels and their correlations with the different parameters mentioned in participants with and without GDM.

2. Methods

2.1. Subjects

A total of 117 Saudi pregnant women identified to be at high risk of GDM (personal history of GDM or polycystic ovarian syndrome, glycosuria, family history of T2DM, severe obesity, macrosomia), recruited at various hospitals in Riyadh, Saudi Arabia were included in this study. In brief, only pregnant Saudi women aged 18–35 were included and were excluded to have a relatively homogenous cohort. Additional criteria for inclusion/exclusion have been described previously (Al-Ajlan et al., 2015). Written informed consent was obtained from each participant prior to inclusion. The study was approved by the Ethics Committee of the College of Science, King Saud University, Riyadh, Kingdom of Saudi Arabia (KSA).

2.2. Anthropometry and blood collection

Pregnant women in their first trimester pre-natal visit were subjected to anthropometric and blood withdrawal procedures as described previously (Al-Ajlan et al., 2015).

2.3. Sample collection and analyses

Blood samples collected from the subjects during their first visit were analyzed for 25(OH)D and various biochemical parameters. Serum glucose, lipid profile, albumin and calcium were measured using a chemical analyzer (Konelab, Espoo, Finland). Serum-free insulin concentration was determined by electrochemiluminescence method (Cobas e411; Roche Diagnostics, Mannheim, Germany). Serum TNF-α, leptin, IL1β and IL6 (human bone magnetic bead panel) [intra-assay variation was 1.4–7.9% and inter-assay variation of <21%. Minimum detectable concentrations (MDC) were as follows: Leptin, 85.4 pg/ml IL-6, 0.4 pg/ml and TNFα, 0.14 pg/ml] as well as adiponectin and resistin (human adipokine magnetic bead panel) [intra-assay variation was 1.4%-7.9% and inter-assay variation of <21%. Minimum detectable concentrations (MDC) for adiponectin was adiponectin was 145.4 pg/ml and 6.7 pg/ml for resistin] were measured using Milliplex Map® (Millipore, Billerica, MA, USA) multiple assays by Luminex® xMAP® (Luminex Corp, Austin, TX, USA). Serum 25(OH)D was determined as described before (Al-Ajlan et al., 2015) with a Roche Elecsys modular analytics (Cobas e411) using an electrochemiluminescence immunoassay (Roche Diagnostics, GmbH, Mannheim, Germany) and commercially available IDS kits (IDS Ltd, Boldon Colliery, Tyne & Wear, UK). Variation for the 25(OH)D ELISA were 5.3 % and 4.6 %, respectively, with 100% cross-reactivity to 25(OH) D3 and 75% cross-reactivity to 25(OH)D2. Circulating SPX measurements were carried out using an enzyme-linked immunoassay (ELISA) following the manufacturer protocol (Phoenix Pharmaceuticals, Inc., Burlingame, CA) with a linear range of 0.11–1.07 ng/ml, intra assay variation of <10% and inter-assay variation of <15%.

2.4. Oral glucose tolerance test (OGTT)

OGTT was conducted with ingestion of seventy-five gram glucose and GDM was diagnosed according to International Association for Diabetes in Pregnancy Society Group (IADPSG) guidelines (Agarwal et al., 2015), if one of the following applies: fasting glucose ≥5.1 mmol/l, 1 h glucose ≥10 mmol/l or 2 h glucose ≥8.5 mmol/l.

2.5. Calculations done

Waist-hip ratio was calculated as waist (cm) circumference divided by hips (cm). BMI was calculated as weight in kg/height in m^2. LDL-cholesterol was calculated using the Friedwald for mula = [Total-cholesterol − HDL-cholesterol − (Triglycerides/2.2)] where all concentrations are given in mmol/L. HOMA-β was calculated using the formula HOMA-β = (20 ∗ fasting insulin)/(fasting g lucose − 3.5). HOMA-IR was calculated using the formula HOMAI R = (fasting insulin ∗ fasting glucose)/22.5. For both HOMA-IR and HOMA-B, glucose was measured in mmol/l and insulin was measured in lU/ml.

2.6. Statistical analysis

Data was entered and analyzed using SPSS version 21 (SPSS Inc., Chicago, IL). Results were presented as mean ± SD for normal variables and median (1st–3rd quartile) for non-normal variables. Differences between groups (Non-GDM and GDM) were tested using Student *t* test for normal variables and Mann–Whitney U-test for non-normal variables. Analysis of covariance (ANCOVA) was used to adjust for age and BMI. Differences across three visits were tested using repeated measures ANOVA for normal variables and Friedman test for non-normal variables. Spearman rank correlation coefficient (R) was used to test correlation between continuous variables. A scatter graph using the linear model was used to display the correlation. Stepwise regression analysis was used to identify the significant predictors for SPX. The results obtained from stepwise regression analysis achieved the power of more than 90% with the effect size of 0.53 at 95% confidence interval. Significance was set at $p < .05$.

3. Results

Baseline characteristics of all participants according to the presence of GDM is shown in Table 1, the mean ages of GDM and non-GDM participants were (29.1 ± 4.8 and 29.9 ± 5.8) and the mean gestational ages were (26.4 ± 3.0 and 26.1 ± 3.6) respectively. As expected, participants under the GDM group had a significantly higher glucose level than the non-GDM group (p = .02). The non-GDM group on the other hand had a significantly higher adiponectin (p < .001) and resistin levels (p = .001) than the GDM group. Anthropometrics, other glycemic parameters such as HbA1c, insu-

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