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Association of Vaspin levels and its SNP rs2236242 with Gestational Diabetes at a tertiary care setting
Karishma Kanhya Lal,1 Rabail Jarwar,2 Sabah Farhat,3 Seyda Sadia Fatima4

Abstract
Objectives: To evaluate and correlate vaspin levels and genotype frequency in gestational diabetes mellitus.

Methods: The case-control study was conducted at Aga Khan University, Karachi, from November 2015 to December 2016, and comprised pregnant women in their second trimester with gestational diabetes mellitus. Healthy pregnant women with similar characteristics were enrolled as the control group. Tetra arms amplification system for vaspin gene was performed. SPSS 21 was used for data analysis.

Results: Of the 112 pregnant women, 67(60%) were normo-glycaemic and 45(40%) had gestational diabetes. Those with gestational diabetes had a higher body mass index (p=0.047) and fasting blood glucose levels (p<0.01). Serum vaspin concentrations were significantly lower in the healthy group compared to the diabetics (p=0.041). Genotype and allele frequencies followed Hardy Weinberg’s Equilibrium but no significant differences were observed in genotype distribution between the groups (p>0.05).

Conclusion: Higher serum vaspin levels were seen in gestational diabetic females, but genotype and allele frequencies showed no association of vaspin with gestational diabetes mellitus.

Keywords: Vaspin, Gestational diabetes, Diabetes, Pregnancy. (JPMA 68: 1734; 2018)

Introduction
Gestational diabetes mellitus (GDM) is characterised by state of insulin resistance (IR) and a marked increase in serum insulin levels.1 GDM usually has its onset in late pregnancy and can have potential adverse effects on a growing foetus, such as macrosomia, preterm delivery low blood glucose levels at birth along with type 2 diabetes mellitus (T2DM) post-term and metabolic disorders.2,3 In Pakistan the prevalence of GDM is 3-8%4 but recently our group reported frequency of 17% in women visiting tertiary care hospitals.5 There have been several hypotheses as to the various contributors to the pathogenesis of GDM in pregnant females. One of these is the presence of excess adipose tissue. Adipose tissue serves to store energy in the presence of excess calories or certain hormones such as insulin. In addition to this basic function, lately adipose tissue was labelled an endocrine organ releasing hormones known as adipokine in the blood stream.6,7 There are more than 200 adipokine, or secretary proteins, released from adipose tissue. These not only help in regulation of the adipose tissue itself but also modulate various body functions such as regulation of appetite and satiety, insulin secretion and sensitivity, endothelial function and inflammation.7 They also affect functions of brain, liver, muscle, vasculature, heart and pancreas.6,9 Therefore, alterations in adipokine secretion may link obesity to inflammation and development of GDM or DM.10 Recent studies have shown GDM to be related with many cytokines and adipokines such as vaspin, C-reactive protein (CRP), tumour necrosis factor-alpha TNF-alpha, adiponectin and visfatin.3,11 Vaspin is one such adipokine that has been implicated in several studies as a possible biomarker for GDM in pregnant females, and its role has been linked to having insulin sensitising effects.12 Vaspin, also known as serpin A12, is a 415 amino acids long hormone derived from adipose tissues. It is a member

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of serine protease inhibitor family.\textsuperscript{13} It has also been reported that vaspin is found in the hypothalamus and its levels can be detected in cerebrospinal fluid (CSF) in all healthy people.\textsuperscript{14} Besides, vaspin also has a protective effect against inflammation, obesity-related skin desquamation and osteoporosis.\textsuperscript{15} Animal studies T2DM led to the discovery of vaspin in visceral adipose tissue and was directly related to obesity and IR.\textsuperscript{12} Vaspin levels increase with obesity, triglyceride (TG) levels, fasting insulin levels and IR.\textsuperscript{12} The correlation between how high or low vaspin levels are in relation to glucose and insulin levels could have potential future therapeutic and management roles. However, several studies have come to very different conclusions regarding the importance and implications of vaspin as a reliable serum biomarker in development of GDM. In an early study of circulating vaspin levels in GDM and its relation with preeclampsia, it was found that serum vaspin levels were not seemingly correlated with GDM.\textsuperscript{14} Another similar comparative study found that pregnant women with GDM regardless of controlling their blood sugars through insulin or diet alone, had higher weight, body mass index (BMI), TG as well as serum vaspin levels.\textsuperscript{3} Vaspin was found to be positively correlated with insulin and negatively correlated with an insulin sensitivity index.\textsuperscript{3} However, these results were only seen with patients of GDM at the third trimester and not in the control groups. Both categories of women with GDM, either using insulin therapy or diet alone, had similar results and thus vaspin was also concluded not to be affected by exogenous insulin administration.\textsuperscript{3} Nevertheless, these results have since been refuted. For example, a study implied that lower vaspin levels could be indicative of GDM along with lipid and carbohydrate metabolism as well as having negative effects on the various developmental stages of a foetus.\textsuperscript{11} A recent study also reported that vaspin was correlated with IR in these women along with leptin and TGs.\textsuperscript{16} This study found a completely different link in that vaspin levels seemed to be higher in those females with GDM when compared to their non-pregnant and non-GDM counterparts.\textsuperscript{16} Until recently, a specific gene of interest, vaspin has been associated with obesity, diabetes and polycystic ovarian syndrome (PCOS),\textsuperscript{17,18} but no association with GDM has been reported. The vaspin gene is located on chromosome 14q32.13 and consists of 6 exons and 5 introns. Recently, the A allele of vaspin rs2236242 polymorphism has been identified to play a protective role against obesity and diabetes and it was reported that individuals with A allele had lower vaspin levels.\textsuperscript{18} Yet another study reported that no difference was found in serum vaspin levels or genotype in metabolic syndrome.\textsuperscript{19} The current study was planned to evaluate and correlate vaspin levels and the genotype frequency in GDM.

**Subjects and Methods**

The case-control study was conducted at Aga Khan University, Karachi, from November 2015 to December 2016, and comprised pregnant women in their second trimester with GDM. Healthy pregnant women with similar characteristics were enrolled as the control group. The sample size was calculated using the Open-Epi website,\textsuperscript{20} with 1:1 distribution of cases and controls, where confidence level of 95\%, power of 80\%, least extreme odds ratio (OR) of 2 and a GDM prevalence of 17\% was taken according to previously published data sources.\textsuperscript{4,5} The minimum sample size calculated was 89. A universal screening using 75g of oral glucose tolerance test (OGTT) was given to the subjects to classify them as GDM females versus normo-glycaemic pregnant females. Any individual with more than 92mg/dl fasting blood glucose (FBG) and/or more than 153mg/dl blood glucose post-prandial was identified as a GDM. Women with twin pregnancies, with a previous history of gestational diabetes, PCOS, hypertension, hepatic and/or renal impairment, diabetes or on hormonal supplements and/or anti-inflammatory drugs were excluded from this study. Any patient who fulfilled the criteria of high risk for GDM i.e. having 2 or more of the following factors; age >25 year, T2DM family history, obesity, previous GDM history, previous adverse pregnancy outcome, and glycosuria, were screened by performing glycosylated haemoglobin (HbA1c) or FBG levels and diagnosed accordingly. Approval was obtained from the institutional ethics review committee and all participants furnished written informed consent. Anthropometric data of each patient was recorded. Also, 10 ml blood samples were taken from each individual and the extracted serum was stored at -80\°C. Sandwich enzyme-linked immunosorbent assay (ELISA) kits were used to measure serum vaspin levels (Kit Cat# 94789 Glory Science). Deoxyribonucleic acid (DNA) was

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**Table-1:** Primer Set used for amplification of Vaspin SNP.

<table>
<thead>
<tr>
<th>Primers</th>
<th>Inner</th>
<th>Outer</th>
</tr>
</thead>
<tbody>
<tr>
<td>Forward</td>
<td>AAGACGCCCTCTCTGCTGACT</td>
<td>GGAAGCAAGACAGAGCACTAGAAA</td>
</tr>
<tr>
<td>Reverse</td>
<td>CACAGGACCCAGGAAACTGGT</td>
<td>CAGTCTCTGGCCTCAAGGCTC</td>
</tr>
</tbody>
</table>
extracted from whole blood samples using QiaGen Genomic DNA extraction kits (Cat. Number 51185, Valencia, USA). Tetra arms amplification system for the vaspin gene rs223642 was implemented using a set of primers (Table-1). Polymerase chain reaction (PCR) was performed using Ruby Taq PCR Master mix 2X (Cat# 71191, Affymetrix, USA) as per the manufacturer’s instructions. The PCR products were visualized on 2.5% Agarose gel (Figure-2). Genotypes were scored by an independent person who was blinded about the case-control status of the study subjects, and each run had five negative controls. Statistical analyses were done using SPSS 21. Descriptive analysis of continuous variables was expressed as mean ± standard deviation (SD). T-test was applied to compare groups. Hardy-Weinberg equilibrium (HWE) was calculated for single nucleotide polymorphism (SNP) data which was analysed for genotype and allele frequency determination by applying chi-squared statistics.

Results
Of the 112 pregnant women, 67(60%) were normo-glycaemic and 45(40%) had GDM. Those with GDM had higher BMI (p=0.047) and FBG levels (p<0.01) (Table-2). Serum Vaspin concentrations were significantly lower in the control group (0.711±0.24 ng/mL) than in the gestational diabetes mellitus (GDM) group (1.68±0.57 ng/mL) p=0.041.

Table-2: Biophysical and Biochemical Characteristics of Study subjects.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Normo-glycemic Gestational Diabetic</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Pregnant Females Females</td>
<td></td>
</tr>
<tr>
<td></td>
<td>(n=67) (n=45)</td>
<td></td>
</tr>
<tr>
<td>Age (year)</td>
<td>25.69 ± 4.54</td>
<td>27.27 ± 5.18</td>
</tr>
<tr>
<td>Body mass index (BMI) at booking (kg/m²)</td>
<td>23.63 ± 4.29</td>
<td>25.80 ± 4.39</td>
</tr>
<tr>
<td>Fasting blood glucose (mg/dl)</td>
<td>87.30 ± 18.06</td>
<td>103.93 ± 11.70</td>
</tr>
<tr>
<td>Random blood glucose (mg/dl)</td>
<td>126.76 ± 65.04</td>
<td>138.43 ± 40.40</td>
</tr>
</tbody>
</table>

Table-3: Genotype and Allele Frequency Distribution of the study subjects.

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Normo-glycemic Gestational Diabetic</th>
<th>Odds Ratio (OR) (95% Confidence Interval [CI])</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Pregnant Females Females</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>(n=67) (n=45)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>AA</td>
<td>8 (11.94)</td>
<td>7 (15.55)</td>
<td>Reference</td>
</tr>
<tr>
<td>AT</td>
<td>29 (43.28)</td>
<td>22 (48.88)</td>
<td>0.867 (0.273-0.754)</td>
</tr>
<tr>
<td>TT</td>
<td>30 (44.77)</td>
<td>16 (35.55)</td>
<td>0.768 (0.247-2.196)</td>
</tr>
<tr>
<td>Allele Frequency</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A</td>
<td>40.0%</td>
<td>33.58%</td>
<td>0.758 (0.436-1.319)</td>
</tr>
<tr>
<td>T</td>
<td>60.0 %</td>
<td>66.42%</td>
<td></td>
</tr>
</tbody>
</table>

Discussion
Vaspin is known to play many roles inside the human body. It has a protective role against inflammation, and skin desquamation related to obesity and osteoporosis. The current study compared levels of vaspin in normal pregnant and GDM females. It found that serum vaspin
Concentrations were significantly higher in the GDM group than in the control group. However, the occurrence of GDM was not significantly associated with elevated vaspin levels \( (p=0.041) \). A study also found that vaspin levels were higher in GDM patients compared to normal pregnant females.\(^1\) Vaspin was found to inhibit a protease responsible for degradation of hormones, indirectly bringing down the glucose levels.\(^{16}\) A significant rise in vaspin levels during pregnancy was also reported which declined within 3 days after delivery in the GDM group, suggesting the source to be placenta.\(^11\) One meta-analysis of 6 studies concluded that vaspin levels were higher in people who were obese and had T2DM.\(^{14}\) Previously, a few studies have reported that there was no significant difference in vaspin levels between normal pregnant females and females with GDM.\(^3,^{15}\) Yet, a study found that lower levels of vaspin were a risk factor for the development of T2DM.\(^{13}\)

The current study did not find any significant difference between the two groups regarding genotype distribution as well as allele frequencies \( (p>0.05) \), suggesting that this may not be related to GDM. On the contrary, very few studies have reported increased risk of developing metabolic syndrome in cases where the AA genotype was predominant.\(^{21,22}\) Further, studies have shown an association of the vaspin gene with coronary artery disease in Chinese population,\(^{23}\) and obesity.\(^{18}\) Therefore, it is difficult to label the lack or the presence of association to be a pathophysiological link. Moreover, an updated guideline was recently introduced by the International Diabetes Federation (IDF) with a revised criteria for GDM diagnosis,\(^{24}\) and very recently a comprehensive guideline was published by the South Asian Federation of Endocrine Societies (SAFES) that specifically caters to the South Asian population.\(^{24}\) These should be extensively used in future studies and in clinics to diagnose GDM. The current study reported higher BMI and FBG levels in GDM females \( (p<0.05) \), though weak association was seen with the vaspin gene and serum levels.

Pregnancy is associated with excess release of many adipokines and cytokines that contribute to IR. These changes in vaspin levels may be observed due to high content of adipose tissue in the GDM-positive females, as seen by the BMI values, leading to excess release of vaspin. A new term, ‘gluco-phenotype’, is now commonly used to classify certain clinical and biochemical aspects of subjects suffering or prone to developing GDM or DM.

Gluco-phenotype is defined as "predominant insulin deficiency or insulin resistance; non-obese or obese; predominant fasting, prandial or combined hyperglycaemia; with or without any predisposing factors."\(^{25}\) These characteristic similarities in blood glucose level and aetiologies of both GDM and DM and the link to adipokine levels may pave a pathway in future to develop therapeutic strategies that may cater to individual needs. Further studies are required to find out the protective role of vaspin during pregnancy.

Conclusion
Higher serum vaspin levels were seen in GDM females. However, no association between SNP rs2236242 and GDM was seen. Further investigations on larger scale are required to analyse the physiological role of vaspin in the context of GDM.

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Conflict of Interest: None.
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References