

## AHSG rs4918 Polymorphism poses a weak predisposition to insulin resistance during pregnancy

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### Abstract

**Objective:** To identify the association between Fetuin-A levels and genetic polymorphism with gestational diabetes mellitus among pregnant women visiting a tertiary care centre.

**Methods:** This cross-sectional case-control study was conducted at Aga Khan University Hospital, Karachi, from December 2015 to September 2016, and comprised pregnant women in their second trimester. Those with gestational diabetes mellitus were considered the cases while the rest acted as controls. The enzyme-linked immunosorbent assay was used to quantify Fetuin-A levels while genotyping for alpha-2-Heremans-Schmid glycoprotein rs4918 was performed using restriction fragment length polymorphism technique. Blood samples were collected and serum and deoxyribonucleic acid were extracted and stored at -80°C. SPSS 21 was used to analyse the findings.

**Results:** Of the 88 subjects, there were 44(50%) in each group. Serum Fetuin-A concentration was higher in cases compared to the controls ( $p < 0.01$ ). The genotype data for the cases was 0.668 and for the controls 0.840 ( $p > 0.05$ ). However, the G allele showed a weak risk or predisposition towards gestational diabetes mellitus ( $p = 0.038$ ).

**Conclusion:** Increased Fetuin-A levels were found to be related to the occurrence of gestational diabetes mellitus, indicating that Fetuin-A possibly contributes towards insulin resistance.

**Keywords:** AHSG polymorphism, Fetuin-A, Gestational diabetes mellitus. (JPMA 68: 698; 2018)

### Introduction

Fetuin-A was first identified in foetal bovine serum and is known as either alpha-2-Heremans-Schmid glycoprotein (AHSG). It works as a key component in numerous physiological and pathological processes.<sup>1</sup> It is known to act as a potent inhibitor of calcification, controls protease activity, keratinocytes migration and initiates breast tumour cell proliferative signalling.<sup>2</sup> Studies suggest that low serum levels lead to multiple complications including cerebrovascular events.<sup>1</sup> Despite a positive correlation between serum Fetuin-A levels and calcium deposition; further research has failed to establish a correlation of Fetuin-A in subjects with a low cerebral vascular risk.<sup>3</sup> Serum Fetuin-A levels also influence the body's defence mechanisms such as inflammatory regulation for macrophage deactivation. Moreover, Fetuin-A can potentially serve as a diagnostic biomarker for metabolic syndromes, liver diseases and cardiovascular diseases.<sup>4</sup>

This 57 kD protein also plays a significant role during normal pregnancy. Higher levels are found in pregnancy,<sup>5</sup> however in neonates with intrauterine growth restriction (IUGR), defective expression and glycosylation of Fetuin-A was

observed, which may be responsible for poor foetal growth.<sup>6</sup> This theory is further supported by the finding that Fetuin-A is a key constituent of the non-collagenous bone matrix, particularly in the foetal age.<sup>6</sup> Correlation of Fetuin-A levels with successful pregnancies in women undergoing assisted pregnancy has also been deduced,<sup>5</sup> indicating Fetuin-A's possible role in normal gametogenesis and fertilisation.

Fetuin-A is encoded by the AHSG gene located at the chromosome 3q27, composed of seven exons and spanning 8.2 kb of deoxyribonucleic acid (DNA).<sup>7</sup> The gene has multiple variants but the specific allele of interest for this study is rs4918. This allele is found at position 256 of Exon 7. The most common polymorphism recorded for this area is the C (Thr)→G (Ser) mutation. The allele is said to be associated with maintaining good body mass index (BMI), body fat and controlling insulin sensitivity. Furthermore, it shows a protective effect against diet-induced obesity,<sup>8</sup> diabetes and metabolic syndrome,<sup>7</sup> and is therefore called the 'lean gene'. Healthy individuals with the G allele have been found to have lower inflammatory cytokines such as the tumour necrosis factor alpha (TNF $\alpha$ ) and adiponectin levels while higher levels of leptin are found.<sup>9</sup> Even in patients with a history of myocardial infarction (MI), the presence of rs4918 G allele renders better anthropometric parameters such as BMI and waist circumference.<sup>9</sup> On the contrary, the allele also predisposes carriers like those with a single G allele to

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certain conditions including insulin resistance (IR). Fetuin-A binds to receptors for insulin in liver and muscles, which function through tyrosine kinase,<sup>10,11</sup> thus contributes to (IR) (Figure). Moreover, it has been linked to causing accumulation of fat in liver cells,<sup>12</sup> which is a predisposing factor for diabetes.

Gestational diabetes mellitus (GDM), described as the diabetes that occurs only during the state of pregnancy, is an illness of increasing prevalence in Pakistan.<sup>13,14</sup> It has been found to be associated with stillbirth and abnormal birth weight, which places new born babies with a higher birth weight at risk of cardiovascular disease.<sup>15</sup> Research has demonstrated that increased Fetuin-A is related to increased GDM risk.<sup>10,16,17</sup> One such study included anthropometric measurements of newborn to relate complications of GDM with increased Fetuin-A and TNF $\alpha$ , among other factors, but their effect as potential confounders was not ruled out.<sup>16</sup> The current study was planned to assess the association of Fetuin-A levels with GDM, and to see whether AHSG rs4918 G allele acts as a risk allele for GDM.

### Subjects and Method

This cross-sectional case-control study was conducted at Aga Khan University Hospital, Karachi, from December 2015 to September 2016, and comprised of pregnant women in their second trimester. Those with gestational diabetes mellitus were considered the cases while the rest acted were labeled as controls. After getting approval from the institutional ethics review committee, the subjects were randomly enrolled from among those who gave informed written consent.

The subjects were given 75g oral glucose (OGTT) solution at 28th week of gestation and were categorised into two equal groups of normoglycemic pregnancies and patients with GDM in accordance with the criteria of the International Association of the Diabetes and Pregnancy Study Groups (IADPSG).<sup>18</sup> Any individual with more than 92mg/dl fasting blood glucose and/or more than 153mg/dl blood glucose post-prandial was identified as a diabetic. Females expecting more than one child, with a past history of chronic illnesses including pre-gestational diabetes, polycystic ovary syndrome (PCOS), hypertension, hepatic and/or renal impairment, on hormonal supplements and/or anti-inflammatory drugs were excluded from this study.

The sample size was calculated by OpenEpi software. Assuming a confidence level of 95%, power of 80%, least extreme odds ratio (OR) of 2 and a GDM prevalence of 17% as per our recent report,<sup>19</sup> the minimum sample size of n=89 was required to have a meaningful result of this study

( $n = [DEFF * Np(1-p)] / [(d^2 / Z^{2-1-\alpha} / 2 * (N-1) + p * (1-p)]$ ) [Where: N= Population size; p = Hypothesized % frequency of outcome factor in the population; d= Confidence limits; DEFF= Design effect (for cluster surveys-); n=Sample Size(n)].

The anthropometric, demographic and personal data of each patient was recorded. After that, 10 ml blood samples were taken from each individual and extracted serum was stored at -80°C. DNA was extracted from whole blood samples stored in ethylenediaminetetraacetic acid (EDTA) tubes using Qiagen Genomic DNA extraction kits (Cat. Number 51185, Valencia, USA). Sandwich enzyme-linked immunosorbent assay (ELISA) kits were used to measure serum Fetuin-A levels (Kit Cat# 95469 Glory science). Primers used for the polymerase chain reaction (PCR) for AHSG rs4918 were designed using the primer 3 software (Forward 5'-GTA AGG CAA CAC TCA GTG A-3'; Reverse 5'-TCA TCT CTG CCA TGT CTA G-3'). PCR was performed using the Ruby Taq PCR Master mix 2X (cat# 71191, Affymetrix, USA) as per the manufacturer's instructions. The amplified product of 1,080 bp was digested with restriction endonuclease SacI (Promega, USA) for 3 hours at 37°C to yield digested products (725 and 355 bp). The digested fragments were electrophoresed in 2% agarose gel. 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 conducted using SPSS 21. Descriptive analysis of continuous variables was expressed as mean  $\pm$  standard deviation. Student t-test was applied to compare groups. Hardy-Weinberg equilibrium (HWE) was calculated for single nucleotide polymorphisms (SNP) data. SNP data was analysed for genotype and allele frequency determination by applying chi-squared statistics. Genotypes were allotted code of 0/1/2 rendering the number of minor alleles under a dominant model of inheritance.

### Results

A total of 88 pregnant women were divided into two

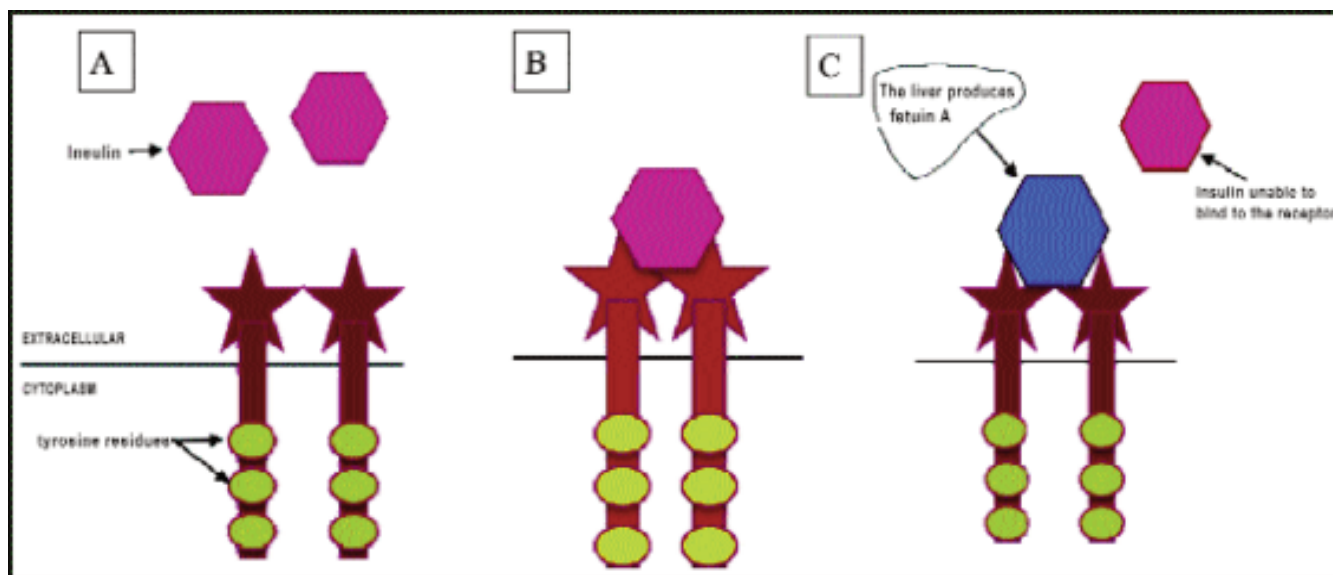
**Table-1:** General Characteristics of the study subjects.

Variable	Gestational Diabetic	Normo-glycemic	P value
	Females (n=44) Mean $\pm$ SD	Pregnant Females (n=44) Mean $\pm$ SD	
Age (year)	27.27 $\pm$ 5.18	25.44 $\pm$ 4.64	0.053
Weight at booking (kg)	64.90 $\pm$ 11.98	59.26 $\pm$ 11.61	0.032
BMI (kg/m <sup>2</sup> )	25.80 $\pm$ 5.63	23.80 $\pm$ 4.65	0.069
OGTT-0 hour (mg/dl)	103.93 $\pm$ 11.70	80.28 $\pm$ 8.33	<0.001
OGTT-2 hour (mg/dl)	176.20 $\pm$ 5.53	119.53 $\pm$ 15.90	<0.001
Fetuin-A (ng/mL)	1856.53 $\pm$ 250.52	1286 $\pm$ 95.44	0.01

BMI: Body mass index.  
OGTT: Oral glucose tolerance test.

**Table-2:** Genotype and Allele Frequency Distribution of the study subjects.

Genotype	Gestational Diabetic Females (n=44)	Normo-glycemic Pregnant Females (n=44)	OR (95% C.I)	P value
CC	18 (40.90)	25 (56.81)	Reference	p= 0.076
CG	21 (47.72)	16 (36.36)	1.823 (0.749-4.434)	
GG	8 (18.18)	3 (6.81)	2.120 (0.917-4.899)	
	OR (95% C.I)	Allele Frequency	P value	
C	57 (60.64%)	66 (75%)	1.947 (1.031-3.677)	0.0385
G	37 (39.36%)	22 (25%)		

**Figure:** Schematic diagram showing insulin and its inactivated receptor (A), insulin binding to its receptor, which works through tyrosine kinase to activate it (B) and Fetuin-A binding to insulin's receptor, which blocks binding of insulin to its receptors (C), contributing to insulin resistance.

equal groups of 44(50%) cases and controls. There was no difference between the groups in terms of age and BMI ( $p>0.05$ ). Serum Fetuin-A concentration was higher in cases than controls ( $p<0.01$ ) (Table-1). Genotype distribution showed no significant difference between the groups ( $p>0.05$ ). However, the G allele showed a weak risk or predisposition towards GDM ( $p=0.0385$ ) (Table-2).

## Discussion

Our study showed that GDM was correlated with higher Fetuin-A levels ( $p= 0.01$ ) in line with literature where several studies have tried to understand the relation of Fetuin-A with IR in GDM. Three such studies found high levels of Fetuin-A in participants with GDM.<sup>16,17,20</sup> whereas one recent study introduced Fetuin-A and B as potential risks for developing GDM.<sup>21</sup> However, one study found that levels of Fetuin-A did not relate with the development of GDM.<sup>10</sup> The different results could be accounted for by the very limited sample size of 20

participants. Yet, we report a positive relation between GDM and higher circulatory Fetuin-A levels. Furthermore, we did not find any significant difference in BMI between the case and control groups, perhaps due to the well-controlled diet plan for these patients prevented the body weight fluctuation. The study results suggest that increased Fetuin-A levels have a strong relation with GDM. This finding strengthens the fact that Fetuin-A may act as an important mediator for development of IR in pregnancy. A possible mechanism of how Fetuin-A acts as an inducer of IR could be by binding to tyrosine kinase receptors and subsequently impeding downstream signalling pathways.<sup>22</sup>

The AHSR gene polymorphism rs4918 has two variants; C and G allele.<sup>23</sup> It has been demonstrated that the C allele correlates with a higher degree of allele expression, as reflected by higher serum Fetuin-A levels in homozygotes with rs4918 C allele.<sup>23</sup> When we assessed the allele

frequencies the G allele was found to be more common in GDM females compared to normoglycaemic pregnant females. This finding implies that the presence of the G allele, whether in a heterozygous or homozygous state, is associated with a higher incidence of GDM. Out of 44 cases, 29 had at least one G allele compared to just 19 out of 44 control participants. Moreover, it has been shown that AHSg is a regulator of body mass as supported by a linear relationship between the G allele and BMI. Contrary to this an earlier study established that the G allele was more common in lean men.<sup>8</sup>

Our study is limited in the fact that we could not perform sequencing of the PCR products and we were unable to recruit more subjects. Yet, this study adds a weak positive link of AHSg and GDM in the existing pool of researches.

## Conclusion

Fetuin-A levels were found to be increased in those with GDM. We found that parameters of IR such as increased responses to OGTT were increased in individuals with G allele of AHSg rs4918 suggesting a link with IR.

**Disclaimer:** None.

**Conflict of Interest:** None.

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