Comparison of Retinol Binding Protein 4 (RBP 4) Levels in Pregnant Females with and without Gestational Diabetes Mellitus (GDM)

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Abstract

Objective: To compare the levels of RBP4 in pregnant females with and without GDM.

Materials and Methods: It was a cross sectional study carried out in Pathology Department KEMU/Mayo Hospital and Lady Aitchison Hospital, Lahore after approval by Institutional Review Board. After informed consent, total 64 pregnant females at 24-28 weeks of gestation undergoing OGTT; 32 in group A with GDM and 32 in group B without GDM were enrolled. Under aseptic conditions, 3ml blood was drawn for RBP-4 to be measured by ELISA.

Results: The means ± SD age of females was 25.5±4.3 and 24.4±4.4 years in group A and B respectively. The age, gestational age, parity, previous history of GDM was not significantly different in 2 groups. The median (IQR) values of RBP4 were 37.3 and 33.2ng/dl in group A and B respectively and were significantly different (p =0.021).

Conclusion: Serum RBP4 concentrations are high in females with GDM as compared to those without GDM.

Keywords: RBP4, GDM

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Introduction

Gestational Diabetes Mellitus (GDM) is defined as any degree of glucose intolerance that is first recognized with the onset of pregnancy.[1] This diagnosis does not apply to pregnant women with previously diagnosed diabetes or overt diabetes.[2] The prevalence of GDM is 1% to 28% with higher ratios in Asian women. A high frequency of GDM (11.8%) has been reported in Pakistan.[1,3] A large number of fetal and maternal complications are caused by GDM. Some of the fetal complications are preterm birth, respiratory distress syndrome, excessive birth weight and hypoglycemia whereas the maternal complications are high blood pressure, pre-eclampsia and risk of diabetes in future.[4] The underlying pathophysiology is failure of pancreas to up regulate insulin secretion relative to insulin resistance created by changes in hyperglycemic hormones (like corticotrophin releasing hormone, growth hormone, placental lactogen and progesterone) during pregnancy.[6] An efficient diagnosis and accurate monitoring of diabetic mothers are important to decrease the risk of diabetic complications. There is disagreement between obstetric, medical and endocrine groups about the effective methods of diagnosis of GDM.[6] The oral glucose tolerance test (OGTT) is currently the recommended method and it is performed worldwide for diagnosis of GDM. But the adequate glucose load amount and cut-off values of OGTT are still controversial.[7,8] HbA1c is also used to diagnose GDM but it has lower diagnostic performance in pregnant women due to anemia and

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biphasic changes in its values.[9,10] Many adipokines for example visfetin, chimerin, adiponectin , leptin, tumor necrosis factor-alpha (TNF-alpha) and Retinol binding protein4 (RBP-4) have been involved in causing insulin resistance and their concentrations in plasma have been investigated for the diagnosis of GDM.[11,12] The cross talk between different adipokines and insulin targeted tissues such as skeletal muscles and the liver plays a significant role in pathophysiology of GDM.[13] RBP-4 is mainly synthesized in hepatocytes and adipose tissues. It is responsible for causing insulin resistance by different pathways; it upregulates gluconeogenesis by inducing the expression on liver's gluconeogenic enzyme phosphoenol pyruvate carboxikinase (PEPCK) and it disrupts glucose transport in muscles and adipose tissues. RBP-4 decreases expressions of glucose transporter-4(GLUT-4) in striated muscles and adipose tissues.[14] In this background, this study is planned to compare the levels of RBP4 in pregnant females with and without GDM.

Materials and Methods

It was a cross sectional study carried out in Pathology Department KEMU/Mayo Hospital and Lady Aitchison Hospital, Lahore after approval by Institutional Review Board. Total 64 pregnant females at 24-28 weeks of gestation undergoing OGTT were included; 32 in group A with GDM and 32 in group B without GDM using non-probability convenient sampling. The pregnant females with history of DM before pregnancy, renal dysfunction, hypertension, hepatic dysfunction were excluded. The relevant information of each patient was recorded in study proforma after informed consent. Under aseptic conditions, 2-3ml venous samples for fasting, one and two hours after 75g glucose were collected from each patient in yellow top vacutainer labeled with Patient's name and ID for the analysis of glucose and RBP4.Serum was separated from samples after clotting through centrifugation at 3000rpm. After ensuring the quality control, the glucose estimation was performed on Beckman Coulter- AU 680 chemistry auto analyzer by Hexokinase method. The patients were labeled as GDM and Non GDM on the basis of OGTT results. The remaining serum was stored in Eppendorf cups labeled with patient's ID at -80°C for RBP-4 analysis. ELISA was performed on samples for RBP-4 using kit by Bioassay Technology Laboratory on Diatrone 710 ELISA plate reader in 2 batches. The data analysis was performed by using SPSS-26). Quantitative variables with normal distribution were presented as mean±SD and skewed data as median (IQR). Qualitative variables were presented as frequency and percentage.

Results

The mean ±SD fasting, 1 and 2 hour blood glucose levels after OGTT were 110±12, 186±45, 149± 35 and 82±7, 124±26, 105±22 mg/dL in group A and group B respectively. Independent sample t test was used to compare these levels between 2 groups that showed significant difference (p value< 0.01). The median (IQR) RBP-4 levels were 37.3 (11) and 33.2 (20) ng/dl in females with and without GDM respectively. Mann Whitney U test was applied to compare RBP-4 levels between two groups that showed significant difference (p value= 0.021).

Table 1: *Demographic and Clinical Characteristics of Study subjects:*

Variables	Group A (GDM) n=32	Group B (Non GDM) n=32	p-value
Age (mean±SD) (years)	25.5 ± 4.3	24.4± 4.4	0.303
Gestational Age (weeks)	26.3 ± 1.8	25.9 ± 1.6	0.421
Parity (PG: MG)	9(28%): 23(72%)	11(34%): 21(66%)	0.590
History of GDM	3(9%)	2(6%)	0.641
Family history of DM	19(59%)	9(28%)	***0.012**

- Independent sample t-test used for age & gestational age.
- Chi Square test used for parity, history of GDM, family history of DM.
- p-Value of < 0.05 was taken as statistically significant.

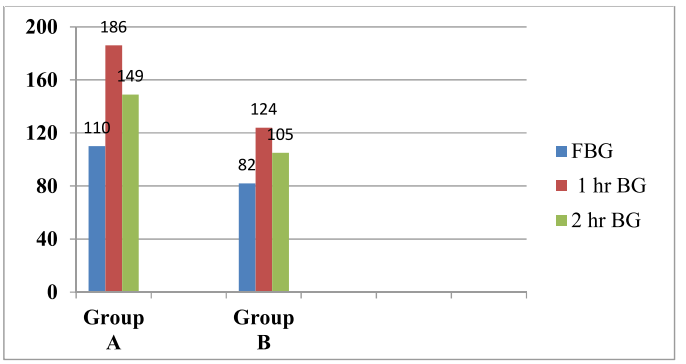

Figure 1: *Comparison of Blood glucose levels in Group A and Group B.*

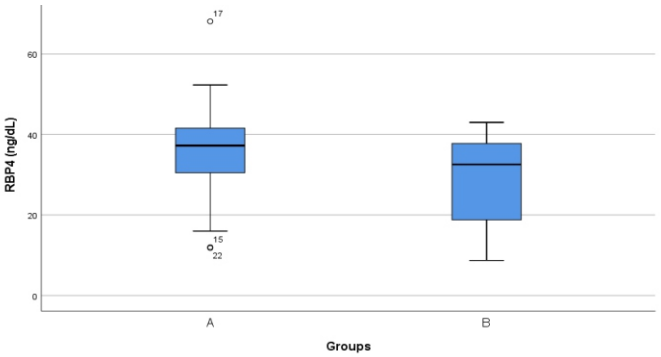

Figure-2: *Comparison of Retinol Binding Protein-4 between Group A and B.*

Discussion

The worldwide prevalence of GDM varies from 1% to 28% and it is 11% in Pakistan.[1,3] Obesity, fatty diet, micronutrients deficiency, advanced maternal age, previous GDM history and history of diabetes in family are some of the risk factors for GDM.[5] The timely diagnosis of GDM is important to reduce maternal and fetal complications The approach to screen GDM varies worldwide.[4] OGTT is used to screen GDM between 24 and 28 gestational weeks in pregnant females. But it has its own limitations like a time-consuming test, needs fasting for at least 8 hours and may result in nausea, vomiting, and headache especially in pregnant women and some participants are unable to complete the test.[2,7] HbA1C is another diagnostic tool but it has some limitations as it is affected by anemia in pregnancy.[9,10] The shortcomings of the existing screening and diagnostic tools raise the need of biomarker that is not affected by conditions mentioned above. Some new biomarkers have been studied for their role in the diagnosis of GDM including adipokines like visfetin, interleukin-6, Leptin, adiponectin , RBP4, Chimerin.[2] RBP4 is one of the adipokine that was investigated for its role in GDM in our study.[2,14] In our study The RBP4 levels were studied in 64 pregnant females; 32 with GDM (group A) and 32 without GDM (group B) diagnosed on the basis of OGTT. The age was comparable between group A and B (p value = 0.303). The findings of our study are in agreement with the study by Chuyao jin. et al and Xiyu Du et al.[14,15] But the results differ from the study of Maghbooli Z et al who showed the age was significant different between study groups (p value=0.001).[16] The frequency of multi parity was 72% for Group A and 66% for Group B and it was comparable to the study by Maghbooli Z et al.[16] The history of GDM was not significantly different between Group A & B (p value= 0.641). This is similar to study done by Fatima S.et al.[17] The findings of our study are not comparable to study done by Xiyu Du at al who showed that previous history of GDM was significant in women with GDM as compared to those without GDM[(14)]. The family history of DM was significantly high in GDM as compared to non GDM (p = 0.01). The findings of our study are in agreement with the study by Fatima S et al.[17] The Fasting blood glucose, blood glucose 1 & 2 hours after 75g glucose was significantly different between group A and B (p value <0.01) in our study. The findings are similar to study by Mengkal Du et al. , Beverly J Tepper et al.[18]

The median (IQR) RBP-4 levels were 37.3 and 33.2ng/dl in GDM and non GDM respectively (p value = 0.02). The results of our study are in accordance with the studies by Xiyu Du et al, Krzysztof C et al, Chiyao Jin et al, Maghbooli Z et al, Mengkai Du et al who showed that RBP4 levels were significantly different between GDM and Non GDM.[14,16,18] The results of our study are not in agreement with the studies of Weerapan K. et al , Asli Yarsi G et al and Khovidhunkit et al that showed no significant difference of RBP4 levels between GDM and Non GDM patients. The difference might be attributed to characteristics of study populations. The study of Weerapan K. et al was performed on Thai women and the difference of ethinicity might be one the reason for this difference.[19,20]

The Role of RBP4 in pathophysiology of GDM is a topic of research worldwide. The relationship of obesity, insulin resistance and DM is due to link between adipokines e.g RBP4 and insulin dependent tissues like liver and skeletal muscles.[13,14,19,20] The relationship of RBP4 levels with GDM was studied in a meta-analyses and it was seen that RBP-4 levels were remarkably high in females with GDM than Non GDM. However, this difference of RBP4 levels beween GDM and non GDM were present particularly in Asian ethnicity. In addition to diagnosis of GDM, RBP4 levels were also found to have a predictive role in GDM. Huag Q-T et al. found that females with GDM had higher values of RBP4 in first trimester than those without GDM.[2,21]

The cut off used to predict GDM varies in different studies. According to a study by Yuan et al. the cut off value of 30.45 µg/mL for RPB4 could diagnose GDM with a sensitivity of 63.6%, specificity of 75% and AUC 0.72 (95% CI 0.64–0.79). Whereas the study by Maghbooli Z et al showed that RBP4 levels equal to 42 µg/ mL could forecast the risk of developing GDM with the sensitivity 0f 75.8%, specificity 65.3%, and p value =

0.001.[16,22] The role of RBP4 in pathophysiology of GDM is also supported by study of Xia sun et al. They studied the effect of Sitagliptin an antagonist of the dipeptidyl peptidase-4 (DPP-4) (an adipokine oversecreted in insulin-resistant obese patients). on the parameters of insulin sensitivity in GDM patients. In addition to increasing insulin sensitivity, reducing fasting blood glucose and insulin levels it also caused marked reduction in RBP-4 levels after 16 weeks of treatment. RBP4 levels reduced from 59.4±16.7 to 42.1±20 (p value=0.023) in the group which was given sitagliptin as compared to the placebo group where it changed from 61.4±17.3 to 57.6±21.8.23

The diagnostic test for GDM in which pregnant females need no fasting and do not require glucose load would not only reduce the nausea and vomiting experienced by pregnant women but also increase compliance of pregnant females for screening of GDM. Moreover, the COVID-19 pandemic has greatly impact the hospital and clinical practices in order to reduce patient undue stay in hospitals and clinics. To perform OGTT in the background of pandemic fear was very challenging. This further emphasizes the importance of a biomarker for GDM that could be practiced in a GP clinic instead of a hospital with minimal stay of female just to get her blood sample drawn. A single blood test would lessen the duration of appointment, help to increase the number of females to be screened and would help to perform test in a non-hospital setting. RBP4 can be a potential biomarker to fulfil the purpose.

Conclusions

RBP4 levels are significantly high in females with GDM as compared to those without GDM. The study had certain limitations that it was performed in a single centre on small scale, the females with GDM were not followed till delivery and postpartum to determine RBP-4 levels and fetal outcome.

Conflict of Interest: **None**

Source of Funding: **None**

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Authors Contribution

RD: Conceptualization of Project
MS: Data Collection
KJ: Literature Search
MA: Statistical Analysis
NK: Drafting, Revision
AN: Writing of Manuscript