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Role of Hepatic and Renal Profile in the Development of Gestational Diabetes Mellitus

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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Original Research Article

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ABSTRACT

Disturbances in hepatic and renal profile are well documented in diabetic patients. Antenatal pregnant women (n=300) selected for blood sampling during the early 2nd trimester (14–18 weeks of gestation) including 176 pregnant women with positive family history of GDM and 124 women without any history of GDM. All the subjects were followed up to the early 3rd trimester (24-28 weeks of gestation) or until the onset of GDM for second sampling. Mean values of ALP, AST and GGT were significantly higher ($p<0.05$), however, mean albumin was found to be significantly lower in early 2nd trimester in those patients who subsequently developed GDM. Levels of ALT, total protein, and total bilirubin did not show significant variations in comparable groups. Uric acid levels were found to be significant in those patients who developed GDM in late trimester but creatinine levels remained almost the same in both trimesters. Among 300 patients, 58 subjects developed GDM, however, in these established cases, 27% were having positive family history while 9% presented negative family history suggesting a stronger relationship of GDM with clinical/family history of pregnant women. The results of our study indicate that abnormal liver and kidney profile

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namely AST, ALP, GGT, albumin and uric acid may adversely affect insulin resistance and if not controlled and treated timely may cause GDM and lead to type 2 diabetes. However, ALT, serum bilirubin, total protein and creatinine appear to have no value in GDM prediction.

Keywords: Gestational diabetes mellitus (GDM); blood serum; liver profile; kidney profile; gamma-glutamyl transferase (GGT).

1. INTRODUCTION

GDM is a state in which women show high blood glucose levels during pregnancy without previously diagnosed diabetes, particularly in their third trimester [1]. It is one of the most frequently occurring medical complication during pregnancy, and can negatively affect the health of both mother and child. Mothers with GDM have a risk of hypertensive disorders during pregnancy, recurrence of post-partum diabetes, and cardiovascular diseases [2]. Pregnancy has been associated with a diabetogenic environment, characterized by hyperinsulinemia and hyperglycemia [3]. After delivery, these variations are rescindable without any impediments in the full term. GDM is characterized by insulin resistance, impaired beta cell function, hyperlipidemia, glucose intolerance and endothelial dysfunction. Annually, GDM affects almost 2-5% of all the pregnancies [4-6].

Liver plays an important role in regulating the metabolism of carbohydrates, since it uses glucose as fuel and energy source, has the ability to reserve glucose as glycogen and also produces glucose from non-carbohydrate sources. The most frequently measured indicator of liver disease is raised serum enzyme activity of aminotransferases such as aspartate aminotransferase (AST) and alanine aminotransferase (ALT) and occurs mostly in diabetics than in healthy populations. Excessive hepatic glucose output in gestational diabetes pays to fasting hyperglycemia. Recent studies indicate that increased gluconeogenesis is a predominant mechanism for increased glucose output, but not involving glycogenolysis, in patients with GDM.

Insulin resistance, GDM and T2DM are associated with hepatocytic enzymes, namely alanine aminotransferase (ALT), gamma-glutamyl transferase (GGT) and aspartate aminotransferase (AST) [7]. Among them, ALT is mainly accumulated in the liver and works as a more specific and improved marker for deposition of liver fat and non-alcoholic liver disease (NAFLD). NAFLD has a high hepatic fat content that increases the risk of T2DM with hepatic insulin resistance. Because GDM and T2DM share many common risk factors and related pathogenesis, it is believed that high levels of ALT may also predict GDM, although this data is relatively scarce and inconsistent [8]. The occurrence of GDM and further incidence of T2DM is associated with increased concentrations of aspartate aminotransferase, alanine aminotransferase, and π-glutamyl transferase [9,10]. The increased π-GT level has been reported to be an independent risk factor for GDM and can detect women at high risk for GDM.

High blood sugar can induce serious health problems in gestational diabetes, particularly, heart disease and nerve and kidney damage [11,12]. Haemodilution and decreased liver functions may change the levels of albumin in GDM [13]. The glomerular filtration rate (GFR) increases by ~50 percent during pregnancy, thus changing the levels of creatinine and uric acid. The elevated levels of uric acid are metabolic syndrome component in GDM that reflects insulin resistance [14].

The objective of our prospective screening study is to examine the relation between various early pregnancy liver and kidney biomarkers, their role in the subsequent development of GDM and the application of results in the provision of useful information for the management of women with GDM.

2. MATERIALS AND METHODS

2.1 Study Participants and Study Site

Out of total 300 pregnant women, 176 with positive and 124 with negative familial background of GDM were selected from different hospitals of Lahore, Pakistan, at their first antenatal visit during early second trimester. Informed consent, clearly mentioning the purpose of the study, was signed by the patient or their attendants. All of the women were

screened for GDM both in early and late stages of their pregnancy.

During the early care visits of gestation, participants were questioned about parity, age, history of previous maternal diabetes, family history of type 2 diabetes mellitus, and habit of smoking. Body mass index (BMI) was determined by recording patient's weight in kilograms and height in meters. Systolic and diastolic blood pressure was recorded.

In this study, blood samples from clinically normal pregnant women with negative and positive family history and those with HbA1c less than 6.5% in 14-18 weeks of gestation were collected and analyzed for different biochemical parameters. All the subjects were followed until the development of GDM. Again blood samples were collected from the individuals in 24-28 weeks of gestation and analyzed. Pregnant women who developed GDM were analyzed to assess the biochemical markers involved in the pathogenesis and development of the disease. A total number of 300 pregnant women (18-40 years of age) were included in study. The subjects who developed GDM were termed as "GDM" group and those who did not develop GDM were treated as "control" group. Blood samples for both groups were collected at predefined intervals; at early second trimester (T2C & T2 GDM) and at early third trimester (T3C & T3GDM) for control group and for GDM group. These groups were further subdivided into two subgroups; positive history group and negative history group on the basis of their family history of GDM and analyzed for biochemical variations.

2.2 Inclusion Criteria

Inclusion criteria was based on positive or negative family history of GDM and T2DM, maternal age between 18 and 40, glycated hemoglobin (HBA1c) of less than 6.5% and without hypertension, renal and cardiac diseases and current medical treatments which could affect patients' hormonal concentration, lipid profile, liver and renal function tests.

2.3 Exclusion Criteria

Subjects with family history of T_2DM, multiple pregnancies, ectopic pregnancy, hypertensive disorders, history of smoking /alcohol abuse, assisted reproductive technology treatment, fetal congenital irregularities and any other confounding pathologies (hyper- or hypothyroidism), polycystic ovarian syndrome, glycated haemoglobin greater than 6.5%, renal or hepatic failure, uncontrolled endocrine or any other metabolic disorder that may influence glucose regulation were excluded. Fasting glycemia was determined and the women with fasting plasma glucose (FPG) >110 mg/dl were considered to possess undiagnosed pregestational diabetes mellitus.

2.4 Blood Sampling

Cases were followed-up until the development of GDM that was diagnosed if any of the glucose level was equal to or greater than 5.1 mmol/l (fasting), 10.0 mmol/l (1 h post-load), or 8.5 mmol/l (2 h post-load). Blood was collected by venipuncture from all of the subjects, at 14 to 18 week of pregnancy. After the determination of fasting glycemia and routine pathology testing, 5cc blood was drawn from all the subjects and serum was separated by centrifugation.

2.5 Sample Analysis

Samples were brought to room temperature before analyses. Alanine aminotransferase (ALT), Alkaline phosphatase (ALP), Aspartate aminotransferase (AST), Total bilirubin, Albumin, Total protein, Gamma glutamyl transferase (GGT), Uric acid and Creatinine were determined using commercially available kits using clinical chemistry analyzer (Crescent diagnostics).

2.6 Data Analysis

Statistical analysis was done by one-way ANOVA and SPSS PROC GLM in SAS software to compare the results in comparable groups. P values (<0.05) were considered as statistically significant.

3. RESULTS

3.1 Overall Biochemical Comparison among GDM Group and Control Group at Early 2nd and 3rd Trimester of Gestation

Overall biochemical comparisons among pre and post GDM group (n=58) and pre and post control group (n=100) were carried out by one-way ANOVA technique. Group means were separated through SNK multiple range test.

3.2 Liver Profile

Variations in the levels of ALT, ALP, AST, Albumin, Total Protein and GGT were analyzed in comparable groups of the study (Table 1).

All the subjects showed no statistical difference (P≤0.05) in ALT, total bilirubin and total protein levels between GDM and control group during early second trimester and early third trimester in hepatic profile. Statistically significant (P≤0.05) increase in ALP, AST and GGT level was observed in GDM group compared to control group. Albumin level was significantly lower in GDM group when compared with controls at early second trimester and early third trimester.

In kidney profile, creatinine concentration for GDM and control group was found to be non-significant ($p<0.0001$) at early second trimester but GDM group showed significant increase at early third trimester in renal profile. Similarly, there was significant increase in uric acid not only in early second trimester but also in early third trimester in GDM group comparison with controls.

All the subjects showed reduced levels of albumin whereas, elevated levels of ALP, AST, GGT and uric acid and no variations were observed in ALT, total bilirubin, total protein and creatinine at early third trimester but the alterations were more distinct in GDM group as compared to control group.

3.3 History Wise Biochemical Comparisons among Risk Group and Control Group

All the subjects were further categorized on the basis of positive and negative family history of GDM or T2DM. Among 300 pregnant women, the percentage of GDM pathogenesis in females with positive family history was found to be 27% while it was 9% for those with negative family history. Hence, all the GDM subjects were further divided into two groups on the basis of positive/negative family history of GDM/T2DM. A comparison was made between these two groups to access the extent of variation.

3.4 Liver and Kidney Profile

Variations among ALT, ALP, AST, total bilirubin, albumin, total protein and GGT were analyzed in comparable groups of the study. In kidney profile, variations in uric acid and creatinine levels were also observed in both comparable groups of the study (Table 2).

Descriptive analysis showed non-significant difference in ALT levels in risk group having positive family history of GDM as compared to control group having negative family history of GDM in early 2nd trimester. While, the risk group showed significant elevation of ALT at early third trimester compared to control group. ANOVA showed significant variance between two groups at 3rd trimester which shows that in risk group, percent increase in ALT was higher at third trimester. Significant increase in ALP levels was observed in risk group having positive family history of GDM compared to control group having negative family history of GDM in early 2nd trimester. The risk group showed significant elevation of ALP at early third. ANOVA showed highly significant difference among two groups. Strikingly, the changes were more pronounced in the group with positive family history. Significant increase in AST and GGT whereas, significant decrease in albumin and no significant difference in total bilirubin and total protein was observed in those with family history of GDM as compared to those without such history, both in early second and third trimester. In renal profile, a significant difference in the levels of uric acid was observed in risk group having positive family history of GDM compared to control group having negative family history of GDM in early 2nd trimester. The risk group showed significant elevation of uric acid at early third trimester. ANOVA showed highly significant difference among two groups. In risk group, the percent increase in uric acid was higher at 2nd as well as at early third trimester. Results showed a significant elevation in creatinine levels in risk group having positive family history of GDM compared to control group having negative family history of GDM in early 2nd trimester. The risk group showed significant rise in creatinine levels at early 3rd trimester. ANOVA showed significant difference among two groups. In risk group, there was non-significant percentage difference in creatinine levels at both trimesters. The results demonstrate that patients with positive family history of GDM or T2DM are more prone to develop GDM as compared to those with negative family history.

Table 1. An overall comparison of liver and kidney profile in GDM group (n=58) and control group (n=100) at early 2nd trimester (14-18 weeks) and early 3rd trimester (24-28 weeks) of gestation

Parameters	Early Second Trimester		Early Third Trimester		*P*-value
	T2C	T2GDM	T3C	T3GDM	
ALT(u/l)	12.63^{b}±0.33	13.84^{b}±0.40	19.06^{a}±0.35	21.11^{a}±0.58	< .0001
ALP(u/l)	52.67^{d}±0.67	63.76^{c}±0.73	168.53^{b}±1.51	212.99^{a}±1.93	< .0001
AST(u/l)	11.43^{d}±0.60	21.10^{c}±1.21	27.77^{b}±0.13	34.83^{a}±0.39	< .0001
Total Bilirubin(mg/dl)	0.34^{b}±0.02	0.37^{b}±0.02	0.49^{a}±0.01	0.53^{a}±0.05	< .0001
Albumin(g/l)	3.38^{a}±0.06	3.20^{b}±0.11	2.68^{b}±0.04	2.34^{c}±0.08	< .0001
Total Protein(g/dl)	6.17^{a}±0.04	6.20^{a}±0.08	5.90^{b}±0.04	5.74^{b}±0.14	< .0001
GGT(u/l)	11.01^{d}±0.47	16.49^{c}±0.69	19.99^{b}±0.56	26.47^{a}±0.94	< .0001
Uric Acid(mg/dl)	3.28^{d}±0.06	5.51^{c}±0.12	6.84^{b}±0.08	8.07^{a}±0.15	< .0001
Creatinine(mg/dl)	0.74^{a}±0.01	0.71^{a}±0.02	0.54^{c}±0.02	0.59^{b}±0.02	< .0001

a,b,c,d on indicate significant differences at p<0.05;
T2C= 2nd trimester control; T3C= 3rd trimester control
T2GDM= 2nd trimester GDM; T3GDM= 3rd trimester GDM

Table 2. An overall comparison of liver and kidney profile and inflammatory marker in negative family history group (n=124) and positive family history group (n= 176) during early 2nd trimester (14-18 weeks) and early 3rd trimester (24-28 weeks) of gestation

Parameters	Early Second Trimester		Early Third Trimester		*P*-value
	Negative History	Positive History	Negative History	Positive History	
ALT (u/l))	14.94 ± 0.22^{c}	15.75 ± 0.14^{c}	20.90 ± 0.19^{b}	24.51 ± 0.17^{a}	<0001
ALP (u/l)	62.96 ± 2.55^{d}	79.54 ± 0.31^{c}	180.06 ± 0.54^{b}	226.77 ± 0.48^{a}	<.0001
AST (u/l)	14.43 ± 0.24^{d}	23.53 ± 0.17^{c}	31.61 ± 0.23^{b}	39.69 ± 0.20^{a}	<.0001
Total Bilirubin(mg/dl)	0.38 ± 0.02^{b}	0.40 ± 0.02^{b}	0.58 ± 0.02^{a}	0.62 ± 0.03^{a}	<.0001
Albumin (g/l	31.30 ± 0.49^{a}	27.01 ± 0.18^{b}	26.69 ± 0.31^{b}	22.78 ± 0.17^{c}	<.0001
Total protein(g/dl)	6.12 ± 0.04^{a}	6.19 ± 0.05^{a}	6.02 ± 0.04^{b}	6.10 ± 0.04^{ab}	0.0401
GGT (u/l)	14.13 ± 0.20^{d}	18.89 ± 0.19^{c}	27.45 ± 0.28^{b}	34.01 ± 0.29^{a}	<.0001
Uric Acid (mg/dl)	4.22 ± 0.06^{d}	6.55 ± 0.09^{c}	7.26 ± 0.09^{b}	9.86 ± 0.09^{a}	<.0001
Creatinine(mg/dl)	0.50 ± 0.00^{d}	0.64 ± 0.00^{c}	0.70 ± 0.00^{b}	0.77 ± 0.01^{a}	<.0001

a,b,c,d indicate significant differences at p<0.05;
Order of significance is as: a>b>c>d

4. DISCUSSION

The insulin/glucagon ratio and altered portal insulin levels in GDM may be influenced by hepatic function and make the patient susceptible to various hepatic disorders [13]. Our results showed that mean values of ALP, AST and GGT, were significantly higher ($p<0.05$) and mean albumin was significantly lower in early second trimester in those patients who subsequently developed GDM in late third trimester as compared to those who didn't develop GDM. The highest number of women who developed GDM were those who had a positive clinical/family history.

The connection between increased AST and GDM risk may be due to shared pathophysiological mechanisms between GDM and T2DM as both diseases share many prevalent risk factors. When there is a liver injury present, such as infection, toxins and ischemia, AST is released from wounded cells of liver, with increased concentrations of AST. However, slight elevation of AST level, often within a normal range may represent fat accumulation, a marker of NAFLD, rather than liver injury [15]. NAFLD is strongly correlated with obesity and insulin resistance [16] leading to T2DM, partially mediated by endothelial dysfunction [8] particularly in subjects with other predisposing factors such as genetics. AST and ALT are the most commonly used hepatocyte injury markers. Hepatocyte necrosis in ischaemic or toxic injury, acute hepatitis leads to enzyme's leakage into the circulation [17].

During the third trimester of pregnancy, the serum ALP level increases due to a type of enzyme generated in the placenta. Serum elevation of ALP could be the manifestation of liver's surplus fat deposition, called non-alcoholic fatty liver disease. It is believed that fatty liver causes resistance to hepatic insulin and contributes to the growth of systemic insulin resistance and hyper insulinemia. Thus, ALP in the pathogenesis of diabetes could serve as a marker of insulin resistance syndrome [18,19]. Levels of ALT, total protein, and total bilirubin were found to differ non-significantly in all patients who developed GDM later in the third trimester and those who didn't develop.

GGT is connected with high insulin resistance. Our data showed an overexpression of hepatic GGT which can cause insulin resistance. Furthermore, unlike ALT, which is predominantly located in hepatocytes and therefore a particular marker for liver injury, GGT is an ubiquitous epithelial enzyme engaged in extracellular catabolism of antioxidant glutathione and hence, a marker of oxidative stress, which in turn may cause insulin resistance [20]. Therefore, distinct cellular processes and downstream pathways can partially explain the stronger association between GGT and ALT with GDM risk [21].

Hyperbilirubinemia is the elevated level of bilirubin in blood and indicates impaired uptake and over production of conjugated and unconjugated bilirubin from hepatocytes to the bile duct. [22]. However, there is no direct correlation of bilirubin levels with GDM. It impacts the protein metabolism and as serum albumin concentrations imply the synthetic function of the liver, a reduction in its concentrations suggests a distortion of the function of the liver in these females. On the other side, microalbuminuria is also an essential element of impaired glucose tolerance of insulin resistance syndrome [23] and this could also lead to the reduced concentrations of albumin in these females. Microalbuminuria alone is an indicator of future renal disease in these females and therefore, periodic monitoring of GDM patients has been suggested to identify other markers of kidney disease [24].

Many conditions can impact kidneys' ability to perform their functions. Some conditions can result in rapid or progressive decline in the kidney functions. Creatinine levels in renal profile, were found to vary insignificantly in patients who developed GDM later in third trimester and those who didn't develop but uric acid levels were found to be significantly higher among GDM patients. Higher levels of creatinine and even those at the upper-limit of the normal range may act as a warning signal of imminent kidney disease in GDM conditions, as chronic kidney disease is usually clinically silent until it is progressed and as creatinine can change to some extent until its clearance drops below 50ml / min. As the current study reported that there were no variations in creatinine levels among patients with GDM.

The connection between uric acid and insulin resistance may be causal. Two mechanisms were hypothesized that could cause uric acid to resist insulin. Uric acid triggers endothelial dysfunction and reduces the endothelial cell's output of nitric oxide. Another mechanism through which uric acid can cause insulin resistance may be that uric acid creates oxidative stress and inflammation in adipocytes, which contributes to metabolic syndrome growth. This research shows a striking link between uric acid in the first trimester and the risk of developing GDM. Women with GDM complex pregnancy have up to 50% opportunity of developing T2DM in their future. Increased levels of uric acid in early gestation indicates that the metabolic state may influence adverse pregnancy consequences [25]. It is possible that of the females who develop GDM, those with high uric acid in the first trimester are the females who are at high risk to develop T2DM, and this warrants future investigation. Thus, we postulated that high serum uric acid in the 2nd trimester helps to predict GDM and also to recognize those at danger of developing type 2 DM and follow-up; and to advise the patient on the short and long term results.

It can, therefore, be concluded that hyperuricemia seen in GDM may be triggered by the impacts of insulin on the kidneys, despite the prevailing resistance to insulin. Uric acid is the ultimate breakdown of adenosine in humans, which plays a significant part in insulin resistance pathophysiology [26-29]. Not many studies in the past have either analyzed uric acid concentrations in GDM. However, in our research, uric acid concentrations in females with GDM were significantly higher compared to controls. Hyperinsulinaemia can activate the sympathetic nervous system and both can be associated with a reduced urinary excretion of uric acid independently [14].

We have excluded all those factors, which could be responsible for above said variations otherwise. Furthermore, those GDM subjects who were found to have some clinical complications other than GDM were also excluded during analysis. So, here we assume that the above mentioned alterations in expectant women have been attributed exclusively by GDM. Further studies are also needed to assess current indicators of GDM.

5. CONCLUSION

It is concluded that early prediction of GDM is possible but if not diagnosed at early stage, it can lead to a greater risk of future development of T2DM. Early diagnosis will help clinicians in the proper treatment of GDM and will improve the quality of life of GDM patients and their offsprings. Pregnant females can be screened in early stages of gestation for these biomarkers to reduce the susceptibility of GDM. If GDM is not controlled and treated in early second trimester, it may adversely affect liver and kidney functions (specifically AST, ALP, GGT, albumin, serum bilirubin and uric acid) in late third trimester. It is important to control and treat GDM in time by screening the at-risk population so as to reduce maternal and neonatal complications. ALT, Total bilirubin, total protein, and creatinine appear to have no value in GDM prediction. If levels of these early biomarkers can be controlled via therapeutic measures at early stages of pregnancy, the onset of said disease can be delayed or prevented in later stages of pregnancy. As clinical history of GDM is associated with susceptibility of T2DM development and/or other metabolic dysfunctions, screening of such pregnancies for hormonal markers in early stages can greatly reduce the chances of GDM development, improve therapeutic efficacy and reduce related complications. Further studies with additional biochemical and clinical markers such as prothrombin time (PT/INR), aPTT, blood urea nitrogen (BUN), cystatin C, urinary NGAL (neutrophil gelatinase-associated lipocalin), urea, etc. can be remarkable.

CONSENT

As per international standard or university standard, patient's written consent has been collected and preserved by the author(s).

ETHICAL APPROVAL

Ethical approval for the study was obtained from Institutional Ethical Review Board of Punjab University.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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