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Biochemical markers of acute kidney injury and hepatic function in gestational diabetes mellitus: A comparative study

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Abstract

One-third of diabetics develop renal and liver disease, which costs global health systems money and resources. The study examines hepatic indices, duration, and glucose levels and assesses neutrophil gelatinase-associated lipocalin (NGAL) as a sensitive marker for acute kidney injury (AKI) in gestational diabetes. This study recruited 30 non-gestational pregnant hospital ante-natal clinic patients and 30 non-diabetic controls without pregnancy. Standard techniques were used to collect and analyze fasting blood sugar, renal, and hepatic biomarkers. Significant findings were determined by conducting statistical analysis with P <0.05. Gestational diabetes (GDM) and pregnant women without gestational diabetes (PNGDM) showed significantly higher levels ($p<0.05$) of NGAL, urea, and creatinine compared to non-pregnant women without diabetes (NPNDM). Compared to women without pregnancy or diabetes (NPNDM), there was a significant difference in mean values of fasting blood sugar (FBS), glycated hemoglobin (HbA1c), aspartate amino transferase (AST), alanine amino transferase (ALT), alkaline phosphatase (ALP), and gamma-glutaryl glutaminase transferase (GGT) among gestational diabetes mellitus (GDM) and PNGDM ($p<0.05$). NGAL had a higher AUROC of 0.684 compared to urea and creatinine. This study shows the significance of plasma NGAL levels as a biomarker for AKI in gestational diabetes. Furthermore, the findings of this study reveal that derangements in hepatic parameters are extensively co-existent in GDM.

Keywords: Acute kidney injury, hyperglycaemia, HbA1c, hepatocytes, or insulin resistance

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INTRODUCTION

Disordered metabolism and hyperglycaemia caused by insulin deficiency or insulin resistance in various body cells characterize diabetes (Fadairo *et al*., 2016). The liver is the main hormone and glucose metabolism site. According to reports, chronic liver disease, especially liver cirrhosis, causes glucose intolerance and diabetes. The link between liver enzymes such gamma-glutaryl glutaminase transferase (GGT), alanine amino transferase (ALT), aspartate amino transferase (AST), and alkaline phosphatase (ALP) and gestational diabetes mellitus (GDM) risk has been contentious (Zhu *et al*., 2018). Hepatocyte function can be affected by gestational diabetes, as it can alter portal insulin levels and the insulin/glucagon ratio, potentially leading to hepatic diseases (Khan *et al*., 2012). Although there is no distinct hepatic ailment linked with gestational diabetes mellitus, abnormal hepatic glucose metabolism may be involved in non-insulin-dependent diabetes (Khan *et al*., 2012). Nevertheless, certain diabetic individuals, particularly those experiencing acute metabolic decompensation, have exhibited abnormalities in liver function tests (LFTs). However, there is ongoing debate regarding the prevalence of abnormal LFT results in gestational diabetes (Khan *et al*., 2012).

AKI is a major global health issue, impacting over 13 million people and causing 2.3 million deaths each year. It is noteworthy that around 85% of these deaths occur in developing nations. Several studies have assessed the epidemiology of AKI in people with diabetes, demonstrating the potential progression to diabetic nephropathy if not treated correctly and promptly (Ponce et al., 2020; Kahindo *et al*., 2022). There is, however, emerging evidence supporting the early identification of renal disease through serial monitoring in pregnancies complicated by GDM. Early detection of damage allows clinicians to implement interventions that delay or halt disease progression (Rawal *et al.,* 2018; Fenna *et al.,* 2019). In humans, LCN2 gene encodes Lipocalin-2 (LCN2), an important member of lipocalin family known oncogene 24p3 or neutrophil gelatinase associated lipocalin (Yang *et al*., 2002. In order to discriminate prerenal from intrinsic causes of AKI, Kjeldsen *et al.* (1993) previously pointed out LCN2 as a novel potential biomarker. More specifically, neutrophils express LCN2 whereas the kidney, prostate and respiratory and gastrointestinal tract epithelial tissues express lower levels (Devarajan, 2010). The aim of this study was to investigate the association of hepatic indices, duration and glucose with NGAL as a reliable predictor for acute renal impairment in gestational diabetic women.

MATERIALS AND METHODS

Study design

This is a comparative cross-sectional study. The research was conducted between January to August, 2021. A total of forty (40) gestational diabetic subjects aged between 20-40 years attending the ante-natal clinic of Federal Medical Centre (FMC), Owo were enrolled for the study.

In this study, gestational diabetes mellitus was defined as having fasting blood glucose levels greater than 7.0mmol/l on two or more occasions, as determined by laboratory findings (WHO, 1999). After obtaining approval from the hospital's ethical committee, the medical histories and personal data of the participants were collected through a comprehensive questionnaire. This study included thirty (30) non-gestational pregnant subjects attending the hospital's antenatal clinic and thirty (30) non-diabetic, non-pregnant control subjects. Informed consent was obtained from all participants.

Consent and ethical clearance

The participants in this study were provided with comprehensive information regarding the research protocols during their visit to the ante-natal clinic. Subsequently, they expressed their consent to participate by signing a written consent form. The ethical review committee of the Federal Medical Centre, Owo granted ethical approval under the reference number FMC/OW/380/VOL.CXVII/195.

Inclusion and exclusion criteria

Inclusion criteria: The research included female participants between the ages of 18 and 50 who were diagnosed with gestational diabetes. The control group consisted of two subgroups: apparently healthy pregnant women without gestational diabetes and non-pregnant women aged 18 to 50 without diabetes. Exclusion criteria: The study excluded individuals with documented comorbidities such as hypertension, HIV, hepatitis, cancer, those undergoing oral anticoagulant therapy, those with bleeding disorders, and lactating women. The same exclusion criteria were applied to the control group as well.

Collection and storage of sample

Five milliliters (5 ml) of venous blood were collected from each subject using aseptic procedure. The blood was dispensed into lithium heparin and a fluoride oxalate anticoagulant bottle. Each sample was spun at 4000 rpm for 5 minutes to obtain plasma which was stored at -20˚C until analysis.

Analytical methods

Plasma creatinine, urea and fasting blood glucose were evaluated using standard spectrophotometric method as described by Akinlade *et al*. (2014) and Atere *et al*. (2021). Plasma activities of AST, ALT, ALP, GGT, and Albumin were determined using reagents supplied by Randox Laboratories Ltd. (UK). Enzyme linked immunosorbent assay (ELISA) kits from Melsin Medical Company, USA were used for determination of plasma neutrophil gelatinase-associated lipocalin and glycated hemoglobin (HbA1c).

Statistical analysis

Data analysis was done using SPSS version 25.0 which is a statistical tool commonly used in social science research. A normality test was applied for each variable, and data distributions with an abnormal distribution were transformed. Analysis of Variance (ANOVA) statistical tool used to compare differences among the groups. To identify relationships between variables, we performed correlation analysis. Additionally, eceiver Operating Curves (ROC) were plotted, and the Area under the ROC (AUROC) for each marker (NGAL, urea, and creatinine) was compared using pairwise comparisons. A p-value of less than 0.05 was considered statistically significant.

RESULTS

Figure 1 compares anthropometric indices in pregnant women with gestational diabetes (GDM), pregnant women without gestational diabetes (PNGDM), and non-pregnant women without diabetes (NPNDM). Pregnant women had significantly greater systolic blood pressure (SBP) than non-pregnant women without diabetes. When the mean renal indices of GDM, PNGDM, and NPNDM were compared using ANOVA, the mean NGAL, urea, and creatinine were significantly higher ($p<0.05$) in both GDM and PNGDM than NPNDM group (Table 1). More so, there was a significant difference in the mean values of FBS, HbA1c, AST, ALT, ALP and GGT among GDM and PNGDM when compared NPNDM ($p<0.05$). Post hoc statistical analysis shows a significant higher in mean values of FBS, HbA1c, AST and ALT, but lower GGT and Alb mean value when GDM group was compared with PNGDM group ($p<0.05$). Similarly, the mean values of FBG, HbA1c, AST, ALT, ALP and GGT were significantly higher in both GDM and PNGDM groups when compared with the NPNDM group ($p<0.05$). Figure 2 showed significant association between NGAL and FBS among GDM. As shown in Figure 3, the diagnostic performance of NGAL, urea, and creatinine was evaluated. With an AUROC of 0.684, NGAL exceeded urea and creatinine. Creatinine had a higher AUROC of 0.640 than urea, which had an area of 0.631.

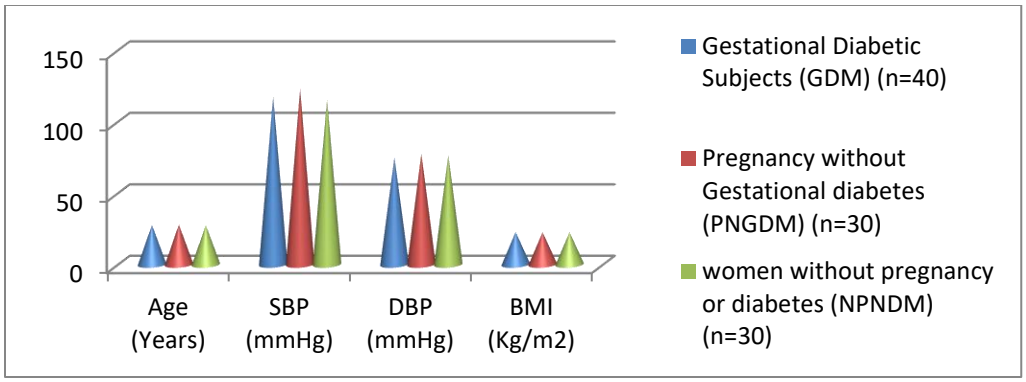

Figure 1: Anthropometric Indices in GDM, PNGDM and NPNDM.

Key: n=sample size, SBP= Systolic blood pressure, DBP= Diastolic blood pressure, BMI= Body mass Index

Table 1: Hepatic indices, Renal indices, FBG and HbA1c in GDM, PNGDM and NPNDM

	Gestational Diabetic Subjects (GDM) (n=40)	Pregnancy without Gestational diabetes (PNGDM) (n=30)	women without pregnancy or diabetes (NPNDM) (n=30)	P-Value
FBG (mmol/L)	6.92±0.49 [a,c]	4.52±0.43 [b]	4.42±0.26 [b]	0.000*
HbA1c (%)	5.96±0.31 [a,c]	4.45±0.28 [b]	4.39±0.17 [b]	0.000*
AST (U/L)	20.88±2.30 [a,c]	19.77±1.59 [a,b]	16.50±2.36 [b,c]	0.001*
ALT (U/L)	17.90±1.78 [a,c]	16.60±1.94 [a,b]	15.23±1.91 [b,c]	0.000*
ALP (U/L)	21.08±2.83 [c]	20.87±2.56 [a,b]	10.43±2.03 [b,c]	0.000*
GGT (U/L)	10.68±1.34 [a,c]	12.23±1.70 [a,b]	9.87±1.48 [b,c]	0.000*
ALB (g/L)	33.72±2.07 [a]	33.94±1.89 [a]	36.62±1.84 [b,c]	0.000*
Urea (mg/dl)	20.47±2.82 [a]	20.75±2.03 [a]	17.03±2.41 [b,c]	0.000*
Creat (mg/dl)	0.93±0.15 [a]	0.94±0.12 [a]	0.79±0.08 [b,c]	0.000*
NGAL (µg/L)	3.25±0.60 [a]	3.44±0.59 [a]	1.66±0.55 [b,c]	0.000*

* significance at $p \leq 0.05$

a = significantly different from NPNDM, b = significantly different from GDM group, c = significantly different from PNGDM group

Key: n=sample size, FBS= Fasting blood sugar, AST = Aspartate amino transferase, ALT = Alanine amino transferase, ALP = Alkaline phosphatase, GGT = alpha glutaryl glutaminase transferase, ALB = Albumin, HbA1c= Glycated haemoglobin, NGAL = Neutrophil Gelatinase Associated Lipocalin, Creat = Creatinine

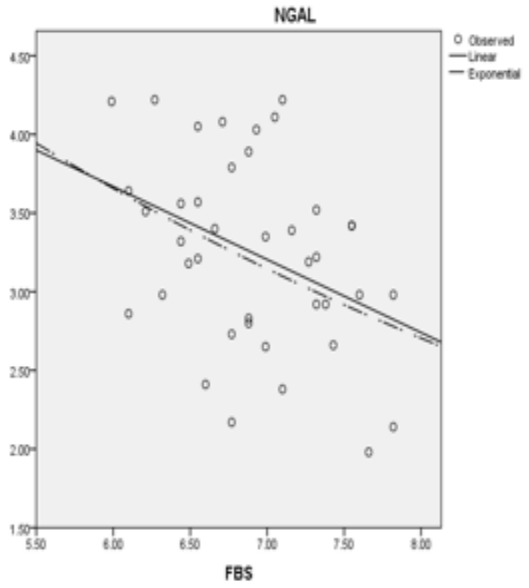

Figure 2: Correlation between NGAL and fasting blood glucose (FBS) in Gestational Diabetic Subjects

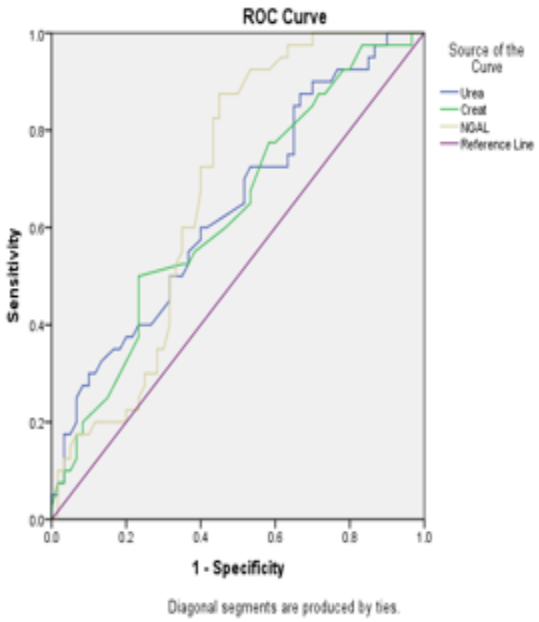

Figure 3: The ROC plasma of NGAL, Urea and Creatinine as diagnostic tool in GDM

DISCUSSION

This study identified a significant difference in systolic blood pressure (SBP) between pregnant and non-pregnant women without diabetes. Pregnancy induces various significant physiological changes in the cardiovascular system (Sanghavi & Rutherford, 2014; Kazma *et al*., 2020). However, these are unavoidable adjustments required to accommodate the increased metabolic demands of both the mother and fetus, ensuring adequate blood flow to the uterus and placenta for normal fetal growth and development. According to Scott *et al*. (2022), high blood pressure during pregnancy is defined as an SBP of 140 mm Hg or more, a diastolic blood pressure (DBP) of 90 mm Hg or more, or both. Such women require more intensive antenatal care and surveillance due to the increased risk of preeclampsia along with other maternal, fetal, or neonatal complications, including death (Butalia *et al.,* 2018).

ANOVA analysis showed that the mean NGAL, urea, and creatinine levels of women with GDM, PNGDM, and NPNDM were significantly ($p<0.05$) higher in both the GDM and PNGDM groups compared to the NPNDM group (Table 1). This observation aligns with previous studies indicating that NGAL, urea, and creatinine levels are elevated in pregnant women (Karampas *et al*., 2014; Atere *et al*., 2018). This is scientifically justifiable because hemodynamic alterations, glomerular endotheliosis, and podocyte impairment can produce renal impairment in hypertensive pregnancies. Due to changed hemodynamics in pregnancy, GFR declines above normal values in healthy non-pregnant women (Garovic *et al*., 2007; Franceschini *et al*., 2008). According to Egwuatu (1983), plasma urea levels increased throughout the second trimester, decreased during the third trimester to levels similar to the first trimester, and then increased once again during the postnatal period. The plasma creatinine levels exhibited a similar trend to that of urea. Nevertheless, it is proposed that these alterations are indicative of a redistribution of fluids rather than a modification in the generation of urea and creatinine.

This study observed a negative correlation between plasma levels of NGAL and FBS in participants diagnosed with gestational diabetes mellitus. In contrast, Kaul *et al*. (2018) discovered statistically significant positive associations between blood NGAL levels and both fasting serum glucose and HbA1c % among individuals with diabetes. Under normal conditions, trace amounts of NGAL can be found in plasma and urine, and this protein has been shown to have a role in Acute Kidney Injury (Singer *et al*., 2013). The filtration of NGAL mostly occurs by glomerular filtration in the plasma. Following

filtration, NGAL is reabsorbed by proximal tubule cells through endocytosis via the megalin system (Van Deursen *et al*., 2014). When the proximal tubule is affected by tubular necrosis, there is a possibility for filtered NGAL to evade tubular reabsorption and be excreted in the urine. It has been proposed that prolonged diabetes duration and inadequate glycemic control may result in elevated levels of NGAL in both serum and urine. The identification of an early indicator of kidney impairment might provide prompt intervention to halt the advancement towards end-stage renal illness (Goldstein and Devarajan, 2008; Goldstein, 2011).

In this study, there was a significant difference in the mean values of FBS, HbA1c, AST, ALT, ALP and GGT among GDM and PNGDM when compared with NPNDM ($p<0.05$). Many studies around the world have reported a varied frequency of derangements in liver function indices in diabetic patients. Our data is in tandem with the earlier studies conducted by Pardhe *et al*. (2018) in Nepal and Balogun *et al*. (2008) in Nigeria reported a high prevalence of deranged liver function indices of about 71.2% and 70% respectively among the diabetic individuals. However, our finding contradicts Ni *et al*. (2012), who found a similar pattern but lower liver function values in Malaysia.

Non-alcoholic fatty liver disease (NAFLD) is common in type 2 diabetes mellitus (T2DM) due to insulin resistance and obesity (Bhatt and Smith, 2015), which may be worse in GDM. Insulin resistance is the main cause of lipolysis, which can lead to non-esterified fatty acids (NFA) buildup. This increased hepatic NFA buildup is directly harmful to hepatocytes (Mandal *et al*., 2018). This is attributed to elevation in transaminases levels and diminished in hepatic synthetic physiological role (Malakouti *et al*., 2017; Pardhe *et al*., 2018).

Additionally, post-hoc analysis showed elevated FBS, HbA1c, AST, ALT, ALP, and GGT in the GDM and PNGDM groups compared to NPNDM ($p<0.05$). Mean FBS, HbA1c, AST, and ALT were significantly higher, whereas levels of GGT and Alb were lower in the GDM group than in the PNGDM group ($p<0.05$). As further detailed by Leng *et al*. (2016), ALT is mainly stored in the liver, while GGT is less specific and a poor indicator for hepatic fat accumulation and NAFLD.

The diagnostic performance of NGAL, urea, and creatinine was further evaluated using the AUROC. In this research, NGAL was significantly superior with an AUROC of 0.684 compared to urea and creatinine levels: creatinine had an AUROC of 0.640, while urea was 0.631. This coincides with earlier studies in diabetics and hypertensives (Baumert *et al*., 2017; Atere *et al*., 2018). As metabolic waste, urea and creatinine are the most common kidney injury indicators. Since impaired kidney function lowers the clearance of urea and creatinine, these substances can be used as kidney function biomarkers. Early renal impairment is not anticipated, as the kidney has considerable reserve capacity (Zappitelli *et al*., 2007).

CONCLUSION

This study demonstrates NGAL as a promising early biomarker for AKI detection in GDM, with significant hepatic enzyme derangements observed among GDM patients. However, limitations, such as the relatively small sample size and cross-sectional design, may impact the generalizability of these findings. Future research should include larger, longitudinal studies across diverse populations to validate these findings and explore additional biomarkers, potentially enhancing diagnostic precision for renal and hepatic complications in GDM.

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