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■ Original Research Article

A Prospective Cohort Study on the Association Between Elevated C-Reactive Protein and Gestational Diabetes Mellitus in a Nigerian Obstetric Population

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ABSTRACT

Background: Gestational Diabetes Mellitus (GDM) is a major public health concern with diverse range of adverse maternal and neonatal outcomes. The global prevalence of GDM varies widely depending on the screening method, diagnostic criteria used, and the characteristics of the population used. Thus, identifying women with GDM, and implementing interventional strategies aimed at controlling glycaemic status would help to reduce maternal and neonatal morbidity and mortality. **Objective**: To investigate the association between first trimester C-reactive protein levels and development of gestational diabetes mellitus later in pregnancy. **Study Design and Setting:** A prospective cohort study was undertaken at University of Abuja Teaching Hospital, Gwagwalada involving 145 eligible consenting pregnant women**. Materials and Methods:** C- reactive protein measurement was done at booking in the first trimester. The women were followed up with a single oral glucose tolerance test (OGTT) between 24-28 weeks for development of gestational diabetes mellitus among women with normal and elevated C- reactive protein. Data collected was analyzed using Statistical Package for the Social Sciences (SPSS) version 20.0 software. Quantitative variables were described using mean and standard deviation; qualitative variables were presented as percentages. The student t-test was used 'to determine association between continuous variables while the Chi-square test was used for categorical variables. A p-value of less than 0.05 was accepted as indicating statistical significance. **Results:** The incidence of gestational diabetes mellitus among women with normal C- reactive protein was 3.3%, while the incidence of gestational diabetes mellitus among women with elevated C-reactive protein was 28.3%. The association between C- reactive protein and gestational diabetes mellitus was statistically significant with a p-value of <0.001 and relative risk of 8.67. **Conclusion:** Findings from the study suggest that elevated C-reactive protein in the first trimester is a good predictor of development of gestational diabetes mellitus later in pregnancy in a cohort of Nigerian pregnant women.

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INTRODUCTION

Gestational diabetes mellitus (GDM) is a medical condition occurring in women characterized by carbohydrate intolerance with onset or first recognition during pregnancy.[1] It is a disease of public health importance that affects a significant number of women, with both short and long-term consequences for the fetus and the mother.[2]

In 2017, the International Diabetes Federation estimated that 21.3 million or 16.2% of live births to women in the reproductive age group (20-49 years) had some form of hyperglycaemia in pregnancy. An estimated 86.4% were due to GDM, 6.2% due to diabetes detected prior to pregnancy, and 7.4% due to other types of diabetes (including type 1 and type 2) first detected in pregnancy.[3] Studies from within Nigeria showed a prevalence of 5.4%, 8,3% and 4.8% in Lagos, Jos, Ebonyi and Ibadan respectively.[4-7]

Women with uncontrolled GDM have a four-fold increase in perinatal mortality rate compared with controls.[8] They are also at a higher risk of gestational hypertension, preeclampsia, caesarean delivery, and of developing diabetes mellitus later in life.[8] The offspring of women with GDM on the other hand are at an increased risk of macrosomia, congenital anomalies, neonatal hypoglycaemia, hyperbilirubinaemia, operative delivery, shoulder dystocia, and birth trauma.[9]

Women who had GDM in a previous pregnancy are at an increased risk of developing diabetes mellitus postpartum.[10,11] Such women are also at an increased risk of developing insulin resistance, metabolic syndrome (characterized by a combination of elevated blood glucose, central obesity, elevated blood pressure (BP), high triglycerides (TG) and low high-density lipoprotein cholesterol (HDL-c)), and cardiovascular disease, which is independent of a diagnosis of type 2 diabetes mellitus later in life.[12] Children of women with previous GDM are likewise at an increased risk of developing diabetes mellitus, obesity, and metabolic syndrome in childhood and adulthood.[12,13]

With the numerous short and long term feto-maternal morbidities associated with GDM, it is imperative to identify women at risk of developing GDM. The different tools that could be employed in screening include use of patient's history, clinical risk factors and glucose challenge test, random blood sugar and fasting blood sugar.[1,14]

The inflammatory cytokines such as tumour necrosis factor (TNF) and interleukin 6 are insulin antagonists which increase resistance to insulin and stimulate the acute phase of inflammatory response.[15]

C-reactive protein (CRP) is an acute - phase protein produced by hepatocytes in response to tissue inflammation, infection and tissue injury. Chronic subclinical inflammation has been proposed to play a key role in the pathophysiology of GDM development.[16]

Early pregnancy CRP levels (a sensitive index of systemic inflammation) have been shown to be significantly higher in women who went on to develop GDM in the course of their pregnancies. C -reactive protein as an inflammatory marker is produced and released by the liver under the stimulation of cytokines mainly tumour necrosis factor- alpha (TNF- α) and interleukin- 6.[17] These cytokines have been shown to inhibit insulin stimulated glucose uptake and also increase resistance to insulin thereby leading to development of diabetes mellitus.

Identifying mothers at risk of GDM is vital to allow early commencement of effective preventive therapies such as exercise and healthy diet, optimal level of surveillance.[18] This will enable reduction in pregnancy complications and improve maternal and fetal outcomes. Few studies that established an association between maternal C-reactive protein as a biomarker in the prediction of GDM, were carried out in high and middle-income countries in North America[19,20], Europe[21] and Asia.[22] with none from the developing countries, as well as the African continent.

Pregnancy complicated by poorly treated diabetes or untreated diabetes is associated with high maternal and perinatal morbidity and mortality e.g. abortions, obstructed labour, macrosomia, shoulder dystocia, gestational hypertension, pre-eclampsia, stillbirths, postpartum haemorrhage, neonatal hypoglycaemia, hyperbilirubinemia, infant respiratory distress syndrome.[9] In Nigeria, prevalence of GDM varies across the different regions. Some workers have reported 2.98 per 1000 pregnancies[23], 0.74 per 1000 deliveries[24] and 1.7% per 1000 pregnancies.[25] In Nigeria, selective screening for GDM is usually based on patient's history or use of clinical risk

factors.[26] The risk prediction based on maternal history has the tendency to be affected by inadequate patient history. Studies have shown that these have failed to identify about 38 – 50% of GDM with low sensitivities (50-69%) and specificities (58-68%). [27,28]

This study seeks to find out whether C-reactive protein, is associated with GDM in a Nigerian obstetric population. The findings and conclusions from this study provides baseline data upon which large studies could be developed to further investigate the phenomenon.

METHODS

This was a prospective cohort study conducted from July 2018 to January 2019 in the departments of Obstetrics and Gynaecology as well as Chemical Pathology, University of Abuja Teaching Hospital, Abuja (UATH) to assess the value of C-reactive protein as a first trimester maternal biomarker for the prediction of GDM. The UATH is a 350-bed health facility which provides healthcare services to the inhabitants of Nigeria's Federal capital and its neighboring states.

Ethical considerations

Approval for the conduct of the study was sort and subsequently granted from the hospital's health research and ethics committee. Clients' anonymity was maintained. Only code numbers were used to identify clients. No client was denied any form of services or promised facilitation of services based on decision to decline or give consent.

Patient recruitment/ counselling and selection/measurements

The study was explained to all pregnant women that presented for antenatal care booking during the first trimester. The explanations included a summary of the background information on gestational diabetes mellitus, risk factors, methods of diagnosis and treatment modalities and a description of the proposed study intervention. Risks and benefits of participation were discussed. Patients were informed that blood samples would be taking for C-reactive protein and glucose estimation at booking and between 24 to 28 weeks respectively. Blood pressure, urinalysis, body weight and height would also be taken at the time of registration for antenatal care. These measurements helped in identifying patients with risk factors for GDM such as patients with hypertensive disorders, pre-gestational diabetes and obesity in pregnancy in those with weight equal or greater than 90kg

All consenting pregnant women with no known risk factor for GDM during the study period, who presented for booking within the first trimester from last menstrual period or an early ultrasound scan were recruited for the study. The following groups of women were excluded: known cases of diabetes mellitus, other associated endocrine diseases like thyroid disease, women on corticosteroid therapy, multiple pregnancy, chronic hypertension, clinical evidence of maternal infections which may affect levels of C-reactive protein.

Pregnant women with the following situations were excluded: known cases of diabetes mellitus, history of diabetes in previous gestations, apparent diabetes mellitus in the first trimester of gestation, family history of type 2 diabetes in first degree relatives, history of recurrent abortion, history of macrosomia or congenital anomalies or stillbirth in previous pregnancy, multiple pregnancy, history of smoking or alcohol use before or during index pregnancy, polycystic ovary syndrome. To control potential confounding factors that could affect the serum CRP levels, patients with the following were also excluded; women with active infection at the time of sampling, women on antibiotics use within 2 weeks before the sampling, women on corticosteroids, women with chronic hypertension or kidney disease, thyroid disease, women with autoimmune and chronic inflammatory diseases and allergic diseases,

This information was determined through interview with clients during recruitment as well as by clinical examination and investigations where necessary such as urinalysis was used to diagnosed urinary tract infection with presence of nitrites in three patients with history suggestive of urinary tract infection which is a cofounding factor and hence were subsequently excluded from the study. Eligible patients were identified for recruitment, the information sheet was given to patients to read and was read out to those that could not read, following which the researcher or research assistants discussed the study with the patient. The counseling was centered on the need for research in

the health sector to improve patients' care. Participants' understanding of the explanation about the research was assessed by asking them to reiterate what they understood. Systematic random sampling was used to select from amongst the eligible pregnant women for the desired sample size. Written informed consent was obtained from the eligible women and enrolment codes were generated and used for each participant in place of identifiers such as names, to ensure confidentiality. Structured interviews with the aid of a proforma was conducted at the first contact to collect information on socio-demographic characteristics, medical and obstetric history of each participant. Gestational age was determined on best available estimate; either by using the patient's last menstrual period or estimation of gestational age from a first trimester ultrasound scan which is reliable.

A total of 145 pregnant women were recruited for this study using the systematic random sampling method from the eligible pregnant women at booking within the first trimester and had blood samples taken at booking within the first trimester (0-13 weeks) for serum C-reactive protein estimation. However, five of the pregnant women recruited had miscarriages before gestational age of 18 weeks respectively. Two of the enrolled patients were lost to follow up before gestational age of 24 weeks as they had transferred their antenatal services elsewhere. The remaining 138 enrolled pregnant women had OGTT done once at gestational ages between 24-28 weeks.

All participating women were tested using the 2-hour 75G oral glucose tolerance test (OGTT) at 24 - 28 weeks gestational age. During the three previous days, the participants were counseled to have an unrestricted diet (containing at least 150G carbohydrates per day) and unlimited physical activity. The test was done in the morning following an 8- to 12 hours overnight fast. The participants remained seated during the test. Following an overnight fast of 8 to 12 hours, about 5ml of venous blood was collected and placed in a fluoride oxalate bottle. The woman was given 75G of anhydrous glucose dissolved in about 300-400ml of water to drink for ≤ 5 minutes. One hour later, a sample was collected, and subsequently, a two-hour sample collected. All the samples were subsequently racked, coded according to the identification numbers on the questionnaires and transported to the chemical pathology research laboratory within the hospital premises for pre-analytical evaluation.

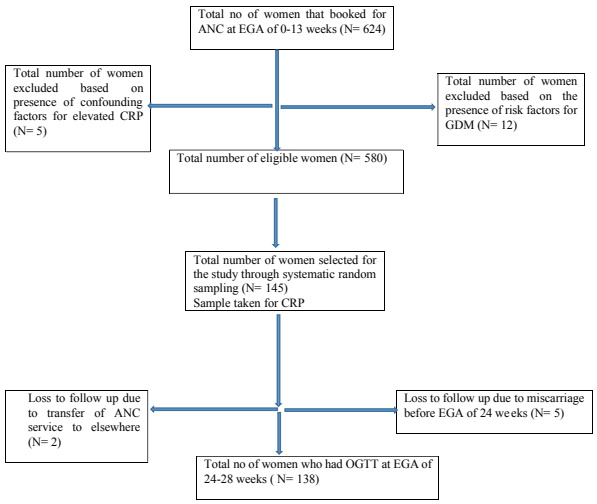

Fig. 1. A flow chart of participants all through the study

The sample size was determined using formula for a cohort study.[29]

$$n = \frac{Z_{1-\alpha/2}^2 \times p(1-p)}{d^2}$$

n = minimum sample size required

$\mathbf{Z_{1-a/2}}$= Is standard normal variate (at 5% type 1 error P< 0.05) which is 1.96

p= Expected proportion in population based on previous study.[30]

d = Absolute error or precision

$$\text{Thus } n = \frac{1.96^2 \times 0.1(1-0.1)}{(0.05)^2}$$

n = 138 for the total sample size

A 5% attrition rate = 7

If the attrition rate is added to the value obtained, the minimum sample size required.

n = 145

Urinalysis

The collection of urine for urinalysis was done in a private bathroom. The participants were provided with a wide mouthed sterile plastic bottle with lid, a sanitary cleansing wipe, and instructions on how to obtain a "midstream clean-catch" sample. This helped in avoiding contamination which could give a false positive result and such participants could be wrongly screened out of the study. Urinalysis was carried out using dipstick (Medi-Test Combi

10 SGL: Machery-Nagel Eurl France) on urine collected by the participants using standard procedures to check for the presence of parameters including glucose, proteins, ketones, nitrites, leucocytes. The presence of significant glycosuria or ketones would suggest diagnosis of diabetes mellitus and such participant would be screened out.

Blood tests

In addition to samples taken for standard antenatal tests, 5ml of venous blood was collected from the ante-cubital vein following aseptic procedures into a plain bottle (SST-gel BD vacutainer) at the antenatal care (ANC) side laboratory for serum C-reactive protein estimation. The samples were transported to the chemical pathology research laboratory, where the samples were processed within 2 hours of collection.

Sample processing

The collected blood samples were centrifuged at 4000 revolutions per minute (rpm) for 10 minutes using the swinging bucket centrifuge.

The samples were separated using a Pasteur's pipette into a supra and infra-natant. The supra-natant was aspirated, delivered into cryovials, and stored at −20 °C for subsequent analysis.

Serum C-reactive protein was measured using immunoturbidimetric method whose principle is predicated on the traditional antigen-antibody reaction. The C-reactive protein reacts with the specific antibody producing insoluble immune complexes. The turbidity caused by these immune complexes is proportional to the C-reactive protein concentration in the sample and this was measured spectrophotometrically at a wavelength of 340 nm. A range of 0-5mg/l was accepted as normal for this study.31

Plasma Glucose Estimation

Glucose estimation was done using the Glucose Oxidase Method (Randox® kits).

Using the WHO recommendations,32 the diagnosis of GDM was made when one or more of the following thresholds were met.

- Fasting plasma glucose = 5.1-6.9 mmol/l (92 -125 mg/dl)
- 1-h post 75g oral glucose load = ≥10.0 mmol/l (180 mg/dl)
- 2-h post 75g oral glucose load = 8.5 – 11.0 mmol/l (153-199 mg/dl)

Data analysis

Data analysis was done using Statistical Packages for Social Sciences (SPSS) version 20 (SPSS inc., Chicago, IL, USA) after coding and inputting of data into the computer.

Continuous variables were described using mean and standard deviation, categorical variables were presented as percentages. Chi-square test was used to calculate the relationship between C-reactive protein and development of GDM. P –value of less than 0.05 was accepted as indicating statistical significance.

RESULTS

A total of 145 pregnant women were recruited and tested for C-reactive protein, however five patients had miscarriages and two were lost to follow up. The remaining one hundred and thirty-eight patients were compliant with follow up and finally had OGTT done at gestational age of 24 – 28 weeks and their results were analyzed.

Table 1: Baseline characteristics of participants

Variable	Study sample n= (138)	GDM
Mean Age (years)	32.04 ± 2.11	
Age group		
≤ 20	1 (0.7)	0
21 – 24	15 (10.9)	1
25 – 29	44 (31.9)	4
30 – 34	61 (44.2)	7
35 – 39	15 (10.9)	3
≥ 40	2 (1.4)	1
Educational status		
Primary	6 (4.3)	0
Secondary	28 (20.3)	0
Tertiary	104 (75.4)	16
Occupation		
Unemployed	37 (26.8)	6
Student	8 (5.8)	1
Employed	93 (67.4)	9
Parity		
0	31 (22.5)	0
1 – 4	84 (60.9)	8
≥ 5	23 (16.6)	8
Mean maternal weight (kg)	69.47 ± 12.44	
Maternal Body weight		
≤ 50	7 (5.1)	2
51 – 69	58 (42.0)	4
70 – 89	63 (45.6)	5
90 – 109	9 (6.5)	3
≥ 110	1 (0.8)	2

The mean age of the participants from the study was 32.04±2.11 years. Most of the women 104(75.4%) had tertiary level of education while women with primary level of education were 6(4.3%). From the study, 93(67.4%) of the women were employed, 37(26.8%) were unemployed while 10(6.2%) were students. Eighty-four (60.9%) of the women were multipara accounting for most of the participants. The average maternal body weight of the participants was 69.47± 12.44 kg with 63(45.6%) of the women having a weight within the range of 70 to 89 kg. These are as shown in table 1.

Table 2: Association between elevated C-reactive protein level in women within gestational age of 0-13 weeks and development of GDM

Characteristic	GDM (n = 16)	No GDM (n = 122)	p-value	RR (95% CI)
Elevated C-reactive protein	13 (28.3)	33 (71.7)	<0.001	8.67 (2.60 – 28.90)
Normal C-reactive protein	3 (3.3)	89 (96.7)		

¥ - Chi-square

The overall incidence of GDM in the study cohort was 11.6%. Assessment of the sub-groups of the cohort showed that the incidence of GDM among women with normal C-reactive protein was 3.3% and this increased to 28.3% among women with elevated C-reactive protein. There was a statistically significant association between C-reactive protein and GDM (P<0.001) with a relative risk of 8.67(table 2). The latter finding implied that women with elevated C-reactive protein had 8.67 times the risk of developing GDM compared to those who had normal C-reactive protein.

Table 3: Association between maternal body weight and development of GDM

Characteristic Maternal weight (kg)	GDM (n = 16)	No GDM (n = 122)	p-value
< 90	11 (68.75)	117 (95.9)	0.18
≥ 90	5 (31.25)	5 (4.1)	

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The association between maternal body weight and GDM using t- test is as shown in table 3. There was no statistically significant relationship between maternal weight at booking and development of GDM in the study population (P= 0.18).

DISCUSSION

This study determined the association between first trimester C-reactive protein levels and GDM in women between 24 to 28 weeks gestational age. The socio-demographic variables were not statistically associated with GDM in this study. This is comparable to findings from previous studies that evaluated the association of C-reactive protein and development of GDM.33,34,35.

Findings from this study showed an association between first trimester inflammation marked by increased CRP levels (a sensitive index of systemic inflammation) and risk of developing GDM later in pregnancy. This is similar to reports from previous studies conducted in USA,35 India,36 and China.37 The relative risk of developing GDM in the presence of elevated CRP was 8.67 compared to those with normal level of C-reactive protein. This was more than twice the 3.6 reported by previous researchers working in Massachusetts, USA .38

In this study in which a cut-off point of 5mg/l for C-reactive protein was used, the incidence of GDM from the study among women with elevated C-reactive protein was 28.3%. Previous studies in which reference values of C-reactive protein of 2.2 mg/l 33 and 3.0mg/l 39 were used showed higher incidence of GDM of 71.7% and 76.0% respectively. From the foregoing, it can be inferred that a much higher incidence of GDM may have been found in our study if the reference value of 2.2mg/l or 3.0mg/l was used. Also unlike in this study where the WHO diagnostic values after a 75g loading glucose was used to make a diagnosis of GDM, in the studies,33,39 the diagnostic method employed for GDM was different. An initial 50g oral glucose loading test between 24-28 weeks of gestation was done, it was women with 1-hour post-loading plasma glucose level > 7.8 mmol/L who then had a diagnostic fasting 100g 3-hour oral glucose tolerance test.

Absolute maternal weight of ≥ 90kg has been used to classify women as been over-weight or obese in pregnancy.40 In this study, there was no statistically significant correlation between increased maternal body weight ≥ 90kg and development of GDM. A previous study that used a higher cut off of ≥ 95kg maternal weight showed a positive correlation between maternal weight in pregnancy and insulin resistance. This contrasting result may be explained by the use of a higher cut-off point of ≥ 95kg and also the measurement of the weights at gestational age of 24 to 32 weeks when there is marked effect of anti-insulin hormones in the latter study.41 However in this study, a cut off ≥ 90kg was used which is widely used as a cut-off value to define overweight or obesity in pregnancy

Risk prediction in GDM has mostly been based on maternal history and clinical risk factors. Studies have shown that these have failed to identify about 38 – 50% of GDM with low sensitivities (50-69%) and specificities (58-68%).26, 27 The addition of C-reactive protein (a low cost and easy to measure biomarker) to the maternal history with clinical risk factors model may significantly increase the risk prediction for GDM. However, the limitation with this may be the lack of capacity to carry out this test in some settings such as some primary and secondary health facilities.

Despite the finding of an association between elevated first trimester C-reactive protein and development of GDM later in pregnancy, this study is limited by its ability to only exclude the presence of clinical infections but not asymptomatic infections, except for urinary tract infection which may affect the levels of C-reactive protein. In this study, women were recruited within the first trimester. However, in real life situations in Nigeria, women tend to book for antenatal care in the second trimester of pregnancy. Studies that would also recruit women in the second trimesters when majority of Nigerian women book for antenatal care to ascertain association between C-reactive protein levels and GDM is needed towards wider applications since studies have shown that C-reactive protein levels remains relatively stable in the trimesters.

CONCLUSION

Findings from the study suggest that elevated serum C- reactive protein in the first trimester is a potentially good predictor of development of GDM later in pregnancy. The utilization of maternal C-reactive protein in the early part of the pregnancy as a screening method may assist the clinician in early identification of women at risk of developing GDM who may benefit from targeted preventive measures.

This study was carried out in a single center, and this may limit the generalization of these results. Although confounders such as clinical infection was excluded from the clinical history and use of urinalysis during the recruitment of these patients, the possibility of residual confounders such as asymptomatic infections that may alter the level of CRP still exist.

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