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Investigation of Some Haematological Parameters in Pregnant Women with Gestational Diabetes at Federal Medical Center, Owerri, Imo State, Nigeria

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Abstract

This study was conducted to investigate some haematological parameters in pregnant women with Gestational Diabetes at FMC, Owerri, Imo state. Seventy (70) pregnant women were recruited in the study of which fifty (50) are suffering from gestational diabetes, while twenty (20) are normal pregnant women serving as the control. Haematoloical parameters were determined using standard haematological procedures, data obtained from the study were analysed using the SSPS package. Haemoglobin concentration was significantly ($p < 0.05$) higher in gestational diabetic women (11.49 ± 1.01g/dl) when compared with the control subjects (normal healthy pregnant women) (10.7 ± 0.75 g/dl)). PCV in gestational diabetic women (33.84 ± 3.26%) was significantly ($p < 0.05$) higher when compared to controls (32.06 + 2.15%). Platelet count was significantly ($p < 0.05$) higher in gestational diabetic women (512.52 ± 106.76 × 10^9/L) when compared to the control subjects (197.60 ± 77.62 × $l0^9$/L). The mean value of TWBC was significantly higher ($p < 0.05$) in gestational diabetic women (8.07 ± 2.01 × 10^9/L) when compared with the control subject (5.38 ± 1.89 × 10^9/L). Neutrophil count was significantly ($p < 0.05$) higher in gestational diabetic women (53.04 ± 9.99%) when compared to controls (43.60 ± 6.24%). There was no statistically significant difference ($p > 0.05$) in the mean value of eosinophil count in gestational diabetic women (0.28 + 0.54%) when compared with the control subjects (0.20 + 0.41%). Basophils were not seen in gestational diabetic women and the control subjects. Lymphocyte count was significantly ($p < 0.05$) reduced in gestational diabetic women (45.78 ± 09.92%) when compared with the control subject (56.70 + 5.32%). There was no statistically significant difference ($p < 0.05$) in the mean value of lymphocyte count in gestational diabetic women (0.10 + 0.00%) when compared with the control subjects (0.30 + 0.00%). In conclusion, gestational diabetes has a significant effect on some haematological parameters.

Keywords: Haematological parameters; Pregnant women; Gestational diabetes

Introduction

Diabetes is a disease of metabolism clinically expressed by chronic hyperglycemia and blood lipid and protein disorders that have been extensively reported as linked to several complications that cause morbidity and mortality [1]. Diabetes and uncontrolled hyperglycemia are known to play a significant role inthe development of cardiovascular disease (CVD) since Framingham study [2].

Gestational diabetes is a condition in which a woman without diabetes develops high blood sugar levels during pregnancy [3]. Gestational diabetes generally results in few symptoms; however, it does increase the risk of pre-eclampsia, depression, and requiring a Caesarean section [3]. Babies born to mothers with poorly treated gestational diabetes are at increased risk of being too large, having low blood sugar after birth, and jaundice. If untreated, it can also result in a stillbirth [3]. Long term, children are at higher risk of being overweight and developing type 2 diabetes.

Additionally, besides the diabetes and classical risk factors, the presences of microvascular complications are also predictor of coronary heart events [4]. In addition to this, statistics show a double risk of GDM in smokers [5]. Polycystic ovarian syndrome is also a risk factor, although relevant evidence remains controversial. Some studies have looked at more controversial potential risk factors, such as short stature [6].

About 40-60% of women with GDM have no demonstrable risk factor; for this reason many advocate to screen all women [6]. Typically, women with GDM exhibit no symptoms (another reason for universal screening), but some women may demonstrate increased thirst, increased urination, fatigue, nausea and vomiting, bladder infection, yeast infections and blurred vision. In addition to atheroma formation, the combination of hypercoagulability, impaired fibrinolysis and

impaired vasodilation likely further increases the risk of vascular occlusion and cardiovascular events in diabetes [7].

In recent years, there has been renewed interest in hematological parameters such as white blood count (WBC), mean platelet volume (MPV), platelet distribution width (PDW), plateletcrit (PCT), platelet count, platelet to lymphocyte ratio (PLR) and neutrophil to lymphocyte ratio (NLR) and are designated as predictors of endothelial dysfunction and inflammation.

Haematological parameters include the red blood cell, white blood cell, Platelets, etc. Elevated white blood cell count (WBC) is a classical inflammatory marker and is associated with several cardiovascular disease risk factors and diabetes [8]. The association of increased MPV, PDW, PCT and platelet count with diseases related to endothelial dysfunction and inflammation as metabolic syndrome, diabetes, coronary artery disease and malignancy have been shown [9]. In the last decades, platelet counts were introduced as potential markers to determine inflammation in cardiac and non-cardiac disorders [9].

There are few studies on the effect of type III diabetes also called gestational diabetes on some haematological parameters. Studies by Levent et al. found out Patients with type 2 diabetes mellitus (T2DM) have an increased risk of coagulation abnormalities and thromboembolic events [10]. Platelets have a key role and increased adhesion, activation, and aggregation of platelets due to dysregulation of several signaling pathways and metabolic disturbances including insulin resistance, hyperglycemia, and dyslipidemia have been noted in diabetic patients [11]. Systematic inflammation, oxidative stress, impaired calcium metabolism, decreased bioavailability of nitric oxide, increased phosphorylation and glycosylation of cellular proteins are responsible for increased platelet activation and increased release of pro-thrombotic and pro-inflammatory agents in diabetes [12]. The study is aimed at determining some haematological parameters of pregnant women with gestational diabetes in Federal Medical Centre (FMC) Owerri.

Materials and Methods

Study area

The study was conducted at the federal medical centre, Owerri, Imo state. The hospital is located along Orlu road, secretariat, Owerri, Imo state. Owerri is the capital of Imo State in Nigeria, set in the heart of Igboland. It is also the state's largest city. Owerri consists of three Local Government Areas including Owerri Municipal, Owerri North and Owerri West, it has an estimated population of about 401,873 as of 2006 census and is approximately 100 square kilometres (40 sq mi) in area. Owerri is bordered by the Otamiri River to the east and the Nworie River to the south [13].

Ethics, advocacy and pre-survey contact

As part of the requirements for conducting surveys on human subjects, a letter of introduction was collected from the HOD department of medical laboratory science, Imo state university. The study was approved by the Research Ethics Committee of the Federal Medical Centre, Owerri, Imo state. Questionnaires were given to the study subjects. In addition, all study participants gave a written informed consent before they were enrolled in this study and samples taken.

Study population

Population for the study were all type 3 diabetic pregnant women coming to the federal medical center Owerri for their regular follow up and willing to participate in the study.

Sample size determination

Arqoye [14], formula for calculating sample size was used in this study.

$$n = \frac{z^2 pq}{d^2}$$

n=Desired sample size

z= The standard normal deviate usually set at 1.96

p=The proportion of the target population estimated to have a particular characteristics at 95% confidence. P is set at 0.025

q=l-p

d= Degree of accuracy set at 0.05

$$n = \frac{1.96^2 x 0.02 x (1 - 0.025)}{0.05^2} = \frac{3.84 x 0.025 x 0.975}{0.0025} = \frac{0.0936}{0.0025} = 37$$

Selection criteria

Inclusion criteria: The pregnant women with type 3 diabetes who were included in this study met the following criteria:

1. Pregnant women who signed informed consent to participate in the study
2. Pregnant women who were aged 18 years and above
3. Pregnant women who were diagnosed of suffering from type-3 diabetes.
4. Pregnant women with no history of diabetes mellitus, liver disease, malignancy, high blood pressure, renal disorder, HIV and endocrine disorder.
5. Pregnant women who are not suffering from any infection.

Exclusion criteria

1. Who did not sign informed consent to participate in the study.
2. Pregnant women with history of diabetes mellitus, liver disease, malignancy, high blood pressure, renal disorder, HIV and endocrine disorder.
3. Pregnant women below age 18 years.
4. Those diabetic patients who were blood transfused within the previous 3 months period were proposed to be excluded from the study.
5. Pregnant women with family history of diabetes

Study design

A cross-sectional study was conducted in the month of June 2018 and all eligible women who filled the questionnaire and gave a written informed consent for the study period were sampled.

The research was grouped into two Group A representing 50 pregnant women with type 3 diabetes and Group B representing 20 pregnant women without type-3 diabetes which served as the control for the study.

Haematological Parameters Determination (Mindray BC-5300)

Determination of packed cell volume

Method: Using microhaematocrit method, a well-mixed anticoagulated whole blood was allowed to enter capillary haematocrit tubes until they were approximately 2/3 filled with blood. Blood filling was done for each tube. One end of each tube was sealed with plastacine and placed in the medial grooves of the haematocrit centrifuge head exactly opposite each other, with the sealed end away from the centre of the centrifuge. All tubes were spun for five minutes at l000 rpm. The tubes were removed as soon as the centrifuge had stopped spinning.

Calculation: PCV was obtained for each tube using microhaematoerit-reader by measuring the height of the RBC column and expressing this as a ratio of the height of the total blood column.

$$PCV(\%) = \frac{Height\ of\ cell\ coloum}{Height\ of\ total\ blood\ column} \times \frac{100}{1}$$

Determination of Haemoglobin (HB) Concentration

Method: Exactly 5.0 ml of Drabkin's reagent was pipetted into two test tubes 1 and 2 and a well-mixed sample of EDTA blood (0.02 ml) was pipetted into the tubes, rinsing the pipette five times with the reagent, until all the blood was removed from the pipette. The solutions were well mixed and allowed to stand at 250 °C for 10 min in order to allow the formation of Cyan-met-haemoglobin. The mixtures were transferred into cuvettes and read in a spectrophotometer at a wavelength of 540nm. The Drabkin's reagent in tube 1 was used as the blank (setting the percentage transmittance at 100%). The readings from each tube were recorded and the actual Hb values in g/dl were determined from a pre-calibrated chart.

Calculation:

$$Hb\ in\ g/dl = \frac{Absorbance\ of\ test}{Absorbance\ of\ standards} \times Conc.\ of\ standards(in\ g/dl)$$

Determination of White Blood Cells (WBCS)

Method: Manual WBC counting method was used as follows.

Dilution of Blood

1. The blood specimen was mixed approximately for one minute; using the aspirator and WBC pipette, blood was drawn to the 0.5 mark in the pipette.
2. Blood was removed from the outside of the pipette with clean gauze.
3. Holding the pipette almost vertically, the tip was placed into the counting diluting fluid to
4. The draw it slowly. While gently rotating the pipette, to ensure proper mixing, the diluting fluid was aspirated until it reached the 11 mark. pipette was placed in a horizontal position and firmly holding the index finger of either hand over the opening in the tip of the pipette, aspirator was detached from the other end of the pipette. This is 1:20 dilution.
5. Having now completed the dilution of blood, the counting chamber and cover glass were cleaned with a lint-free cloth.

Filling the counting chamber: Approximately, 0.02ml of well mixed EDTA- anticoagulated venous blood sample was added to 0.38ml of diluted fluid and dispensed into a small container. One of the grids of the counting chamber was filled with re-mix of the diluted blood sample using a Pasteur pipette, taking care not to overfill the area. The filled area was left undisturbed for two minutes to allow time for the white blood cells to settle, after which the underside of the chamber was dried and placed on the microscope stage.

Counting the white blood cells: Using the l0x objective, with the condenser iris closed sufficiently to give good contrast, the ruling of the chamber and white cells were focused until the cells appeared as small black dots. The cells in the four large squares of the chamber werethen squarely counted.

Calculations

1. The number of white cells per liter of blood was calculated as follows:
2. The total number of cells counted was divided by 2
3. The number obtained was then divided by 10
4. The result was then multiplied by 109 to give the white cell count.

Determination of Differential White Blood Cell Counts

Method: Thin blood films were made on slides and allowed to air- dry. The blood smears were fixed by flooding with methanol after which the slides were flooded with Wright's stain and after four minutes mixed with an equal volume of phosphate buffer and allowed to stand for seven minutes. The slides were rinsed using distilled water and stood up on one end to dry.

Counting the differential white blood cells: The stained blood smears were examined using the l0x objective lens. A drop of oil

immersion was placed on the slides and counting began in areas where RBCs were slightly overlapping; moving the slides, each white cell was counted and recorded on a differential cell counter until 100 white blood cells have been counted (Monocyte, lymphocytes, neutrophils, basophils and eosinophils).

Platelet Count

Test procedure

A 1: 200 dilution of the blood and the diluting fluid was prepared using Thoma pipette. The Neubauer chamber was charged with well mixed blood and the platelet was allowed to settle in a moist chamber for 5 minutes. The ruled area in the chamber was located and platelet counted using the 40x objectives.

Calculation

Total number of platelets/ul= Number of platelet counted 500

Statistical Analysis

Results were presented as the mean ± standard deviation. A two-sided p <0.05 was considered statistically significant for t-test (used to determine the differences between the groups).

Results

Haemoglobin concentration was significantly (p=0.004) higher in gestational diabetic women (11.49 ± 1.01g/dl) when compared with the control subjects (normal healthy pregnant women) (10.73 ± 0.75g/dl)). PCV in gestational diabetic women (33.84 ± 3.26%) was significantly (p=0.028) higher when compared to controls (32.06 ± 2.15%). Platelet count was significantly (p=0.000) higher in gestational diabetic women (512.52 ± 106.76 x 10^9/L) when compared to the control subjects (197.60 ± 77.62 × 10^9/L). The mean value of TWBC was significantly higher (p=0.000) in gestational diabetic women (8.07 ± 2.01 × 10^9/L) when compared with the control subject (5.38 ± 1.89 × 10^9/L). Neutrophil count was significantly (p=0.000) higher in gestational diabetic women (53.04 ± 9.99%) when compared to controls (43.60 ± 6.24%). There was no statistically significant difference (p=0.551) in the mean value of eosinophil count in gestational diabetic women (0.28 ± 0.54%) when compared with the control subjects (0.20 ± 0.41%). Basophils were not seen in gestational diabetic women and the control subjects. Lymphocyte count was significantly (p=0.039) reduced in gestational diabetic women (45.78 ± 09.92%) when compared with the control subject (56.70 ± 5.32%). There was no statistically significant difference (p=0.146) in the mean value of lymphocyte count in gestational diabetic women (0.10 ± 0.00%) when compared with the control subjects (0.30 ± 0.00%) **(Table 1).**

Table 1 Mean values of HB, PCV, TWBC, platelets, neutrophils, eosinophils, lymphocytes and monocytes in preeclampsia women and normal pregnant women.

Haematological	parameters Test (n=50)	Control (n=20)	P-value
HB (g/dl)	11.49 + 1.01	10.73 + 0.75	0.004*
PCV (%)	33.84 + 3.26	32.06 + 2.15	0.028*
Platelets (× l0^9/L)	512.52 + 106.76	197.60 ± 77.62	0.000*
TWBC (× 10^9/L)	8.07 + 2.01	5.38 + 1.89	0.000*
Neu (%)	53.04 + 9.99	43.60 ± _6.24	0.000*
Eos (%)	0.28 + 0.54	0.20 + 0.41	0.551
Lymph (%)	45.78 + 9.92	56.70 ± _5.32	0.000*
Monocyte (%)	0.10 + 0.00	0.30 + 0.00	0.146

HB: Haemoglobin
PCV: Packed cell volume
TWBC: Total white blood cell count
Neu: Neutrophil count
Eos: Eosinophil count
Lymp: Lymphocyte count
%: percentage
p<0.05 (* Significant); P>0.05 (Not significant).

Discussion

Increase in blood glucose level in pregnancy is one of the factors that change the erythrocyte morphology. The extent of change in shape of erythrocyte depends on the level of blood glucose level. All this affects the flow property of blood due to alteration and deformation [15].

There is abundant evidence that haematological parameters vary considerably in gestational diabetic women when compared with normal pregnant women [15]. These changes

may be due to several factors, which includes, the level of insulin, placental hormones secreted in pregnancy, increased erythropoietin production and increased plasma volume [16]. Reports from all over the World have been published on haemoglobin, packed cell volume, erythrocyte sedimentation rate, platelets and white cell count variables in pregnant women but finding on these parameters in gestational diabetes is scare.

Result from the present study reveals that the level of haemoglobin and PCV in gestational diabetes was significantly ($p < 0.05$) higher when compared to normal pregnant women. The increase in haemaglobin and PCV level in gestational diabetes might probably due to an increase in blood glucose level which probably favors the proliferation of red blood cells. The result agrees with the study carried out by Dhirendra et al. [17] but the study carried out by Asmah et al. did not find any significance difference in haemoglobin and PCV level in gestational diabetes and normal pregnancy. Geographical location, time of collection of the blood sample might be a predisposing factor for the variation of result.

The present study reveals a significant ($p < 0.05$) increase in platelet count in gestational diabetes when compared to controls. Pregnant women with gestational diabetes have an increased risk of coagulation abnormalities and thromboembolic events [18]. Platelets have a key role and increased adhesion, activation, and aggregation of platelets due to dysregulation of several signaling pathways and metabolic disturbances of insulin. Systematic inflammation, oxidative stress, impaired calcium metabolism, decreased bioavailability of nitric oxide, increased phosphorylation and glycosylation of cellular proteins are responsible for increased platelet activation and increased release of prothrombotic and proinflammatory agents in diabetes [19]. Biadgo et al. also reported that platelet indices such as mean platelet volume and platelet distribution width were significantly higher in gestational diabetic women [20].

Total white blood cell count and neutrophil count was significantly ($p < 0.05$) increased in gestational diabetes when compared to control. Inflammation isclosely associated with both secretory function of beta cell and insulin resistance [20]. Circulating inflammatory molecules can decrease beta cell functions directly by secretory dysfunction or uncontrolled apoptosis [20]. As a result glucotoxicity and lipotoxicity occurs and causes enhanced inflammatory process and a vicious cycle. Elevated WBC is a classical inflammatory marker and reveals association of inflammation with impaired glucose metabolism, insulin resistance and diabetes mellitus [8]. Lymphocyte count was significantly ($p < 0.05$) reduced in gestational diabetic women when compared to control. The decrease might be due to glucose toxicity affecting the proliferation of the B and T lymphocyte cell [8]. There was no significant difference in eosinophil and monocyte count in gestational diabetes when compared with that of the control subjects. Eosinophils are mainly increased in parasitic infection and do not have any relationship with blood glucose level.

Conclusion

Pregnant women with gestational diabetes had significant higher levels of haemoglobin, PCV, TWBC, neutrophils and platelet count. Gestational diabetes is associated with low level of lymphocyte, whereas there is no significant difference in eosinophil and monocyte count in gestational diabetic women when compared with normal pregnant women.

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