



Haematological and Anthropometric Profile in Gestational Diabetes Mellitus with Non-diabetic Pregnant Women in Port Harcourt, Nigeria

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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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ABSTRACT

Aim: Haematological and anthropometric profile of pregnant women who had gestational diabetes and non-diabetic pregnant women attending antenatal clinic in a Tertiary Hospital in Rivers State, Nigeria were evaluated,

Methods: This was a cross sectional study involving 70 pregnant women comprising of 40 diabetic and 30 non-diabetics for the purpose of comparing haematological and anthropometrical parameters in diabetic and non-diabetic pregnant women. Using standard venipuncture technique, 5ml blood sample was aseptically drawn from the subjects and 2.5ml dropped into each of two different anticoagulant sample bottles -Ethylene Diamine Tetra acetic Acid(EDTA) and fluoride-oxalate bottles respectively. The methods of choice for estimation of parameters were Enzymatic Colorimetric method for glucose and Automation using Sysmex KX-21N Haematology Analyzer for full blood count. Results were statistically analyzed using Graph Pad prism version 5.0 and statistical significance set at $P < 0.05$.

Results: Mean \pm SD results for diabetic and non-diabetic pregnant women respectively showed Haemoglobin concentration (10.99 \pm 0.69g/dl versus 10.69 \pm 1.01g/dl), Packed cell volume (31.7 \pm 1.96% versus 32.89 \pm 3.89%), Red blood cell (3.99 \pm 0.29 $10^6/\mu\text{l}$ versus 3.85 \pm 0.39 $\times 10^6/\mu\text{l}$), Mean

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cell haemoglobin (27.51 ± 1.22 pg versus 27.54 ± 2.22 pg), Platelets count ($223.3 \pm 52.67 \times 10^3/\mu\text{l}$ versus $205.5 \pm 45.09 \times 10^3/\mu\text{l}$), Mean cell haemoglobin concentrations (34.49 ± 1.19 g/dl versus 32.54 ± 3.17 g/dl) these results were not statistically significantly different ($P > 0.05$). The mean \pm SD of white blood cell ($9.73 \pm 0.49 \times 10^3/\mu\text{l}$ versus $7.27 \pm 1.66 \times 10^3/\mu\text{l}$) and mean cell volume (79.3 ± 4.16 fl and 85.24 ± 0.39 fl) were statistically significantly different ($P < 0.05$). Mean \pm SD of subject's blood pressure showed, Systolic (116 ± 11.52 mm/Hg and 105 ± 5.72 mm/Hg), Diastolic (77 ± 17.86 and 68 ± 1.11 mm/Hg), results were statistically significantly different ($P < 0.001$). Age did not show statistical significant difference ($P > 0.05$). Body mass index (BMI) 28.33 ± 3.81 kg/m², 32.86 ± 5 kg/m², 37.96 ± 3.44 kg/m² showed statistically significant difference ($P < 0.05$) at the Gestational ages of (1-3), (4-6) and (7-9) months respectively while that of the non-diabetic were obviously not statistically significantly different ($P > 0.05$).

Conclusion: Gestational diabetes is capable of causing changes in haematological parameters; but it could improve or disappear after child birth. Factors such as lifestyle activities and diet can predispose pregnant women to gestational diabetes mellitus and this can lead to complications for both the mother and the baby.

Keywords: Diabetes; gestation diabetes; haematological parameters.

1. INTRODUCTION

Pregnancy is a state characterized by many physiological haematological changes, which may be seen to be pathological in the non pregnant state. Haematological parameters such as the haemoglobin concentration, packed cell volume, red blood cells, mean cell volume, mean cell haemoglobin, mean cell haemoglobin concentration, platelets and white blood cells usually undergo some changes which may range from moderate to severe during pregnancy [1]. The severe changes may be due to some underlying conditions which may include gestational diabetes, gestational thrombocytopenia, pregnancy induced hypertension [2]. The haemoglobin concentration of women during the period of gestation have always been shown to be at a decreased level [3]. This is because the blood volume increases by 50% in the course of pregnancy for providing essential nutrient for the developing baby. It is quite normal that the haemoglobin concentration for a pregnant mother should range from 11.0g/dl to 14.0g/dl [4]. Even though a decreased level of haemoglobin is observed in pregnant women (with a mild variation), a decrease below 10.5g/dl causes mild anaemia and several other health issues that affect the mother and child as the pregnancy progress [5]. The haemoglobin level starts to increase around the 8th week of conception. This is because the body of a pregnant woman requires more oxygen and the oxygen-carrying capacity of the blood is proportional to the circulating haemoglobin concentration [6]. A normal adult platelet count ranges from 145×10^3 to $450 \times 10^3/\mu\text{l}$, pregnancy can make the count dip to between 100×10^3 and

$150 \times 10^3/\mu\text{l}$, this is when mild gestational thrombocytopenia is diagnosed. It affects about one pregnant woman out of ten and usually develop from mid to late pregnancy [7]. There is an increase in white blood cell count in impaired glucose tolerance as seen in diabetes in pregnancy [8], however, the blood glucose level of a woman may become higher than normal during pregnancy. This is known as gestational diabetes. At this point, the fasting blood glucose of the pregnant women exceeds normal level.

Gestational diabetes mellitus is a condition in which there is a development of carbohydrate intolerance which leads to an increase in the blood glucose level above normal during pregnancy. Gestational diabetes mellitus (GDM) is similar to type 2 diabetes mellitus in several manners, involving a combination of relatively inadequate secretion of insulin and responsiveness. It occurs in about 2–10% of all pregnancies and may improve or disappear after child birth. After pregnancy approximately 5–10% of mothers with gestational diabetes are seen to have diabetes mellitus, most commonly type 2. Gestational diabetes is fully treatable, but requires careful medical supervision throughout the period of gestation.

If there is a prolonged circulation of excess sugar in the mother's blood, it enters the fetal circulation through the placenta. This leads to complications for both the mother and the baby. If gestational diabetes is left unmanaged, it can affect the baby at birth and right after birth in the following ways; Excess Growth (macrosomia): the baby becomes very big with extra fat. This bigger baby is more likely to become wedged in

the birth canal. This can make delivery challenging and a Cesarean section may be needed for safe delivery. Respiratory Distress Syndrome: babies born of women with gestational diabetes may sometimes have trouble breathing on their own right after birth, trouble with breathing may stop after the lungs become stronger. Low Blood Glucose (Hypoglycaemia): the blood glucose level may fall very low right after the baby is born because of too much insulin in their bodies. The extra glucose in the mother's body actually stimulates the body of the baby to make more insulin, so after delivery the extra insulin poses a threat to the newborn.

Gestational diabetes can affect the child later on by causing development problem such as language development skill and motor skill development [9]. There is also a higher risk of developing type 2 diabetes later in life.

After confirmation of gestational diabetes mellitus, treatment starts with medical nutrition therapy, physical activity and weight management depending on the pre-gestational weight. Fortunately, careful control of the blood sugar level can eliminate virtually all of the risk factors associated with gestational diabetes. Medical practitioners and researchers recommend the following for women diagnosed with gestational diabetes:

- Meet with a dietician that can help you review healthy food options and plan your meal for you.
- Always check your blood sugar level in the morning to obtain your fasting rate and then an hour after each meal to ensure your blood glucose remains in a healthy range.
- Keep a food log, and write down everything you have eaten along with your blood glucose number after each meal. This gives you a better knowledge of the kind of food that is spiking your glucose so that they can be avoided.
- Go for a walk or other mild exercise after each meal to lower your glucose level.

Supplementary insulin may be required in cases where the above mentioned is not enough to control gestational diabetes. Initiation of Pharmacologic therapy is required for women with greater initial degrees of hyperglycemia. Treatment has been demonstrated to improve perinatal outcomes [10].

2. MATERIALS AND METHODS

2.1 Study Design

This was a cross sectional study designed to involve pregnant and non-pregnant subjects. Demographic and clinical data were obtained using a designed questionnaire to include the following information: name, age, height, weight, body mass index, blood pressure, use of blood supplement drugs/diet, gravidity, parity, age of pregnancy and history of diabetes.

2.2 Study Area

The study was carried out at Braithwaite Memorial Specialist Hospital (BMSH) now Rivers State University Teaching Hospital, Port Harcourt. The Hospital has a well-managed antenatal care clinic.

2.3 Ethical Consideration

Due permission was obtained from the hospital management of Rivers State University Teaching Hospital, Port Harcourt and informed consent obtained from pregnant women attending antenatal clinic in the hospital.

2.4 Study Population

This study was carried out on pregnant women suspected to be diabetic and control samples were obtained from non-diabetic pregnant women attending antenatal clinic in Rivers State University Teaching Hospital. Subjects comprised of seventy pregnant women, forty women with gestational diabetes who served as test subject and 30 non diabetic pregnant women who served as control subject.

2.5 Subject Selection

Convenient sampling technique was employed.

2.6 Sample Collection

After the withdrawal of 5ml of fasting venous blood sample from each of the subjects, the samples were aseptically shared 2.5ml into fluoride-oxalate anticoagulant sample bottles and labeled 'fasting blood' and the remaining 2.5ml into an ethylene diamine tetra acetic acid (EDTA) anticoagulant sample bottles.

2.7 Estimation of Serum Glucose Level

Method: Enzymatic colorimetric method

Principle of glucose oxidase-peroxidase : Glucose oxidase catalyses the oxidation of glucose to give hydrogen peroxide and gluconic acid in the presence of the enzyme peroxidase, the hydrogen peroxide is broken down and the oxygen released reacts with 4-aminoantipyrine and phenol to give a pink colour. The absorbance of the colour produced is measured in a colorimeter at a wavelength of 520nm.

2.8 Estimation of Full Blood Count

Using the automated Sysmex KX-21N Haematology Analyzer, haematologic parameters analyzed were; Haemoglobin concentration (HB), Packed cell volume(PCV), Red blood cell (RBC), Mean cell haemoglobin (MCV), Platelet's count (PT), Mean cell haemoglobin concentrations(MCHC), white blood cell (WBC) and mean cell volume (MCV).The results displayed on the LCD screen after automatic analyses were printed out.

2.9 Statistical Analysis

The results were expressed as mean standard deviation. The data were analyzed using Graph Pad prism version 5.0, ANOVA and statistical significance set at $P < 0.05$.

2.10 Eligibility Criteria

Non-pregnant women, Pregnant women who are cigarette smokers and alcohol consumers, and pregnant women with history of renal and liver dysfunction were excluded. Pregnant women with diabetes mellitus and non-diabetic pregnant women were included in the study.

3. RESULTS

3.1 Comparison of Haematologic Parameters and Blood Pressure and Age of Diabetic and Non-Diabetic Pregnant women

The mean \pm SD of the Haemoglobin Concentration for the diabetic pregnant women and the non-diabetic pregnant women were (10.99 \pm 0.69g/dl and 10.69 \pm 1.01g/dl)respectively, Packed Cell Volume were (31.7 \pm 1.96% and 32.89 \pm 3.89%) respectively, Red Blood Cell

were(3.99 \pm 0.29 $\times 10^6/\mu\text{l}$ and 3.85 \pm 0.39 $\times 10^6/\mu\text{l}$) respectively, Mean Cell Haemoglobin were(27.51 \pm 1.22pg and 27.54 \pm 2.22pg) respectively, Mean Cell Haemoglobin Concentration were (34.49 \pm 1.19 g/dl and 32.54 \pm 3.17g/dl) respectively and for Platelet Count, it was(223.3 \pm 52.67 $\times 10^3/\mu\text{l}$ and 205.5 \pm 45.09 $\times 10^3/\mu\text{l}$) respectively. These results showed no statistically significant difference($P > 0.05$).The mean \pm SD for Mean Cell Volume for diabetic and non- diabetic pregnant women were (79.3 \pm 4.16fl and 85.24 \pm 0.39fl) respectively .White Blood Cell were (9.73 \pm 0.49 $\times 10^3/\mu\text{l}$ and 7.27 \pm 1.66 $\times 10^3/\mu\text{l}$)respectively. These results were statistically significantly different ($P < 0.05$). The mean \pm SD for diabetic and non- diabetic pregnant women, for Systolic blood pressure(116 \pm 11.52mm/Hg and 105 \pm 5.72 mm/Hg) respectively, Diastolic blood pressure (77 \pm 17.86mm/Hg and 68 \pm 1.11 mm/Hg) respectively showed statistically significant difference ($P < 0.05$). Mean \pm SD of Age for diabetic and non- diabetic pregnant women respectively (31.7 \pm 4.60years and 29.9 \pm 4.16years) showed no statistically significant difference.(Table 1)

3.2 Comparison of Haematologic Parameters and Body mass index of Diabetic Pregnant Women by Gestational Age at three different trimesters

The mean \pm SD for haemoglobin concentration for 1-3months, 4-6months and 7-9months respectively were (11.95 \pm 0.37 g/dl, 11.25 \pm 0.28 g/dl and 10.67 \pm 0.70 g/dl), Packed cell volume were(33.98 \pm 1.41%, 32.33 \pm 0.92% and 31.1 \pm 2.13%) and Red Blood Cell (4.28 \pm 0.021 $\times 10^6/\mu\text{l}$, 4.13 \pm 0.211 $\times 10^6/\mu\text{l}$ and 3.87 \pm 0.30 $\times 10^6/\mu\text{l}$), These results showed statistically significant difference ($P < 0.05$). The mean \pm SD of the diabetic pregnant women at the three different trimesters for Mean Cell Volume were (76.75 \pm 5.70fl, 78.41 \pm 3.21fl and 80.37 \pm 4.24fl) respectively, Mean Cell Haemoglobin (27.88 \pm 0.92pg, 27.26 \pm 0.8809pg and 27.58 \pm 1.43pg), Mean Cell Haemoglobin Concentration (35.2 \pm 0.86 g/dl, 34.78 \pm 0.80 g/dl and 34.2 \pm 1.36g/dl), White Blood Cell (9.52 \pm 0.17 $\times 10^3/\mu\text{l}$, 9.77 \pm 0.41 $\times 10^3/\mu\text{l}$ and 9.75 \pm 0.57 $\times 10^3/\mu\text{l}$) and Platelet Count (219.3 \pm 40.66 $\times 10^3/\mu\text{l}$, 241.9 \pm 74.67 $\times 10^3/\mu\text{l}$ and 213. \pm 36.63 $\times 10^3/\mu\text{l}$) did not show a statistically significant difference ($P > 0.05$). The mean \pm SD of BMI at different gestational age

Table 1. Comparison of Haematological Parameters, SBP, DBP and Age of Diabetic and Non-diabetic Pregnant Women

Parameters	DPW	NDPW	t-value	P-value	Remarks
HGB (g/dl)	10.99±0.69	10.69±1.01	1.476	0.1445	Ns
PCV (%)	31.7±1.96	32.89±3.89	1.552	0.1252	Ns
RBC (×10 ⁶ µl)	3.99±0.29	3.85±0.39	1.748	0.0849	Ns
MCV (fl)	79.37±4.16	85.24±11.68	2.942	0.0045	S
MCH (pg)	27.51±1.22	27.54±2.22	0.0849	0.9291	Ns
MCHC (g/dl)	34.49±1.19	32.54±3.17	3.578	0.006	S
WBC (×10 ³ µl)	9.73±0.49	7.27±1.66	8.837	<0.001	S
PLT (×10 ³ µl)	223.3±52.67	205.5±45.09	1.48	0.1436	Ns
SBP (mm/Hg)	116±11.72	105±5.72	4.728	<0.0001	S
DBP (mm/Hg)	77±17.86	68±1.11	2.643	0.0102	S
AGE(years)	31.7±4.60	29.9±4.16	1.686	0.0964	Ns

Keys: HGB = Haemoglobin. PCV = Packed cell volume. RBC = Red blood cell. MCV = Mean cell volume. MCH = Mean cell Haemoglobin. MCHC = Mean cell haemoglobin concentration. PLT = Platelet WBC = White Blood Cell. SBP = Systolic blood pressure. DBP = Diastolic blood pressure. DPW = Diabetic pregnant women. NDPW = Non-diabetic pregnant women. S = Significant difference. Ns = No significant difference.

Table 2. Comparison of haematological parameters and BMI in diabetic pregnant women by gestational age

Gestational Age	1-3 months n=4	4-6 months n=13	7-9 months n=23	F-value	P-value	Remarks
HGB (g/dl)	11.95±0.3695	11.25±0.2757	10.67±0.695	10.570	0.002	S
PCV (%)	33.98±1.406	32.33±0.9214	31.1±2.134	5.406	0.0087	S
RBC(×10 ⁶ /µl)	4.28±0.022	4.131±0.2127	3.87±0.3039	6.562	0.0036	S
MCV (fl)	76.75±5.695	78.24±3.205	80.37±4.236	1.885	0.1662	Ns
MCH (pg)	27.88±0.917	27.26±0.8809	27.58±1.43	0.4743	0.6261	Ns
MCHC (g/dl)	35.2±0.875	34.78±0.804	32.2±1.362	1.826	0.1752	Ns
WBC(×10 ³ /µl)	9.52±0.170	9.769±0.4131	9.75±0.5736	0.3916	0.6787	Ns
PLT(×10 ³ /µl)	219.3±40.66	241.9±94.67	213±36.63	1.247	0.2992	Ns
BMI(Kg/m ²)	28.33±3.81	32.86±6.561	37.96±3.44	9.783	0.0004	S

Keys: HGB = Haemoglobin. PCV = Packed cell volume. RBC = Red blood cell. MCV = Mean cell volume. MCH = Mean cell Haemoglobin. MCHC = Mean cell haemoglobin concentration. PLT = Platelet WBC = White Blood Cell. BMI = Body mass index. S = Significant difference. Ns = No significant difference

Table 3. Comparison of haematological parameters, SBP, DBP, age and BMI in non-diabetic pregnant women by trimester

Gestational Age	1-3 months n=11	4-6 months n=19	t-value	P-value	Remarks
HGB (g/dl)	10.82±0.606	10.61±1.193	0.5357	0.5964	Ns
PCV (%)	32.83±3.762	32.33±0.9214	0.6593	0.9479	Ns
RBC (×10 ⁶ µl)	3.865±0.3064	3.849±0.4414	0.1093	0.9137	Ns
MCV (fl)	83.55±1.87	86.22±11.77	0.5969	0.5554	Ns
MCH (pg)	27.41±1.901	27.63±2.453	0.2524	0.8027	Ns
MCHC (g/dl)	33.21±2.807	32.15±3.381	0.879	0.3869	Ns
WBC (×10 ³ /µl)	7.109±2.037	7.378±1.448	0.4153	0.6812	Ns
PLT (×10 ³ /µl)	219.3±46.71	197.6±43.37	1.284	0.2096	Ns
SBP (mm/Hg)	104.5±6.876	105.3±5.13	0.3258	0.7470	Ns
DBP (mm/Hg)	68.18±6.03	67.89±6.306	0.122	0.9037	Ns
AGE (years)	31.09±4.277	29.21±4.049	1.201	0.2398	Ns
BMI (kg/m ²)	27.07±3.159	30.2±2.968	2.717	0.0112	Ns

HGB = Haemoglobin. PCV = Packed cell volume. RBC = Red blood cell. MCV = Mean cell volume. MCH = Mean cell Haemoglobin. MCHC = Mean cell haemoglobin concentration. PLT = Platelet, WBC = White Blood Cell. SBP = Systolic blood pressure. DBP = Diastolic blood pressure. BMI=Body Mass Index, S = Significant difference. Ns = No significant difference

(1-3), (4-6) and (7-9) months respectively were $28.33 \pm 3.81 \text{ kg/m}^2$, $32.86 \pm 5 \text{ kg/m}^2$ and $37.96 \pm 3.44 \text{ kg/m}^2$, the result showed statistically significant difference ($P < 0.0004$). This result is shown in (Table2).

3.2 Comparison of Haematological Parameters and Body Mass Index in Non-diabetic Pregnant Women by Gestational Age at three different trimesters

Comparison of the mean \pm SD for all haematologic parameters in the first and second trimesters 1-3months, 4-6months respectively of the non-diabetic pregnant women showed no statistically significant difference ($P > 0.05$). Haemoglobin concentration were ($10.82 \pm 0.61 \text{ g/dl}$ and $10.61 \pm 1.91 \text{ g/dl}$), Packed cell volume ($32.83 \pm 3.76\%$ and $32.93 \pm 4.07\%$), Red Blood Cell ($3.87 \pm 0.31 \times 10^6/\mu\text{l}$ and $3.85 \pm 0.44 \times 10^6/\mu\text{l}$), Mean Cell Volume ($85.55 \pm 1.87 \text{ fl}$ and $86.22 \pm 1.77 \text{ fl}$) and Mean Cell Haemoglobin ($27.41 \pm 1.90 \text{ pg}$ and $27.63 \pm 2.45 \text{ pg}$), Mean Cell Haemoglobin Concentration ($33.21 \pm 2.81 \text{ g/dl}$ and $32.15 \pm 3.38 \text{ g/dl}$), White Blood Cell ($7.11 \pm 2.03 \times 10^3/\mu\text{l}$ and $7.38 \pm 1.45 \times 10^3/\mu\text{l}$) and Platelet count ($219.3 \pm 46.71 \times 10^3/\mu\text{l}$ and $197.6 \pm 43.33 \times 10^3/\mu\text{l}$). The mean \pm SD for systolic blood pressure were $104.5 \pm 6.88 \text{ mm/Hg}$ and $105.3 \pm 5.13 \text{ mm/Hg}$, Diastolic Blood Pressure $68.18 \pm 6.03 \text{ mm/Hg}$, $67.89 \pm 6.31 \text{ mm/Hg}$, The both results showed no significant difference ($P > 0.05$). Also the mean \pm SD for age ($31.09 \pm 4.28 \text{ years}$ and $29.21 \pm 4.05 \text{ years}$) and that of BMI which were ($27.07 \pm 3.16 \text{ kg}$ and $30.2 \pm 2.97 \text{ kg}$) showed no significant difference. This result is shown in (Table 3).

4. DISCUSSION

Pregnancy is a physiological condition. However, some women develop problems during pregnancy period, which puts both the mother's and the foetus health at risk. Hypertensive disorders, gestational diabetes, gestational thrombocytopenia are the types of the maternal diseases that can cause the most detrimental effects to the mother and foetus [2]. Diabetes mellitus in pregnancy is similar to type 2 diabetes mellitus in several manners; it involves a combination of relatively inadequate secretion of insulin and responsiveness. It may improve or disappear after putting to birth [11]. Factors such as lifestyle, physical in activities and improper

diet can predispose one to gestational diabetes mellitus. This can lead to complications for both the mother and the baby. Different studies have been carried out to evaluate the changes in haematological parameters in pregnant women, but, little is known about the relationship between gestational diabetes and haematological parameters in pregnant women in Nigeria especially Rivers State. This Study was carried out with the aim to evaluate the likely changes that may occur as a result of diabetic conditions and comparing the values obtained with those gotten from normal pregnant women. According to recent reports, 9.2% of women with pregnancy have gestational diabetes [10]. From the studies, there was a significant increase in mean cell volume, mean cell haemoglobin concentration and white blood cell when comparing diabetic and non-diabetic pregnant women; with P-values =0.0045, =0.006 and = 0.001 respectively. This could be attributed to Preeclampsia leads to the enhancement of physiological blood changes like neutrophilic leucocytosis, Hyperlipidaemia, pro-coagulant property and inflammation Another fact observed was a Statistically significant difference in systolic and diastolic blood pressure with P-values =0.001 and =0.0102 respectively as shown in table 1. The increase in systolic and diastolic blood pressure may be due to physiological rise in blood volume leading to dilution anaemia and a dip in blood pressure maximum at 20th week gestation which could be an indication of Preeclampsia. This study is in agreement with a study which showed that haematological parameters in the assessment of pregnancy induced hypertension shows that there is a significant difference in the haemoglobin concentration, packed cell volume, red blood cell and with P-values of 0.002, 0.0087 and 0.0036 respectively [12]. The decrease in these parameters observed between the second and the third trimester of pregnancy may be as a result of continuous expansion in plasma volume by 10-15%.

5. CONCLUSION

Gestational diabetes may cause some changes in some haematological parameters and may lead to pregnancy induced hypertension which can cause significant maternal and perinatal morbidity and mortality [13]. Preeclampsia is a maternal pathology involving placental modification, and it is one the most common and important hypertensive disorder of pregnancy which could be attributed to poor management. These modifications can lead to placental

abnormalities which can cause haematological abnormalities and foetal complication [14]. The increase in some haematological parameter in diabetic pregnant women especially during the third trimester and in this study, is an indication of preeclampsia and its complication in gestational diabetes mellitus can be averted if properly managed.

DISCLAIMER

The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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