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Research Article

Homocysteine, Red Cell Distribution Width and Platelet Indices among Normal Pregnant Women at Federal Medical Center, Katsina, Nigeria

ABDULQADIR, Ibrahim[1*] and ISYAKU, Mujittaba Mashi[2]

[1]Department of Haematology and Blood Transfusion, Usmanu Danfodiyo University Teaching Hospital (UDUTH), Sokoto, Nigeria
[2]Department of Haematology and Blood Transfusion, Federal Medical Centre (FMC), Katsina, Nigeria

*Corresponding Author
ABDULQADIR, Ibrahim

Abstract: Homocysteine, red cell distribution width and platelet indices are emerging as simple but important markers with diagnostic and prognostic potentials in obstetrics practice. This necessitates the need for local references to guide appropriate intervention to prevent fetal and maternal complications. This study aimed to determine the homocysteine, red cell distribution width and platelet indices of normal pregnant women at various trimesters. Two hundred women were enrolled to represent subjects and controls of the study. Demographic and clinical data were collated. Six (6) milliliters of venous blood was obtained from each participant for full blood count and homocysteine assay with Sysmex XT 2000i and ELISA method respectively. The subjects had higher red cell distribution width (p = <0.001) and platelet large cell ratio (p = <0.001) and lower platelets count (p = 0.02) in comparison to the controls and there was no difference between subjects and controls with respect homocysteine (p = 0.26). Among the subjects, there was no difference in any of the parameters on account of trimester of pregnancy or gravidity (p >0.05) and their platelet count shows significant positive correlation with Plateletcrit (r = 0.36, p = 0.02) and negative correlation with platelet large cell ratio (r = -0.13, p = 0.03). Local reference source for emerging markers of diseases has been established for pregnant women and their values are different from those of non pregnant women in the same environment. We therefore recommend that, these differences should be taking in to consideration to guide appropriate interventions.

Keywords: platelet indices, red cell distribution width, homocysteine, reference values, pregnant women, Katsina, Nigeria.

INTRODUCTION

Non invasive markers of disease are continuously been explored and have gained scientific interest because of their ability to guide diagnosis, choice of treatment and prognosis in various specialties of medicine. (Inchingolo R *et.al.* 2019; Panagiotis P *et al.*, 2018; Mariano M *et al.*, 2014; Ustundag YB *et al.*, 2016; Sun IO *et al.*, 2016; Melissa KSL *et al.*, 2018). Homocysteine, red cell distribution width (RDW) and platelet indices (PIs) are some of these markers emerging as predictors of both complications and outcomes of pregnancy such as pre-eclampsia, gestational diabetes, placental abruption, recurrent miscarriages, preterm labour, still birth, very low birth weight and neural tube defect among others. (Panagiotis P *et al.*, 2018; Stein EV *et al.*, 2000; Bergen NE *et al.*, 2012; Alsheeha MA *et al.*, 2016; Abdurrahman AA *et al.*, 2018; Burcu AU *et al.*, 2014). High serum homocysteine level in addition to its known effect on cardiovascular disease; was shown to be associated with hypertensive disorders in pregnancy, oligohydramnios and meconium stained amniotic fluid while variations of PIs like mean platelet volume (MPV), platelet distribution width (PDW) and plateletcrit (PCT) can be used in predicting the onset of pre-eclampsia and recurrent pregnancy loss (Mariano M *et al.*, 2014; Abdurrahman AA *et al.*, 2018; Dhakre R *et al.*, 2018). Although, there is need for additional researches specifically design to evaluate the benefit of such markers in the management of pregnancy, their putative role should not be discarded at this stage. This is particularly true in low resource setting since some of the markers are generated with no extra effort from cheap and most widely requested investigation during the course of pregnancy. However, in spite of their potential benefit in rationalizing obstetrics intervention,

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their kinetics such as variations with genetic and acquired factors like age, cigarette smoking, alcohol consumption and physical activity, make it difficult to adopt a single reference value for general use and thus highlighted the need for local reference (Ustundag YB *et al.,* 2016; Giovanetti TV *et al.,* 2011).

This study was conducted to determine serum homocysteine, RDW and PIs namely platelet count, MPV, PDW PCT and platelet large cell ratio (PLCR) among normal pregnant women in Federal Medical Center, Katsina, North-Western, Nigeria.

MATERIALS AND METHOD

The study was a comparative cross sectional, conducted among 100 pregnant women (subjects) recruited at various stages of pregnancy at antenatal clinic of Federal Medical center, Katsina and 100 non pregnant women (controls) recruited at family planning clinic of the same hospital between April and December, 2017. Exclusion criteria in this study were non consent, haemoglobin concentration <10g/dL, breastfeeding, sickle cell disease, hypertension, diabetes, HIV and viral hepatitis. Additional exclusion criteria for non pregnant women were hormonal contraception, infertility, bleeding and diagnosis of any form of malignancy.

Clinical data of participants comprising of evidence of pregnancy, sickle cell disease, hypertension, diabetes and use of medication were extracted from their case file. Following standard venepuncture procedure, 6ml of venous blood was collected from each participant. Three (3) milliliters of venous blood sample each were dispensed into Ethylene Diamine Tetra Acetic acid (EDTA) and plain bottles for determination of full blood count and serum homocysteine respectively.

Full blood count was performed within 2 hours of sample collection with Sysmex XT 2000i haematology analyser while serum harvested from clotted samples in plain bottles were stored at -20^{0}C. Serum homocysteine was determined with Elisa kit for homocysteine (Wkea Med Supplies Corp. Changchun, Jilin, China) according manufacturer's instruction. Serologic tests for HIV, Hepatitis B and C were conducted using serum of the participants with Determine^{TM} HIV-1/2 Ag/Ab Combo, Ascon and Healgen respectively while pregnancy test was done on early morning urine specimen of non-pregnant women using SURESIGN pregnancy test strip.

Data obtained was analysed with Statistical Package for Social Sciences (SPSS) version 20 (IBM Corp. Armonk, NY). Data set was tested for normality and homogeneity of variance using Kolmogorov-Smirnov and Levene's tests respectively. Student *t* – test and ANOVA were employed for comparison between groups as appropriate whereas Pearson correlation analysis was used to determine association between platelet count and PIs. The results are presented as median value and inter quartile (IQ) range at 2.5% and 97.5% and the level of significant statistical relationship was set at $p < 0.05$.

Informed written consent was obtained from each participant and the study was approved by Hospital Committee on Research Ethics and Patients Protection.

RESULTS

The data set was homogeneously and normally distributed ($p > 0.05$). The mean ± SD of subjects and controls were; for age (26.65±5.75years and 33.05±9.35years, p = <0.001), weight (71.62±15.86kg and 66.15±12.10, p = 0.03) and height (157.90±5.45cm and 157.63±4.80cm, p = 0.68) respectively.

The subjects had higher RDW (p = <0.001), MPV (p = <0.001) and P-LCR (p = <0.001) and lower platelet count (p = 0.02) in comparison to the controls and there is no difference between subjects and controls with respect homocysteine (p = 0.26) as depicted in the Table 1.

Among the subjects, there is no difference in any of the parameters on account of trimester or number of deliveries in the past (p >0.05) as shown in Tables 2 and 3. Platelet count shows significant positive correlation with PCT (r = 0.36, p = 0.02) and negative correlation with P_LCR (r = -0.127, p = 0.03) as presented in Table 4.

Table 1: Comparison of homocysteine, RDW and PIs between subjects and controls

S /Number	Parameters	Subjects N = 100	Controls N = 100	*P* - value
		Median (IQ Range)	Median (IQ Range)	
1	Homocysteine (µmol/L)	11.84 (7.50 – 13.05)	11.39 (8.85 – 13.46)	0.26
2	RDW_CV (%)	39.43 (16.43 – 50.70)	18.53 (9.68 – 30.61)	<0.001
3	RDW_SD (fL)	38.30 (17.19 – 56.48)	17.63 (14.50 – 46.22)	<0.001
4	PLT x 10^9/L	266. 20 (86.00 – 447.00)	297.50 (76.00 – 431.00)	0.02
5	MPV (fL)	9.97 (7.60 – 15.65)	8.56 (6.00 – 16.85)	<0.001
6	PDW (%)	13.92 (7.14 – 23.43)	11.39 (7.60 – 18.44)	0.03
7	PCT (%)	0.22 (0.06 – 1.64)	0.38 (0.19 – 1.64)	<0.001
8	P_LCR (%)	38.75 (21.33 – 54.84)	20.10 (10.10 – 37.90)	<0.001

Statistically significant $p < 0.05$

RDW_CV = red cell distribution width cumulative value, RDW_SD = red cell distribution width standard deviation, PLT = platelet count, MPV = mean platelet volume, PDW = platelet distribution width, PCT = plateletcrit, P_LCR = platelet large cell ratio

Table 2: comparison of homocysteine, RDW and PIs on basis of gestational age

S /No	Parameters	First trimester N = 22	Second trimester N = 41	Third trimester N = 39	*P* - value
		Median (IQ Range)	Median (IQ Range)	Median (IQ Range)	
1	Homocysteine (µmol/L)	11.27 (7.51- 13.36)	11.55 (6.85 – 13.40)	11.32 (7.45 – 13.46)	0.36
2	RDW_CV (%)	18.26 (9.68 – 24.85)	18.60 (14.43 – 27.43)	18.50 (15.85 – 30.70)	0.23
3	RDW_SD (fL)	38.02 (30.07 – 44.40)	38.38 (35.56 – 46.48)	38.24 (27.19 – 43.70	0.33
4	PLT x 10^9/L	259.00 (99.00 – 407.00)	250.00 (80.00 – 377)	257.00 (76.00 – 308.00)	0.44
5	MPV (fL)	10.02 (7.76 – 14.09)	10.05 (8.37 – 15.86)	9.80 (7.60 – 14.57)	0.28
6	PDW (%)	11.95 (7.56 – 17.92)	11.85 (7.14 – 15.86)	9.80 (7.60 – 14.57)	0.38
7	PCT (%)	0.24 (0.11 – 1.39)	0.30 (0.09 – 0.58)	0.26 (0.06 – 0.46)	0.52
8	P_LCR (%)	37.99 (21.33 – 59.25)	39.64 (28.39 – 54.84)	38.63 (22.28 – 56.16)	0.19

Statistically significant $p < 0.05$

RDW_CV = red cell distribution width cumulative value, RDW_SD = red cell distribution width standard deviation, PLT = platelet count, MPV = mean platelet volume, PDW = platelet distribution width, PCT = plateletcrit, P_LCR = platelet large cell ratio

Table 3: comparison of homocysteine, RDW and PIs on basis of gravidity

S /No	Parameters	Primigravidae N = 24	Multigravidae N = 40	Grand multipara N = 38	*P* - value
		Median (IQ Range)	Median (IQ Range)	Median (IQ Range)	
1	Homocysteine (µmol/L)	11.63 (6.85 – 13.36)	11.26 (6.45 – 13.40)	11.39 (7.85 – 13.46)	0.45
2	RDW_CV (%)	18.52 (15.60 – 25.99)	18.51 (15.40 – 27.43)	18.68 (9.68 – 26.61)	0.57
3	RDW_SD (fL)	48.30 (34.97 – 55.34)	48.28 (34.73 – 56.48)	48.30 (37.19 – 54.40)	0.53
4	PLT x 10^9/L	252.00 (86.00 – 401.00)	250.40 (97.00 – 404.00)	249.00 (90.00 – 401.00)	0.17
5	MPV (fL)	10.06 (8.40 – 15.79)	10.01 (8.03 – 16.85)	9.66 (7.60 – 16.10)	0.38
6	PDW (%)	11.58 (7.23 – 15.79)	11.89 (7.14 – 15.43)	12.35 (7.15 – 17.92)	0.32
7	PCT (%)	0.27 (0.09 – 1.86)	0.31 (0.10 – 2.64)	0.32 (0.05 – 2.24)	0.15
8	P_LCR (%)	40.83 (29.59 – 57.51)	38.86 (22.28 – 54.84)	36.87 (21.33 – 51.41)	0.45

Statistically significant $p < 0.05$

RDW_CV = red cell distribution width cumulative value, RDW_SD = red cell distribution width standard deviation, PLT = platelet count, MPV = mean platelet volume, PDW = platelet distribution width, PCT = plateletcrit, P_LCR = platelet large cell ratio

Table 4: Correlation between platelet count and platelet indices among subjects

S /No.	Parameters	Correlation coefficient (*r*)	*P* - value
1	MPV	-0.23	<0.001
2	PDW	-0.21	<0.001
3	PCT	0.36	<0.001
4	P_LCR	-0.13	0.03

Statistically significant $p < 0.05$

MPV = mean platelet volume, PDW = platelet distribution width, PCT = plateletcrit, P_LCR = platelet large cell ratio

DISCUSSION

Reference values in conjunction with clinical details of the individual are essential in transforming any result of laboratory investigation to vital information needed to guide life saving interventions. Yet, reference values are highly variable and frequently affected by multiple factors like, age, sex and pregnancy among others (Abdulqadir I *et al.,* 2017).

The findings of this study with respect to the absence of significant difference in serum level of homocysteine between pregnant and non pregnant women as well as among pregnant women across the three trimesters and parity were in contrast to the findings other studies (Osunkalu VO *et al.,* 2009; Walker MC *et al.,* 1999). Walker et.al reported lower values among pregnant women compared to controls with lowest value recorded in second trimester of the pregnancy while Osunkalu et.al shows a progressive decline of serum homocysteine with increasing gestational age among primigravidae. However, the finding of this study is in keeping with that of Dorothy et.al when they reported that, the homocysteine levels did not differ base on trimester among pregnant women (Dorothy JV *et al.,* 2007). There is no clear explanation for the observed differences between our finding and other studies but it is worthy to note that, several factors such as assay procedure, folate and vitamin B12 status,

drug usage and variation in metabolic enzymes arising from genetic polymorphism among others were reported to influenced the serum homocysteine level and may be responsible for these differences and it further emphasizes the need for local reference to guide decision making. (Hague WM, 2003). Normal. Normal pregnancy is associated with fall in plasma concentration of homocysteine due to increased uptake by foetus, glomerular filtration rate, plasma volume and physiologic haemodilution. (Hague WM, 2003). Elevated homocysteine concentration has consistently been shown to be an independent risk for cardiovascular disease and importantly, high serum concentration of homocysteine during pregnancy is emerging as an index of adverse foeto-maternal outcome (Mariano M *et al.*, 2014).

Red cell distribution width (RDW); a measure of the variability in size of circulating red blood cell was found to be significantly high among pregnant women in this study, similar to the findings of Raychaudhuri et.al and Mohammed who independently reported high RDW among normal pregnant women in Haryana, India and Omdurman, Sudan respectively (Raychaudhuri S *et al.*, 2017; Mohammed MOM, 2015). The high RDW among pregnant women in our study may be a consequence of multiple factors such as erythropoietin surge, haematenics deficiency and black ethnicity as all these were reported to influence RDW (Melissa KSL *et al.*, 2018). Erythropoietin is increased in pregnancy to support red cell production to counter the haemodilution caused by increased plasma volume while haematenics deficient erythropoiesis could arise as a result of poor nutrition and/or excessive fetal demand of nutrient for its growth. Although, there are varied reports on the pattern of RDW as pregnancy progresses with some studies reported no significant difference across the three trimesters, (Raychaudhuri S *et al.*, 2017; Musa AU *et al.*, 2016) similar to the present study and others reported either significant increase in third trimester (Shehata HA *et al.*, 1998; Purohit G *et al.*, 2015) or rise and fall with peak in second trimester. (Rayis DA *et al.*, 2017). However, irrespective of how it varies in various trimester of the pregnancy, RDW has potentials of predicting some complications of pregnancy such gestational diabetes, hypertensive disorders and miscarriage (Panagiotis P *et al.*, 2018; Melissa KSL *et al.*, 2018).

Although, majority of our participants had platelet count above 100 x 10^9/L except 5 (5%) and 2 (2%) of pregnant and non-pregnant women respectively, but the platelet count of the pregnant women was lower than that of control group. This finding is similar to the finding of other studies, where Saad et.al, Raychaudhuri et.al and Patrick et.al in Morocco, Haryana, India and Warri, Nigeria independently reported lower platelet count among pregnant women in comparison to their non pregnant counterpart (Raychaudhuri S *et al.*, 2017; Saad B *et al.*, 2018; Patrick CII *et al.*, 2013). This low platelet count in pregnancy have been previously attributed to pregnancy associated haemodilution and increased consumption of platelets within the utero-placental circulation (Musa AU *et al.*, 2016; Gebreweld A *et al.*, 2018). Most studies that previously accessed platelet count in pregnancy reported some variations across different trimesters, even though there is no consistency in the variation pattern (Saad B *et al.*, 2018; Gebreweld A *et al.*, 2018; Azab EA *et al.*, 2017). This is not in keeping with our finding as we did not find a significant difference in platelet count across the three trimesters. Both Musa et.al, Raychaudhuri et.al and Patrick et.al also did not report significant difference in platelet count according to trimesters of the pregnancy (Raychaudhuri S *et al.*, 2017; Musa AU *et al.*, 2016; Patrick CII *et al.*, 2013). The correlations between platelet count and indices have been well established both in and out of pregnancy (Alsheeha MA *et al.*, 2016; Dhakre R *et al.*, 2018; Giovanetti TV *et al.*, 2011). The MPV, PDW and P_LCR which measures average size of platelets, variations in sizes of platelets and proportion of platelets larger than 12fL respectively shows significant negative correlation with platelet count among pregnant women. This is in line with the findings of previous studies from Benin City, Nigeria and Northwest, Morocco ((Saad B *et al.*, 2018; Omorogiuwa A *et al.*, 2016). Also, it has further buttressed increased platelet consumption as one of the important mechanisms mediating platelet count in normal pregnancy. Increased platelet consumption will lead to compensatory increase platelet turn out from bone marrow with release of young platelets in to circulation which are usually larger and give rise to high MPV, P_LCR and together with old platelets in circulation causes platelet anisocytosis leading to high PDW as demonstrated in this study. On the other hand PCT; a measure of proportion of whole blood occupied by the platelets, shows a significant positive correlation with platelet count and this finding is expected and also in keeping with other studies (Raychaudhuri S *et al.*, 2017; Omorogiuwa A *et al.*, 2016). However, the lack of variation in any of the platelet indices with respect to trimester (gestational age) and parity of the pregnant women reported in this study, may have arisen because there is no significant difference in platelet count and both groups of pregnant women had a normal platelet count. Platelet and its indices are been employ in monitoring pregnancy and when utilized judiciously can prevent some pregnancy related feto-maternal complications (Alsheeha MA *et al.*, 2016; Abdurrahman AA *et al.*, 2018; Burcu AU *et al.*, 2014; Dhakre R *et al.*, 2018).

In conclusion, local reference source for emerging markers of diseases with potential of rationalizing of obstetrics practice in our environment has been established for pregnant women. The values of these markers are different between pregnant and non

pregnant women in the same environment. We therefore recommend that these differences should be taking in to consideration to guide appropriate interventions.

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