

Effects of Hepatitis B Infection on Haematological Parameters in Pregnancy in Port Harcourt, Nigeria

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Abstract: The effect of hepatitis B infection on the haematological parameters of 3,465 apparently healthy pregnant women attending antenatal clinic was studied over a period of 3 years in Port Harcourt, Nigeria. Fifty-seven were HBsAg positive (test) while the remaining 3,408 were HBsAg negative (control) representing a prevalence rate of 1.6%. The mean±SD obtained for Packed Cell Volume (PCV), Haemoglobin concentration (Hb conc.), platelets, total White Blood Cell count (WBC), neutrophils, lymphocytes, eosinophils, basophils and monocytes for hepatitis B positive pregnant women (test) were 22.77±2.86%, 7.26±0.56 g dL⁻¹, 144.23±3.60 ×10⁹ L⁻¹, 8.08±0.89×10⁹ L⁻¹, 78.88±2.11%, 16.68±1.87%, 1.49±0.71%, 0.04±0.19% and 2.91±1.27% in the same order. The corresponding values for hepatitis B negative pregnant women (control) were 31.96±2.78%, 10.36±0.76g L⁻¹, 159.84±20.70×10⁹ L⁻¹, 6.73±1.47×10⁹ L⁻¹, 69.12±3.33%, 24.02±4.01%, 2.96±1.50%, 0.04±0.20% and 3.84±1.60%. The differences between the PCV, Hb conc, platelets, TWBC, lymphocyte, eosinophil and monocyte counts of test were statistically significant from that of the control (p<0.05). There were no significant differences in the neutrophil and basophil counts of the test and control (p>0.05). There was no significant correlation (R<0.50, p>0.01) between the haematological parameters and maternal age, trimester and gravidity in the control subjects. There was however, a strong positive correlation between gravidity and PCV (R = 0.54, p<0.001) and haemoglobin concentration (R = 0.61, p<0.01) in the test.

Key words: Hepatitis B, pregnancy, haematological parameters, infection, Nigeria

INTRODUCTION

Hepatitis B is a viral infection, a DNA hepadna virus type 1 and it is one of the most common serious liver diseases encountered in pregnant women. It is endemic worldwide. Approximately, 350 million people are chronic carriers and only 2% will spontaneously develop antibodies enough to clear the virus from their system (Pyrsoopoulos and Reddy, 2001). Hepatitis B is transmitted sexually and through blood and blood products and by other less understood means. Sexual transmission occurs through unprotected penetrative vaginal and anal sex (Davies *et al.*, 1989; Hart *et al.*, 1993; Osella *et al.*, 1998; Gilson *et al.*, 1998; Hahne *et al.*, 2004). Haematogenous transmission may be parenteral or vertical (Xiao *et al.*, 2007).

Various studies have shown that haematological parameters in pregnancy show variation from normal non-pregnant women (Onwukeme and Uguru, 1990; Osoagbaka *et al.*, 2000; Kei, 2004). Neutrophilia is a

feature of pregnancy, while neutropenia is common among non-pregnant Africans (Arinola *et al.*, 2004). Various studies have also shown that hepatitis B infection causes diverse haematological changes in pregnancy (Boxall *et al.*, 1994; Chaisiripoomkere *et al.*, 1994; Okada *et al.*, 1995; Di *et al.*, 1999; Metha and Hoffbrand, 1999; Seeger and Mason, 2000). Apart from the haematological changes brought about by Hepatitis B infection on the mother, both perinatal and maternal deaths are substantially increased in Hepatitis B infection more especially in underprivileged populations and developing countries (Tse *et al.*, 2005). There appears to be a high incidence of low birth weight among infants born to mothers with acute infection during pregnancy. It may also cause abortion or premature labour as well as intrauterine death in late pregnancy (Tse *et al.*, 2005). About half of the babies born to mothers who have hepatitis B during pregnancy will show hepatitis B antigen in their blood and a proportion of them will develop hepatic lesions (Tucker, 2001).

Routine examinations and investigations are carried out on the expectant mother to monitor the progress of the pregnancy and to ensure the wellbeing of both the mother and the fetus. This routine examination and investigations include among others the assessment of the haematological status by way of estimating the haematological parameters. Haematological parameters are measurable indices of blood and their values can be used as guide for disease diagnosis such as Hepatitis B infection as well as monitoring of physiological conditions such as pregnancy.

MATERIALS AND METHODS

Setting/study population: A total of three thousand, four hundred and sixty five apparently healthy pregnant women visiting the antenatal clinic with varying maternal and gestational ages and gravidity constituted the study population. The subjects were informed of the study objectives and informed consent was obtained from all the participants. Both the HBsAg positive and the HBsAg negative pregnant women studied were all on routine 5 mg folic acid and 200 mg fersolate tablets daily medication and none was homozygous for sickle cell gene.

Sample collection: A standard clean venepuncture technique as described by Dacie and Lewis (2006) was used to collect 5 mL of venous blood from each participant from the antecubital vein. Four milliliters of the venous blood was dispensed into dipotassium EDTA anticoagulant tube and mixed, while the remaining 1 mL was dispensed into a dry plain tube without anticoagulant. The anticoagulated samples were used for the determination of the haematological parameters while the sera derived from the plain tubes were used to screen for the hepatitis B status. All the analyses were done on the same day of sample collection.

Hepatitis B screening: Hepatitis B surface Antigen (HBsAg) was screened with the HBs-Antigen Latex Slide screening technique. Serum containing the HBsAg caused the latex particles to agglutinate representing a positive reaction. On the contrary, in the absence of HBsAg, no agglutination occurred and the latex particles remained in homogenous suspension.

Determination of Haemoglobin Concentration (Hb Conc.), Packed Cell Volume (PCV), Total White Blood Cell count (TWBC) and platelet count.

The anticoagulated venous blood samples collected were analyzed for the haemoglobin concentration, packed cell volume, total white blood cell count and platelet count

using QBC Reader System, a semi-automated machine accompanied with QBC Centrifuge, QBC Venous-Blood Pipetter and QBC Work Station.

Differential leucocyte count: Thin blood film was prepared by standard manual technique on clean glass slides as described by Dacie and Lewis (2006) and stained with Leishman's stain. The differential leucocyte count was carried out by the longitudinal technique.

The values of the haematological parameters for the test were compared with that of the control using student t-test to ascertain whether there was a statistically significant difference between the two groups of pregnant women studied while, simple linear correlation was used to find the relationship between the age, trimester and gravidity on one hand and the haematological parameters on the other hand.

RESULTS

The mean and standard deviation of the values obtained for the hepatitis B positive and negative pregnant women are shown in Table 1. The correlation between the maternal age and the haematological parameters in the test group were: positive fair correlation with PCV ($R = 0.44, p < 0.01$) and Hb ($R = 0.49, p < 0.01$), a positive very slight correlation with monocytes ($R = 0.20, p < 0.01$) and lymphocyte ($R = 0.11, p < 0.01$), a negative slight correlation with TWBC ($R = -0.39, p < 0.01$) and a negative very slight correlation with platelet ($R = -0.10, p < 0.01$), neutrophil ($-0.17, p < 0.01$), eosinophil ($R = -0.10, p < 0.01$) and basophil ($-0.18, p < 0.01$). The correlation between the maternal age and the haematological parameters in the control group were: positive slight correlation with PCV ($R = 0.32, p < 0.01$) and Hb ($R = 0.39, p < 0.01$), a positive very slight correlation with lymphocytes ($R = 0.14, p < 0.01$), a negative very slight correlation with platelets ($R = -0.13, p < 0.01$) and monocytes ($R = -0.10, p < 0.01$) and a negligible correlation with TWBC ($R = 0.01, p > 0.01$), neutrophil ($R = -0.07, p > 0.01$), eosinophil ($R = -0.10, p > 0.01$) and basophil ($R = -0.01, p > 0.01$).

Table 1: Mean value±SD of haematological parameters in hepatitis B positive (test) and hepatitis B negative (control) pregnant women

Haematological parameter	Test	Control	Probability
PCV (%)	22.77±2.86	31.96±2.78	<0.05
Hb. conc. (g dL ⁻¹)	7.26±0.56	10.36±0.76	<0.05
Platelets (×10 ⁹ L ⁻¹)	144.23±3.60	159.84±20.70	<0.05
Total WBC (×10 ⁹ L ⁻¹)	8.08±0.89	6.73±1.49	<0.05
Neutrophils (%)	78.88±2.11	69.12±3.33	>0.05
Lymphocytes (%)	16.68±1.87	24.02±4.01	<0.05
Eosinophils (%)	1.49±0.71	2.96±1.50	<0.05
Basophils (%)	0.04±0.19	0.04±0.20	>0.05
Monocytes (%)	2.91±1.27	3.84±1.60	<0.05

The correlation between gravidity and the haematological parameters in the test group were: a positive moderate correlation with PCV ($R = 0.54$, $p < 0.01$) and Hb ($R = 0.61$, $p < 0.01$), a negative fair correlation with TWBC ($R = -0.44$, $p < 0.01$), a negative slight correlation with neutrophil ($R = -0.32$, $p < 0.01$), a positive very slight correlation with lymphocytes ($R = 0.27$, $p < 0.01$) and monocytes ($R = -0.16$, $p < 0.01$), a negligible correlation with eosinophil ($R = -0.05$, $p > 0.01$), monocyte ($R = 0.16$, $p > 0.01$) and no correlation with platelets ($R = 0.00$, $p > 0.01$). The correlation between gravidity and the haematological parameters in the control group were: a positive slight correlation with PCV ($R = 0.35$, $p < 0.01$) and Hb ($R = 0.36$, $p < 0.01$), a negative very slight correlation with eosinophil ($R = -0.13$, $p < 0.01$) and a negligible correlation with platelets ($R = -0.05$, $p > 0.01$), TWBC ($R = 0.06$, $p > 0.01$), neutrophil ($R = -0.07$, $p > 0.01$), lymphocytes ($R = 0.07$, $p > 0.01$), basophil ($R = 0.06$, $p > 0.01$) and monocyte ($R = 0.09$, $p > 0.01$).

The correlation between trimester and the haematological parameters in the test group were: positive fair correlation with Hb ($R = 0.41$, $p < 0.01$), a positive slight correlation with PCV ($R = 0.32$, $p < 0.01$), a positive very slight correlation with platelets ($R = 0.10$, $p < 0.01$) and lymphocytes ($R = 0.24$), neutrophil ($R = -0.25$, $p < 0.01$) and basophil ($R = -0.11$, $p < 0.01$), a very slight negative correlation with TWBC ($R = -0.19$, $p < 0.01$) and a negligible correlation with eosinophil ($R = 0.06$, $p > 0.01$) and monocytes ($R = 0.05$, $p > 0.01$). The correlation between trimester and the haematological parameters in the control group were: a negligible correlation with PCV ($R = -0.03$, $p > 0.01$), Hb ($R = -0.01$, $p > 0.01$), neutrophil ($R = -0.07$, $p > 0.01$) and eosinophil ($R = 0.07$, $p > 0.01$), a positive very slight correlation with TWBC ($R = 0.19$, $p < 0.01$) and lymphocytes ($R = 0.10$, $p < 0.01$).

DISCUSSION

The haemoglobin concentration of 7.26 ± 0.56 g dL⁻¹ obtained for the hepatitis B positive pregnant women were significantly lower than the 9.0 g dL⁻¹ given by Isibor *et al.* (2003) as the value below which true anemia sets in pregnancy. The mean haemoglobin concentration and PCV for the test of 7.17 ± 0.30 g dL⁻¹ and $22.60 \pm 1.84\%$ in the first trimester, 7.11 ± 0.51 g dL⁻¹ and $21.97 \pm 2.82\%$ in the second trimester and 8.10 ± 0.44 g dL⁻¹ and $26.50 \pm 1.41\%$ in the third trimester as shown in Table 2 are all within the values ascribed to true anemia by Khuroo *et al.* (1981), Taylor *et al.* (1982), Okada *et al.* (1995) and Di *et al.* (1999).

Correlation coefficients $R \geq 0.5$ were considered to indicate a significant relationship between any two

Table 2: Mean values \pm SD of haematological parameters in hepatitis B positive (test) and hepatitis B negative (control) pregnant women with respect to trimester

Haematological parameter	Trimester	Test	Control	Probability
PCV (%)	1	22.60 \pm 1.84	32.60 \pm 2.56	<0.05
	2	21.97 \pm 2.82	31.36 \pm 2.42	<0.05
	3	26.50 \pm 1.41	33.00 \pm 4.16	<0.05
Hb. conc. (g dL ⁻¹)	1	7.17 \pm 0.30	10.55 \pm 0.73	<0.05
	2	7.11 \pm 0.51	10.15 \pm 0.67	<0.05
	3	8.10 \pm 0.44	10.74 \pm 1.00	<0.05
Platelet ($\times 10^9$ L ⁻¹)	1	144.13 \pm 3.18	167.93 \pm 26.48	<0.05
	2	143.94 \pm 3.36	157.18 \pm 18.04	<0.05
	3	145.63 \pm 5.26	153.14 \pm 12.32	<0.05
Total WBC ($\times 10^9$ L ⁻¹)	1	8.06 \pm 0.83	6.34 \pm 1.76	<0.05
	2	8.28 \pm 0.90	6.82 \pm 1.38	<0.05
	3	7.28 \pm 0.52	7.17 \pm 1.07	<0.05
Neutrophils (%)	1	79.33 \pm 2.06	69.53 \pm 3.23	>0.05
	2	79.03 \pm 2.12	68.96 \pm 3.58	>0.05
	3	77.28 \pm 1.69	68.86 \pm 2.79	>0.05
Lymphocytes (%)	1	16.13 \pm 1.77	23.33 \pm 3.79	<0.05
	2	16.71 \pm 1.95	24.29 \pm 4.21	<0.05
	3	17.63 \pm 1.51	24.43 \pm 4.08	<0.05
Eosinophils (%)	1	1.47 \pm 0.64	2.878 \pm 1.25	<0.05
	2	1.47 \pm 0.75	2.93 \pm 1.59	<0.05
	3	1.63 \pm 0.75	3.29 \pm 1.80	<0.05
Basophils (%)	1	0.07 \pm 0.26	0.07 \pm 0.26	>0.05
	2	0.03 \pm 0.17	0.04 \pm 0.19	>0.05
	3	0.00 \pm 0.00	0.00 \pm 0.00	>0.05
Monocytes (%)	1	3.00 \pm 1.31	4.13 \pm 1.92	<0.05
	2	2.76 \pm 1.26	3.79 \pm 1.52	<0.05
	3	3.38 \pm 1.30	3.43 \pm 1.13	<0.05

variables. There was no significant correlation between age, gravidity and trimester on one hand and any of the haematological parameters on the other hand among the hepatitis B negative population. There was however, a significant positive correlation between PCV and gravidity ($R = 0.54$) and Hb and gravidity ($R = 0.61$) among the hepatitis B positive pregnant women. This implies that as the gravidity increases, the PCV and the Hb. Conc. also, increase and invariably the degree of anaemia reduces in hepatitis B pregnant women.

CONCLUSION

This study has established that there is an association between hepatitis B infection, pregnancy and haematological parameters. The prevalence rate of hepatitis B among pregnant women in Port Harcourt was established as 1.6%. The PCV, Hb Conc., lymphocyte and platelet counts were lower in the hepatitis B positive pregnant women than in the normal pregnant women. Total white cell count was higher in the infected cases than in the normal cases. However, the values in both cases were within the normal range. There were no significant differences in the neutrophil and basophil counts of the test and control ($p > 0.05$). There was no significant correlation ($R < 0.50$, $p > 0.01$) between the

haematological parameters and maternal age, trimester and gravidity in the control. There was however, a significant positive correlation between gravidity and PCV ($R = 0.54$, $p < 0.01$) and haemoglobin concentration ($R = 0.61$, $p < 0.01$) in the test.

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