

Original Article

Prevalence of malaria parasites, Hepatitis B and C viral infections in pregnant women attending ante-natal clinic.

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ABSTRACT

The prevalence of Hepatitis B virus (HBV), Hepatitis C virus (HCV) and malaria parasites (MP) was studied amongst pregnant women. One hundred pregnant women on routine visit to the ante-natal clinic were randomly recruited into the study. Analysis of their blood samples showed that 2(2%) out of the 100 pregnant women were sero-positive for HBV and 77(81%) out of 94 of pregnant women were positive for malaria parasites. However, all of the pregnant women were HCV sero-negative. The alanine amino transferase (ALT) values are not definitive for any particular infection. This study revealed that malaria parasites were the most prevalent infection among pregnant women. HBV infection in pregnant women is a cause for concern as it may result in congenital infection.

Keywords: Hepatitis B and C, Malaria, Pregnant women

INTRODUCTION

Nigeria is one of the countries situated in malaria endemic area of Sub-Saharan Africa. The transmission of malaria is believed to be stable throughout the year. Therefore report of symptomatic and asymptomatic cases of malaria infection is not uncommon finding in pregnant women¹. However, the emergence of Hepatitis B virus (HBV) and Hepatitis C virus (HCV) in an area of malaria endemicity calls for concern since any of these infections have high mortality and morbidity rate,^{2,3,4}. In our earlier report, we observed 7% and 3% prevalence rates of HBV and HCV respectively amongst the blood donors⁵. In continued search to actually know the prevalence of these infectious agents in different population groups, we decided to screen pregnant women for HBV and HCV and possible role of alanine amino transferase (ALT) in such infections in malaria endemic area of south Eastern Nigeria.

MATERIALS AND METHODS

Subjects: One hundred pregnant women aged between 18-39 years, with no history of HBsAg

vaccination, attending ante-natal at Nnamdi Azikiwe University Teaching Hospital, Nnewi, were recruited for this study. A structured standard questionnaire was used to collect medical and maternal data such as; fever, malaise, nausea, chills, fatigue or weight-loss, headache, and history of previous blood transfusion, abortion and still-birth and current age and gestational age were taken. Blood samples were collected for determination of serum alanine amino transferase (ALT) activity, Hepatitis B surface antigen (HBsAg), and Malaria Parasites and Hepatitis C virus (HCV) screening. The serum samples were stored at -20°C until analyzed. The participants in the present study gave informed consent before being recruited for the study.

METHODS

Hepatitis B surface antigen (HbsAg) screening:

The principle is based on detection of Hepatitis B surface antigen (HbsAg) antibodies in the serum using one step HbsAg strip (ACON

Laboratories incorporated USA). One step HbsAg strip is a qualitative, lateral flow immunoassay. The membrane is pre-coated with anti-HbsAg antibodies on the test line region of the strip. The procedure for the detection of HBV was as described by the manufacturer (ACON Laboratories, Incorporated, USA). The test strip was immersed vertically in the serum specimen for 15 seconds, thereafter; the test strip was placed on a non-absorbent flat surface, and allowed to incubate for 15 minutes. This allows for reaction between the pre-coated anti-HbsAg antibodies and the HbsAg antibodies present in the serum and generate a coloured line. For HBV sero-positive sera, two distinct red lines appeared; one line on the test region (T) and the other on the control region (C). However for HBV sero-negative, one red line appeared on the control region (C) but no red line appeared on the test region (T). The appearance of red line at the control region validates the result and test strip.

Hepatitis C Virus (HCV) Screening

The principle is based on detection of HCV antibodies in the serum using HCV rapid test device (Core Diagnostics UK). HCV rapid test device is a qualitative membrane based antigen-antibody immunoassay. The membrane is coated with anti-HCV antibodies on the test line region of the device. The procedure is as described by the manufacturer (CORE Diagnostics United Kingdom) Two drops (0.1ml) of serum sample was added using pasture pipette, into appropriately labeled sample well of the rapid test device and allowed to incubate for 10 minutes. This will allow for complete reaction between the pre-coated anti-HCV antibodies and the HCV antibodies if present in the serum will generate a coloured line in the test region. For HCV sero-positive sera, two distinct lines appeared, one on the control region (c) and the other on the test region (T), while only one red line appeared on the control region (C) and non on the test region (T) for negative sera. The appearance of red line at the control region validates the result and test strip.

Determination of Alanine Amino transferase (ALT) activity: ALT was determined as described by Reitman et al⁶. The principle of the test was based on the measurement of pyruvate hydrazone formed by the reaction between alpha oxoglutarate and L-alanine in the presence of alanine amino transferase. The procedure followed was as supplied by the manufacturer of the kit (Randox Laboratories limited, UK.). In brief, 0.1ml of sera were added into appropriately labeled test tubes containing 0.5ml of solution-1 (comprising phosphate buffer, L-alanine, α -oxoglutarate). The reaction mixtures were allowed for incubation for 30 minutes at 37°C before the addition of 0.5ml of 2, 4-dinitrophenylhydrazine into the respective sample test tubes. The tubes were allowed to incubate for further 20 minutes at 20°C. Subsequently, 0.5ml of sodium hydroxide was then added into the respective test tubes and mixed. After 5 minutes, the contents of test tubes were mixed and the absorbance of the samples read against the reagent blank at 530nm wavelength. For the reagent blank similar procedure was followed except that 0.1ml of distilled water replaced the 0.1ml of sample. A calculating chart that accompanied the kit was used to deduce the serum activities of ALT using the observed absorbance.

Detection of Malaria Parasites:

Thick blood films were made on clean grease free slides. The films were air dried and allowed to stain in 1/20 diluted Giemsa stain for 30 minutes. The blood stained films were screened for *P. falciparum* malaria parasites.

STATISTICAL ANALYSIS

The quantitative variables were expressed as mean (\pm SD) while the incidences of the various infections were expressed in percentage. The student t-test was used to determine significance difference in mean.

RESULTS

Out of the one hundred pregnant women recruited for this study, 2 (2%) were sero-positive for Hepatitis B virus (HBV), while 98 (98%) were sero-negative. No sero-positive

result was recorded for Hepatitis C virus (HCV). However, 77 (82%) of the pregnant women had positive malaria parasites blood smear while 17 (18%) had no detectable malaria parasites in their blood smears. See table 1

The two subjects who were HBV sero-positive as well as MP positive, had mean Alanine amino transferase (ALT) activity of 68.5 ± 25.5 (iu/l) ^(a), while those who were MP positive but HBV sero-negative had mean ALT value of 50.3 ± 24.8 (iu/l) ^(b). Whereas subjects who were HBV sero-negative as well as MP negative, had ALT value of 75.5 ± 18.8 (iu/l) ^(c). The difference in mean ALT between HBV sero-positive and MP positive subjects vs. MP positive and HBV sero-negative ^(ab) subjects was significantly different ($P < 0.05$). No significant

difference in mean ALT values was observed between HBV sero-positive and MP positives vs. HBV sero-negative and MP negatives ^(ac) ($P > 0.1$) or between MP positive and HBV sero-negative vs. MP negative and HBV sero-negative ^(bc) subjects ($P > 0.1$). See table 2

Out of 77(82%) subjects who were MP positive, 41(53%) were asymptomatic while 18(23.3%) had symptoms and 18(23.3%) had their history not recorded. Among 22 subjects with raised ALT, 15(68%) were asymptomatic, 5(22.7%) had history of previous abortion and 1(4.5%) had malaise. The two pregnant women who were positive to HBV were asymptomatic. A total of 18(18%) had history of previous abortion, 2(2%) had history of previous blood transfusion. See table 3.

Table 1: Prevalence of Hepatitis B virus (HBV), Hepatitis C virus (HCV) and Malaria parasites (MP) amongst pregnant women attending antenatal clinic

Variables	Positive (%)	Negative (%)	Total
HBV	2(2%)	98(98%)	100
HCV	0 (0%)	100(100%)	100
MP	77(82%)	17	94

Table 2: ALT levels in pregnant women with different status of HBV, HCV and MP.

Variables	ALT iu/ul	P-Value
HBV and MP positive (n = 2)	68.5 ± 25.5 a	$P > 0.1$ ^(ac)
Positive MP & HBV negative (n=46)	50.3 ± 24.8 b	$P < 0.05$ ^(ab)
HBV & MP negative (n=8)	75.5 ± 18.8 c	$P > 0.1$ ^(bc)

Key:

ac = HBV & MP positives vs. HBV & MP negatives

ab = MP Positive & HBV negative vs. HBV & MP positives

bc = MP positives & negative HBV vs. MP negatives & HBV negatives

MP = Malaria Parasite

HBV= Hepatitis B virus

ALT = Alanine aminotransaminase

n = number of subjects.

Table 3: presentations of pregnant women during recruitment at the ante-natal clinic.

Variables	Vomiting	Malaise	Fever	+Abortion	Still Birth	Headache	Chills	*Transfusion	Asymptomatic	No History
MP positive	5	6	6	10	2	9	2	2	41	18
HBV pos	-	-	-	-	-	-	-	-	2	-
HCV	-	-	-	-	-	-	-	-	-	-
ALT raised	-	1	-	5	-	-	-	-	15	2

+previous abortion

*previous blood transfusion

DISCUSSION

The result of this work showed low prevalence of HBV amongst pregnant women in Nigeria. The 2% positive cases of HBV infection amongst pregnant women may be considered a significant report considering that it also represents 20 out of every 1000 pregnant women in Nigeria. In the largest study performed so far, investigating more than 15 000 pregnant women from northern Italy over a period of four years, a prevalence of 2.4% was found⁷. This gives a count of 360 sero-positive pregnant women detected within 4 years. The zero prevalence of hepatitis C viral infection in pregnant women in this study calls for further investigation with higher number of pregnant women participating. Although with the same sample size we have reported incidences of HCV infection amongst apparently healthy blood donors⁵. Since our previous study and this present study were conducted within same locality, it could be that blood donors might be active source of reservoir for HCV transmission. Report elsewhere, has shown that the prevalence of HCV specific antibodies varies geographically and in different population groups⁸. For instance, 5% cases of HCV have been observed in another study in another location in Nigeria⁹. Although the population used for the study were normal blood donors and multiple transfused sickle cell anaemia patients.

The essence for using ALT was to see if there could be any evidence of active hepatitis. However, only 1 out of the 2 HBV positive pregnant women had increased serum activity of

ALT above the confidence interval for the study population. Conclusion may be difficult to draw from the present study with regard to the number of patients positive for HBV. Hence we could not attribute increased ALT activity as a marker for HBV infection. In their study, Acquave and Tetty⁸ could only detect raised ALT activity in 23 (33.85%) out of 68 serum samples of subjects with positive Hepatitis B viral infection. Thus suggesting that raised ALT activity may not be a consistent finding in Hepatitis B viral infection.

In the present study, the two pregnant women positive for HBV on presentation were asymptomatic. Report has shown that 15% of asymptomatic carriers exist in developing countries, and HBV is frequently associated with asymptomatic conditions². It is true that asymptomatic conditions can escape diagnosis and then increase the chances of congenital infection and infection of unsuspecting health workers in cases of exposure.

Because most pregnant women were asymptomatic, the possibility of missing the diagnosis may portend grave consequences to their children who if congenitally infected may present with hepatocarcinoma in future. The strong possibility of vertical transmission lends importance to diagnosing acute or chronic HBV infection in pregnant women and justifies mandatory ante-partum serum HBsAg screening. By doing so, previously unsuspected chronic HBV infection is diagnosed in young otherwise healthy individuals^{10,11}. This has the added benefit of making it possible to refer them for appropriate antiviral therapy before

development of significant liver damage and associated functional insufficiency. The infants of potentially infectious mothers are treated with HBV Human Hyperglobulin (HBIG) at delivery and simultaneously active immunoprophylaxis is initiated¹⁰. This approach is effective in preventing chronic HBV in approximately 85% of neonates¹⁰. It has been reported that childhood infection can easily lead to chronic stage of the HBV and HCV infections¹²

82% positive cases of malaria parasite infection in pregnant women showed high prevalence. High prevalence of malaria parasites infection was previously reported among pregnant women in Nigeria¹. This is possible because the study location is endemic to malaria. Maternal anemia contributes significantly to maternal mortality and causes an estimated 10,000 deaths per year most of which are as a result of infection with *Plasmodium falciparum*¹⁵. Thus Pregnant women in malaria endemic areas may experience a variety of adverse consequences from malaria infection including maternal anaemia, placental accumulation of parasites, low birth weight (LBW) from prematurity and intrauterine growth retardation (IUGR), foetal parasite exposure and congenital infection, and infant mortality (IM) linked to preterm-LBW and IUGR-LBW¹³.

Considering the grave consequences of HBV infection on pregnant women and pregnancy outcomes, we are advocating for routine screening for all individuals within the reproductive age as this will help identify asymptomatic cases and proper management instituted. Malaria parasite screening should also be routinely performed on pregnant women in this part of the world.

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