Seroprevalence of Hepatitis B Virus using HBV-5 Rapid Panel Test and Associated Factors amongst Pregnant Women Attending Antenatal Care in Garoua, North-Cameroon

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ABSTRACT

**Background:** In Cameroon, prevalence of Hepatitis B Virus (HBV) is high and varies from different places in general population and vulnerable persons such as pregnant women. We performed this survey to determine seroprevalence of HBV using HBV-5 Rapid panel test and associated factors amongst pregnant women attending antenatal care (ANC) in Garoua.

**Methods:** This was a cross-sectional study conducted from February, 15th to April, 15th, 2016 amongst 102 pregnant women attending ANC in city of Garoua. Data were obtained using a structured questionnaire by interview. The blood samples were collected and tested by the immuno-chromatographic panel method (OnSite HBV-5 Rapid Panel Test) for the detection of HBV biomarkers. Statistical analyses were performed by EPI Info™ version 7 software, with P<0.05 considered significant.

**Results:** Overall seroprevalence of HBV (HBsAg) was 10.78% (11/102) and the other HBV biomarkers were 15.68% (16/102), 9.80% (10/102) and 26.47% (27/102) for anti-HBsAb, anti-HBeAb and total anti-HBcAb respectively. According to general information, marital status (P=0.001) was statistically associated with HBsAg seroprevalence. Bivariate analysis logistic regression recorded that, scarification (OR= 30.10; 95% CI 6.55-138.15; P=0.00000) and piercing or tattoo (OR= 11.80; 95% CI 2.77-50.18; P=0.00008) were statistically associated with seroprevalence of HBsAg.

**Conclusion:** Seroprevalence of HBV biomarkers is high amongst pregnant women attending ANC in Garoua. Associated factors such as scarification and piercing or tattoo were associated with HBV infection. The awareness of pregnant women about vaccination in routine against HBV were necessary to prevent the transmission of Mother-to-Child Transmission (MTCT).

**Keywords:** Associated factors, Garoua, HBV, pregnant women, seroprevalence

I. INTRODUCTION

HBV is an important worldwide public health problem and known to cause chronic hepatitis with high risk of death subsequent after cirrhosis and primary liver cancer. An estimated in 2015, 257 million persons were living with chronic HBV infection worldwide [1]-[3]. The widespread caused by HBV affects typically African and Western pacific areas with more than 68% of those infected in the world [2], [4]. Amongst African areas more affected by HBV infection, sub-Saharan Africa (SSA) has the second largest global weight of chronic carriers of HBV infection after Asian continent [5]. Greatest of the load of disease from HBV infection comes from infections acquired before, during or after birth. Then babies born to untreated HBV-infected mothers can acquire infection from the mother, commonly during birth [6]. Well-known, transmission from mother to its child or horizontal transmission in youthful remain a principal route of transmission mostly found in high endemic area of HBV like SSA [6]. Therefore, several pregnant women living in SSA are infected with or exposed to HBV that can be transmitted vertically to its new-borns [7]. Thus, babies born to mothers who are positive for both HbsAg and hepatitis B e antigen (HBeAg) are at a higher risk of acquiring infection (transmission risk for HBsAg-positive and HBeAg-positive mothers: 70–100% in Asia and 40% in Africa) than those born to HbsAg-positive mothers who have lost the HBeAg (5–30% in Asia and 5% in Africa) [8]-[10]. Moreover, HBsAg-negative mothers have a near 0% risk of transmitting HBV to their offspring vaccinated at birth [11],
whereas HBeAg-positive mothers have a 20% risk of transmitting the virus despite vaccination at birth [12]. Management of pregnant mothers with antivirals, which is being introduced as a new intervention to prevent mother-to-child transmission (MTCT) of HBV, should more reduce the risks of transmission [13].

Nevertheless, of whether they have been beforehand tested or immunized, screening of all pregnant women for HBV infection at the primary antenatal care appointment is imperative in understanding of the morbidity and mortality of pregnant women, its upshot on the gravity result, and the risk of vertical transmission from mother to child [14]. HBsAg is the first detectable serological marker to appear in acute HBV infection. It is most commonly used in laboratory to screen for the presence of HBV infection and its perseverance for further than 6 months suggests chronic infection. Nonetheless, early in an infection, this antigen may not be present, and it may be undetectable later in the infection as it is being cleared by the host [15].

In Cameroon, the prevalence of HBV (HBsAg) varies according to regions and ethnic groups with an average estimated more than 8% (HBsAg> 8%) [5], [16] like many countries situated in SSA. Several studies conducted by Noubiap and collaborators [17] in far north, Frambo and collaborators [18] in health district of Buea and Fomulu and collaborators [19] in Yaoundé found a prevalence of HBsAg of 10.20%, 9.7% and 7.7% respectively in pregnant women. The previous studies carried out in Cameroon found only the prevalence of HBsAg among pregnant women. Despite the variability of this prevalence (HBsAg), there is a rareness information concerning the HBV biomarkers. There are five serological biomarkers for HBV (HBsAg, anti-HBsAb, HBeAg, anti-HBeAg, anti-HBcAb) that can be used to screen, diagnose, manage, and monitor HBV infection. In view of the above, it is therefore rational to conducted new investigation in a context of high endemicity like Cameroon. The survey was designed to determine seroprevalence and associated factors of HBV amongst pregnant women attending ANC in Garoua using HBV-5 Rapid panel test.

II. METHODOLOGY

A. Study Design and Sitting

During February, 15th to April, 15th 2016, a multicenter cross-sectional study was carried out amongst pregnant women attending ANC at the regional hospital and 03 health Integrated centers (Kollere, Foulbere and Souari) of Garoua, in far North of Cameroon.

B. Ethical Considerations

The ethical clearance for this study was issued from the National Ethics Committee of Research on Human Health of Cameroon (Reference: N°2016/04/333/CE/CNERSH/SP). Administrative authorizations were obtained from the Regional Delegation of Public Health for the North Region (Reference: N°0491NS/GS/DRSPN/SAGE/GRA) and the director of Garoua regional hospital. All participant received detailed information with an information sheet. Furthermore, all was signed written informed consent approved by the National Ethics Committee. Each participant was identified by a single code to guarantee the confidentiality.

C. Sample Size Determination

We obtained sample size using the following formula: n = \( z^2 \times \frac{P \times (1 - P)}{\epsilon^2} \) with “\( z \)” = 1.645 (90% confidence interval), “\( P \)”= prevalence of HBV (HBsAg) found in Cameroon (10.20%) [17], “\( Q \)” = 1-P and E = random error (5%). We obtained a minimum size n of 97.88≈98 participants but 102 were enrolled. Participants were recruited consecutively by interview with a structured questionnaire including general data such as age group, marital status, education level, occupation, gestational age, parity, history of blood transfusion, scarification, piercing or tattoo, previous surgery and knowledge of the disease.

D. Sample Collection and Detection of HBV Biomarkers

Five milliliters of blood were collected at the bend of the elbow in the dry tube previously labeled using a vacutainer needle. After centrifugation of whole blood, sera obtained were separated in the cryotubes previously labeled and stored at -20°C in the freezer. The samples were analyzed by the “OnSite HBV Rapid Panel Test” kit (from the CTK Biotech USA laboratory; Lot No F0624K7D00, date of manufacture: 10-08-2015 and expiration date: 09-02-2017) according to the manufacturer’s instructions.

OnSite HBV Rapid Panel Test is the lateral flow chromatographic immunoassay consisting of 5 test panel strips for the qualitative detection of HBsAg, HbsAb, HBeAg, HBeAb, and HBcAb assembled in one cassette. Each strip of the panel member is composed of a sample pad, colloidal gold conjugate pad, nitrocellulose membrane (NC membrane) strip pre-coated with control band (C band) and test band (T band), and absorbent pad. The HBeAb, HbsAb, HBsAg are based sandwich immunoassay and the HBeAb, HbcAb are based competitive immunoassay [20]. Negative result shows the appearance of one red line only in control zone “C” on the HBsAg, HbsAb, HBeAg strip, or both the “C” and the “T” lines are developed on either the HBeAb or the HbcAb strip. The positive result was reported two distinct red lines appear in the “C” and the “T” on the HBsAg, HbsAb, or the HBeAg strip or only the “C” line is developed on the HBeAb or the HbcAb strip.

E. Data Collection and Statistics

Data collected by a questionnaire and the results obtained after analysis of the samples were reported in a MS excel spreadsheet. Then transported into, and analyzed with, EPI Info™ software version 7. Statistical association between HBV biomarkers, pregnant women characteristics and associated factors were performed using chi-square, Fisher’s exact test corrected by Yates test and bivariate logistic regression. P-values were significant for all P <0.05.

F. Study Limitations

Some limitations were described in this present study: The type Rapid Diagnostic Test (RDT) used to detect HBV biomarkers in the collected sera. Furthermore, the lack of HBV-DNA PCR assay did not allow us to determine the contribution of occult HBV infection (OBI) on the epidemiological burden of HBV during gravidity in this locality. Moreover, the small sample size did not allow us to have roughly statistically significant results due to the lack of financial resources.
III. RESULTS

A. General Information of the Participants

| Variables | Total number of participants N=102 | Frequency (%) |
|-----------|------------------------------------|---------------|
| Age groups (in years) | | |
| 15-24 | 47 | 46.08 |
| 25-34 | 52 | 50.98 |
| 35-44 | 03 | 02.94 |
| Marital Status | | |
| Married | 94 | 92.15 |
| Single | 08 | 07.85 |
| Education level | | |
| Primary | 88 | 86.27 |
| Secondary | 03 | 02.94 |
| University | 11 | 10.79 |
| Occupation | | |
| Pupils | 02 | 01.96 |
| Students | 08 | 07.85 |
| Housewives | 85 | 83.33 |
| Trade | 02 | 01.96 |
| Seamstress | 02 | 01.96 |
| Nurses | 03 | 02.94 |
| Gestational age | | |
| First Trimestre | 23 | 22.55 |
| Second Trimester | 49 | 48.04 |
| Third Trimester | 30 | 29.41 |
| Parity | | |
| Primiparous | 24 | 23.53 |
| Paucaiparous | 32 | 31.37 |
| Multiparous | 46 | 45.10 |

Out of one hundred and thirty-five (135) pregnant women were met but only one hundred and two (102) approved to participate with the participation rate of 75.55% (102/135). The average age of the pregnant women was 24.11±5.56 years (minimum: 15 years and maximum: 44 years). The predominant age group was 25-34 years with 50.98% (n=52), followed by 15-24 years old with 46.08% (n=47) and pregnant woman aged between 35-44 years with only 2.94% (n=3). Among one hundred and two (102) pregnant women recruited, 92.15% (n=94) were married and only 7.85% (n=8) were single. Eighty-eight (86.27%) study participants were primary education level, 83.33% (n=85) were housewives, forty-nine (48.04%) were secondary gestational age, and forty-six (45.10%) were multiparous.

B. Seroprevalence of HBV Biomarkers

Among one hundred and two pregnant women tested, seroprevalence of HBV were 10.78% (n=11; 95%CI: 5.51-18.48), 15.68% (n=16; 95%IC: 6.78% - 27.58%), 9.80% (n=10; 95%IC: 2.52% - 17.08%), 26.47% (n=27; 95%IC: 15.67% - 37.27%) for HBsAg, anti-HBs antibodies (anti-HBsAb), anti-HBe antibodies (anti-HBeAb) and total anti-HBc antibodies (anti-HBcAb) respectively. Moreover, no HBeAg was found in the current study (Fig. 1).

### TABLE II: GENERAL INFORMATION OF PARTICIPANTS ASSOCIATED TO HBV BIOMARKERS

| Variables | Total N=102 | HBsAg Positive (%) | Anti-HBsAb Positive (%) | Anti-HBeAb Positive (%) | Anti-HBeAg Positive (%) |
|-----------|-------------|--------------------|-------------------------|-------------------------|-------------------------|
| Age groups (in years) | | | | | |
| 15-24 | 47 | 04 (8.51) | 07 (14.89) | 0.96 | 04 (8.51) | 0.95 | 04 (21.27) | 0.58 |
| 25-34 | 52 | 06 (11.54) | 09 (17.64) | 0.01 | 06 (11.76) | 0.01 | 16 (31.37) | 0.58 |
| 35-44 | 03 | 01 (33.33) | 0 (0.00) | 0 (0.00) | 0 (0.00) | 0 (0.00) | 01 (25.00) | 0.58 |
| Marital status | | | | | | | | |
| Married | 94 | 07 (7.45) | 14 (14.89) | 0.001 | 06 (6.38) | 0.0007 | 23 (24.46) | 0.24 |
| Single | 08 | 04 (50.00) | 02 (25.00) | 0.001 | 04 (50.00) | 0.0007 | 04 (50.00) | 0.24 |
| Education level | | | | | | | | |
| Primary | 88 | 11 (12.50) | 16 (18.18) | 0.52 | 10 (11.36) | 0.74 | 25 (28.40) | 0.16 |
| Secondary | 03 | 0 (0.00) | 0 (0.00) | 0 (0.00) | 0 (0.00) | 0 (0.00) | 02 (6.67) | 0.66 |
| University | 11 | 0 (0.00) | 0 (0.00) | 0 (0.00) | 0 (0.00) | 0 (0.00) | 0 (0.00) | 0.99 |
| Occupation | | | | | | | | |
| Pupils | 02 | 0 (0.00) | 0 (0.00) | 0 (0.00) | 0 (0.00) | 0 (0.00) | 02 (100) | 0.24 |
| Student | 08 | 0 (0.00) | 0 (0.00) | 0 (0.00) | 0 (0.00) | 0 (0.00) | 0 (0.00) | 0.99 |
| Housewives | 85 | 09 (10.59) | 16 (18.82) | 0.08 | 08 (9.41) | 0.009 | 23 (27.05) | 0.24 |
| Trade | 02 | 02 (100.00) | 0 (0.00) | 0 (0.00) | 02 (100) | 0 (0.00) | 2 (100) | 0.24 |
| Seamstress | 02 | 0 (0.00) | 0 (0.00) | 0 (0.00) | 0 (0.00) | 0 (0.00) | 0 (0.00) | 0.99 |
| Nurses | 03 | 0 (0.00) | 0 (0.00) | 0 (0.00) | 0 (0.00) | 0 (0.00) | 0 (0.00) | 0.99 |
| Gestational age | | | | | | | | |
| First Trimestre | 23 | 01 (4.35) | 02 (8.66) | 0.006 | 02 (8.66) | 0.006 | 03 (13.04) | 0.08 |
| Second Trimester | 49 | 06 (12.25) | 14 (28.57) | 0.001 | 04 (8.16) | 0.13 | 12 (24.48) | 0.08 |
| Third Trimester | 30 | 04 (13.33) | 0 (0.00) | 0 (0.00) | 06 (20.00) | 0.13 | 03 (10.00) | 0.08 |

Seroprevalence of HBV biomarkers

- **anti-HBcAb**: 73.53%
- **anti-HBeAb**: 90.20%
- **HBeAg**: 100.0%
- **anti-HBsAb**: 84.32%
- **HBsAg**: 89.22%

Fig. 1. Seroprevalence of Hepatitis B Virus biomarkers.
The overall seroprevalence of HBV (HBsAg) was 10.78%. Among pregnant women HBsAg-positive, higher seroprevalence, 11.54% (6/52), was observed in age of 25–34 years. The HBsAg seroprevalence of 7.45% (7/94) was recorded in age of 25 to 29 years. The finding can be explained through small sample size, a socioeconomic status and lifestyle of pregnant women who were vaccinated (anti-HBsAb positive), the higher rate (26.47% [27/102]) of those who had no vaccine, higher rate (26.47% [27/102]) of pregnant women enrolled were asymptomatic [19]. This result joins the prevalence of HBsAg in the high endemicity zone like SSA [21, 22]. Many studies conducted in Cameroon and SSA reported high prevalence of HBsAg among pregnant women [23]-[26] suggest that prevalence of HBsAg varies from place to place. The result obtained may explained through small sample size (15.68% [16/102]) of pregnant women who were vaccinated (anti-HBsAb positive), the higher rate (26.47% [27/102]) of those who have previous contact with hepatitis B virus (anti-HBcAb) and the knowledge about HBV-infection because fifty percent of study participants have no information about HBV. In the group of population who did not know disease, the higher seroprevalence of HBsAg was more observed (14.55% [8/102]). This higher HBsAg seroprevalence could be also explained by a small sample size, a socioeconomic status and the diagnostic technique used (RDTs) [19, 27, 28].

Concerning characteristics of pregnant women, higher HBsAg seroprevalence was more recorded in those who aged 25 to 34 years (11.54%). Similar findings were reported by [28] in Ethiopia who have observed the higher HBsAg prevalence amongst pregnant women aged 20 to 24 years and 25 to 29 years. The finding can have explicated by the fact that the age group associates with the uppermost age of sexual activity, signifying the role of sexual intercourse in HBV transmission [29]. HBsAg positive was only recorded in those who had scarification (HBc antibodies) and the presence of HBsAg after bivariate testing (P=0.01). In this study, more than 90% of individuals with HBsAg had HBc antibodies and anti-HBe antibodies. Six on twelve (50.00%) study participants who had scarification vs 4.00% (4/90) who had no scarification were anti-HBcAb positive (OR= 21.50; 95%CI 4.30-107.12; P=0.00001). Furthermore, we recorded higher seroprevalence of anti-HBcAb antibodies among women who did not know HBV seroprevalence with 36.36% (20/55) vs 14.89% (7/47) for those who know HBV disease (P=0.01).

### IV. DISCUSSION

The study was performed to determine prevalence of HBV using HBV-5 Rapid panel test and associated factors amongst pregnant women attending ANC in Garoua. A total of 102 pregnant women were recruited consecutively in this study with an average age of 24.11 years. Seroprevalence of HBsAg was 10.78% (95%CI: 5.51-18.48). This is reasonably because several pregnant women enrolled were asymptomatic [19]. This result joins the prevalence of HBsAg in the high endemicity zone like SSA [21, 22]. Many studies conducted in Cameroon and SSA reported high prevalence of HBsAg among pregnant women [23]-[26] suggest that prevalence of HBsAg varies from place to place. The result obtained may explained through small rate (15.68% [16/102]) of pregnant women who were vaccinated (anti-HBsAb positive), the higher rate (26.47% [27/102]) of those who have previous contact with hepatitis B virus (anti-HBcAb) and the knowledge about HBV-infection because fifty percent of study participants have no information about HBV. In the group of population who did not know disease, the higher seroprevalence of HBsAg was more observed (14.55% [8/102]). This higher HBsAg seroprevalence could be also explained by a small sample size, a socioeconomic status and the diagnostic technique used (RDTs) [19, 27, 28].

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of women were married and among them, 7.45% (7/94) were HBsAg positive compared to 50.0% (04/8) in single pregnant women. The HBsAg prevalence was reported in the two studies conducted by Bayo and collaborators in Uganda and Tegegne and collaborators in Ethiopia who found 10.6% and 2.64% respectively in pregnant women who were married [31], [32].

In this study, two variables (scarification and piercing or tattoo) were associated with HBsAg. Study participant with scarification had 30 times (OR = 30.10; 95%CI: 6.55-138.15; P<0.0001) high chance to contact the virus. In the other hand, women with piercing or tattoo had 12 times (OR = 11.80; 95% CI: 2.77-50.18; P<0.0001) high chance to carried HBV. Comparable results were recorded by [28] in Eastern Ethiopia (AOR= 8.9; 95%CI: 1.3-59.39; P=0.025), Baye and collaborators [33] in Ethiopia (AOR= 18.1; 95%CI: 2.9-114; P<0.002) and [34] in Northwest Ethiopia (AOR= 3.0; 95%CI: 1.17-7.80; P=0.02) who recorded that piercing or tattoo was statistically associated with the HBV infection. Otherwise, the seroprevalence of HBsAg was not significantly related to statistically associated with the HBV infection. Otherwise, the seroprevalence of HBsAg was not significantly related to the Knowledge about HBV. These could be explained by the small sample size.

V. CONCLUSION

The findings of this study reveal a high seroprevalence of HBV biomarkers amongst pregnant women in Garoua, North-Cameroon. The higher seroprevalence of HBsAg was more recorded in pregnant women in age group of 25-34 years, who were married and who were housewives’ occupation. Associated factors such as scarification and piercing or tattoo were statistically associated with HBV infection.

VI. RECOMMENDATION

The systematic screening of HBV using HBV-5 Rapid panel test and the awareness of pregnant women about vaccination in routine against HBV were necessary to prevent the transmission of Mother-to-Child Transmission (MTCT).

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