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Research Article

Prevalence of Ac Anti-Hbc Alone Among Pregnant Women at the Yaounde University Teaching Hospital

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Abstract

Introduction: Infection with the Hepatitis B virus (HBV) is a major public health issue; however, Asia and sub-Saharan Africa are the most affected regions. Occult hepatitis B Infection (OBI) refers to the presence of HBV DNA in the serum and/or liver of a patient despite negative HBsAg. It is typically an asymptomatic clinical form. Its reactivation is rare and generally occurs in immunocompromised individuals, such as those infected with the Human Immunodeficiency Virus (HIV) and pregnant women. *Objective:* The objective of this study was to determine the prevalence of anti-HBc antibodies (anti-HBc Ab) among pregnant women at the Yaound éUniversity Teaching Hospital (YUTH). *Methodology:* A prospective, cross-sectional, single-center study was conducted from September 17, 2018, to February 25, 2019, involving 102 pregnant women aged 15 to 44 years. Sociodemographic, clinical data, and risk factors were collected; samples were also taken and analyzed using immunochromatographic methods and PCR. A total of 102 pregnant women were tested for HBV markers (HBs antigen and anti-HBc antibody). Only samples containing anti-HBc antibodies were used for the search for HBV DNA. Statistical analysis was performed using Microsoft Excel 2019 and he statistical analysis used the Statistical Package for Social Sciences (SPSS) version 22.0. A p-value of <0.05 was considered statistically significant. *Results:* Out of the 102 pregnant women recruited, 91 tested negative for the HBs antigen, among which 17 were carriers of only the anti-HBc antibody. Of these, 8 were positive for HBV DNA, and 8 pregnant women presented with occult hepatitis B. *Conclusion and Suggestions:* The presence of the anti-HBc antibody and HBV DNA should be considered as markers of occult hepatitis B. It is imperative to implement systematic screening for these HBV markers to improve the management of pregnant women and their newborns, in order to prevent vertical transmission (mother-to-child).

Keywords

Anti-HBc Antibody, Hepatitis B Virus, OBI, Pregnant Women, YUTH

1. Introduction

Hepatitis B virus (HBV) is the most prevalent cause of viral inflammation of the human liver [1]. HBV infection is a public health issue causing more than a million annual deaths worldwide, [2] with a prevalence of 8% in sub-Saharan Africa [3]. Occult hepatitis B is a clinical form of this infection described in the early 1980s. It corresponds to the presence of

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HBV DNA in the serum and/or liver of a patient in whom the HBV surface antigen (HBsAg) is undetectable in the serum by standard serological tests [4]. It is usually asymptomatic and characterized by a very low level of HBV DNA. However, rare cases of reactivation of occult hepatitis B have been reported in the literature in immunocompromised individuals, such as those undergoing immunosuppressive treatment, [5] individuals infected with Human Immunodeficiency Virus (HIV), and during pregnancy [6].

According to national algorithms, the diagnosis of HBV infection typically focuses on the detection of HBsAg in biological samples [7]. The absence of HBsAg is considered a marker of hepatic clearance [4, 5]. Previous studies have shown that HBsAg may not be detectable in the serum of certain patients, which can be related to chronicity with an undetectable HBsAg level [8-10]. The undetectability of HBsAg is associated with mutations in the S gene region that encodes envelope proteins, including a major surface protein targeted by neutralizing antibodies as well as preS1 and preS2 proteins [11, 12].

The presence of anti-HBc antibodies (anti-HBc) is often linked to the absence of surface antibodies and antigens and is observed in various population groups, including pregnant women, individuals infected with HIV, drug users, and those co-infected with HBV and Hepatitis C Virus (HCV) [11]. Despite the fact that the presence of anti-HBc alone remains poorly understood, it could represent an occult HBV infection during which HBV DNA is present in the liver and/or blood while the serum level of HBsAg is below the threshold [13-15]. As a special form of HBV infection, occult hepatitis, although accounting for a relatively low proportion, may exacerbate disease transmission due to missed diagnoses, especially in resource - limited, highly endemic regions (such as Cameroon).

However, it is under-evaluated in our context, as HBsAg is almost the only serological test used for screening blood donations, premarital testing, examinations of pregnant women, organ transplantation procedures, and diagnostic investigations. particularity pregnant women is a high - risk group due to immune changes during pregnancy may increase the risk of viral reactivation and mother - to - child transmission occult HBV infection [16].

In Cameroon, there is a lack of data regarding the prevalence of markers of occult hepatitis. To improve the monitoring of pregnant women, the objective of this study was to determine the prevalence of isolated anti-HBc antibodies among pregnant women and associated risk factors to occult hepatitis at the Yaoundé University Teaching Hospital.

2. Materials and Methods

The study was a cross-sectional type conducted over a period of 6 months from September 17, 2018, to February 25, 2019, at the Gynecology Department of the Yaoundé University Teaching Hospital (YUTH). The participants were pregnant women attending consultations at YUTH.

The sampling was non-probabilistic and consecutive.

Each participant was required to sign a consent form prior to enrollment. A questionnaire was then administered, and a blood sample was collected and sent to the Hematology/Blood Bank Laboratory of the Yaoundé University Teaching Hospital, located in the Center region of Cameroon, which served as the site for biological analysis of the samples. Any missing information during the interview could be supplemented using the medical records. The standard measures necessary to guarantee the confidentiality of the information collected in the files have been taken. Only patient file numbers were recorded and access to the data was secured by an encrypted password. The study has been approved by the Ethics Committee of the Yaoundé Hospital and University Center before the start of the work and the study received authorization from the Yaoundé University Teaching Hospital under reference N/Ref: (N °245/AR/CHUY/DG/DGA/CAP RC).

2.1. Study Subjects

A total of 102 participants were included in the study. The inclusion criteria included: being pregnant women; providing assent for adolescents aged 12 to 20 years; obtaining parental consent for children aged 0 to 20 years; and providing informed consent for individuals aged 21 years and older. The exclusion criteria included: Excluded were any patients already on corticosteroids or immunosuppressants, and those unable to answer the investigator's questions.

2.2. Definition of Occult Hepatitis B

Occult Hepatitis B is defined as the presence of HBV DNA in the liver and the presence or absence of HBV DNA in the total blood of individuals who test negative for the HBs antigen. There are two types of occult hepatitis B: seropositive occult hepatitis B and seronegative occult hepatitis B, depending on the presence or absence of HBV markers in the serum of the subjects. Any person who has antibodies against HBV core antigens (anti-HBc antibodies) and/or anti-HBs antibodies in their serum is considered to have occult hepatitis B. An individual is considered seronegative for occult hepatitis B if they are negative for all HBV markers, including anti-HBc antibodies and anti-HBs antibodies, in whom the only detectable marker of HBV infection is intrahepatic (and more rarely circulating) HBV DNA [17].

2.3. Sample Collection

The collection of blood samples took place within the gynecology department of the YUTH. For each participant, five millilitres of blood were collected in tubes. Blood sampling was performed from the veins in the elbow crease, and blood samples were collected in dry tubes without anticoagulant.

2.4. Laboratory Analyses

All sera that were positive for both HBs antigen and HBc antibody, as well as those that were negative for HBs antigen and positive for anti-HBc, were tested for the presence of anti-HBs antibodies.

All these tests were conducted using cassettes based on the principle of immunochromatography with a Combtest (S/P) kit *(Nantong, Diagnos Biotechnology Co., China)* [18]. Anti-HBc antibody sera alone were stored at for HBV DNA testing. Viral was isolated using MagNA Pure Compact Nucleic Acid Isolation Kit (Roche Diagnostics, Mannheim, Germany), and quantitative real-time PCR was performed on the LightCycler instrument using LC-FastStart DNA Master HybProbes kit (Roche Diagnostics, Mannheim, Germany), targeting the HBV surface gene as previously described [19].

2.5. Statistical Analyses

For each participant, data on parameters of interest gathered through interviews and by blood analysis were recorded and processed using Excel 2019, and the statistical analysis was done using IBM Statistical Package for Social Science Version 22.0. On one hand, we used the Fischer exact and the Chi-square tests to compare qualitative variables between groups, while on the other hand, we used the Mann-Whitney test to compare quantitative variables. The threshold for statistical significance was set at $P < 0.05$ for a 95% confidence interval.

3. Results

3.1. Socio-demographic and Obstetric Characteristics

A total of 102 pregnant women were included in the study. They were aged 15 to 44 years with an average age of 24.11 ± 5.58 years; with a median of 25 years. The most represented age group was [25-35] years with a prevalence of 50% (95% CI: 40.11% - 59.8%). Concerning the marital status of our participants, 92.15% (95% CI: 85.13% - 96.55%) lived as a couple compared to 7.85% (95% CI: 3.45% - 14.87%) for singles. Regarding profession, pregnant housewives were significantly more represented with a proportion of 83.33% (95% CI: 74.66%-89.98%) followed by students, 7.94% (95% CI: 3.69%-21.77%; For parity, multiparous women had a proportion of 45.10% (95% CI: 35.22% - 55.26%; 46/102) compared to 31.37% (95% CI: 22.55% - 76.47%; 78/102) and 23.53% (95% CI: 15.69% - 32.96%; 24/102) respectively for primiparous. The sociodemographic characteristics of the study population are represented in Table 1 below.

Table 1. *Distribution of the study population according to socio-demographic characteristics.*

Socio-demographic and obstetric characteristics	Number n (%)	($IC_{95\%}$)
Matrimonial status		
Live in couple	94 (92.15)	85.13% - 96.55%
Bachelor	8 (7.85)	3.45% - 14.87%
Age		
[15-25]	47 (46.08)	36.23%-55.93%
[25-35]	51 (50)	40.11%-59.89%
[35-45]	4 (3.92)	0.2%-7.64%
Profession		
Students	10 (7.94)	3.69%-21.77%
Housewives	85(83.33)	74.66%-89.98%
Sellers	2 (1.96)	0.24%-6.90%
Seamstresses	2 (1.96)	0.24%-6.90%
Nurses	3 (2.94)	0.61%-8.36%
Parity		
Primipares	24 (23.53)	15.69% - 32.96%
Multipares	78 (76.47)	57.77% - 96.57%

3.2. Distribution of the Study Population According to Risk Factors

Depending on the risk factors, we noted that 80.4% (95% CI: 70.52%-93.49%) of pregnant women reported having transfusion, 11.76% (95% CI: 6.23%-19.65%) said they had scarifications, 10.78% (95% CI: 5.51%-18.41%) said they had tattoos. All these risk factors are represented in Table 2 below.

Table 2. *Distribution of the study population according to risk factors.*

Risks factors	Number n (%)	(IC95%)
Transfusion		
Yes	82(80.4)	70.52%-93.49%
No	20(19.6)	17.23%-19.65%
Scarification		
Yes	12(11.76)	6.23%-19.65%
No	90(88.24)	80.35%-93.77%
Piercing or Tattoo		
Yes	11(10.78)	5.51%-18.41%
No	91(89.22)	81.52%-94.49%

3.3. Presence of Markers for Hepatitis B Virus Infection

In this study, 91 out of 102 pregnant women tested negative for HBsAg, among whom 17 out of 91 were carriers of only anti-HBc antibodies. Out of these 17 pregnant women, 8 tested positive for HBV DNA, with an average of 4.6E+07 copies/ml.

4. Discussion

According to the report from the World Health Organization, in countries endemic for Hepatitis B Virus, the severity of HBV infection has been categorized into low (<2%), moderate (2-8%), and high (>8%) prevalence by WHO [20]. The objective of this study was to determine the prevalence of isolated anti-HBc antibodies among pregnant women at the Yaoundé University Teaching Hospital.

Our study reported that the most represented age groups are between 25 and 35 years old. This could be explained by the fact that the population is predominantly young, sexually active and likely to become pregnant. These results are similar to that of the work carried out by Omatola et *al*. (2019) in Anyigba, Nigeria, who found that the most represented age groups were 20-25 years, 25-30 years and 30-35 years [21].

In the present study, the prevalence of participants positive for anti-HBc antibodies was 18.7% (n=17). This result is higher than those reported by studies conducted on blood donors in Egypt and Italy, which were 10.3% (18) and 4.8% [22], respectively. These differences may be attributed to the varying endemicity of HBV in these different countries [23]. This antibody does not provide any protection against Hepatitis B virus; it indicates that a person may have been infected with HBV at some point. The presence of anti-HBc antibodies alone can be associated with the resolution of infection in the absence of anti-HBs antibodies, or with the formation of the HBs antigen-antibody complex, but cannot be considered a marker for occult Hepatitis B [12]. Cannot alone be considered evidence of viral infectivity. There are several mechanisms that have been hypothesized to lead to development of OBI. These include development of HBV S gene mutants that affect the detectability of the virus by conventional HBsAg assays, strong suppression of viral replication and reduced expression of HBsAg, epigenetic mechanisms and co-infection with other viruses [24]. Although the isolated presence of anti-HBc antibodies cannot directly diagnose occult hepatitis, the combined detection of anti - HBc antibodies and HBV DNA can significantly improve the diagnostic accuracy.

HBV DNA was detected in 47.05% of pregnant women who tested positive for anti-HBc antibodies. The higher the viral load, the greater the risk that the child will be infected and that chronic disease will occur; similarly, an increased viral load can raise the risk of maternal-fetal transmission to as high as 90% [16]. This result is higher than those reported by Gachara et al [25]. It should be noted however that the prevalence of OBI is dependent on the sensitivity of the DNA assay used, demography and the population studied [26]. Thus, the study used a more sensitive real time PCR method compared to the conventional PCR method used in other study. This result demonstrates the importance of conducting tests for anti-HBc antibodies and HBV DNA when the mother tests negative for HBs antigen.

These findings underscore the urgency of systematic screening for anti-HBc antibodies and HBV DNA in pregnant women and the need to revise the follow-up algorithm for pregnant women to reduce the risk of transmission to newborns.

However, our study showed that anti-HBc antibodies alone cannot be considered an indicator of occult Hepatitis B [27].

There is a lack of data concerning the epidemiology of occult Hepatitis B in Africa and Cameroon. However, some studies conducted on different populations report highly variable figures. For instance, in Cameroon, a prevalence of occult Hepatitis B of 5.9% was found among patients living with HIV, [28] while this rate was 17.2% among blood donors in Egypt [24]. And in Uganda, this rate was 11, 8% among patients [29]. The prevalence of occult Hepatitis B in our study was 7.8%. Our results are higher than the first prevalence but lower than the second prevalence. These differences in prevalence may be explained by the heterogeneity of study populations as well as by differences in serological tests and

molecular biology techniques used for the respective detection of HBsAg, anti-HBc antibodies, and HBV DNA. The prevalence of occult Hepatitis B is likely underestimated in our regions, highlighting the need for systematic screening in any woman with liver disease and a negative HBsAg.

Since we are in a region with limited resources, we suggest that organizations such as the WHO finance the implementation of genomic tests for the detection of HBV DNA.

Limitations: The prevalence of occult Hepatitis B among pregnant women in our study does not reflect the burden of occult Hepatitis B in our study area. The small sample size which does not allow robust statistical analysis and short duration of the study do not provide sufficient statistical power to generalize these results to all pregnant women in Cameroon, the monocentric and cross-sectional type of the study. Despite these limitations, this study has provided valuable information on occult Hepatitis B among pregnant women at the Yaoundé University Teaching Hospital.

5. Conclusions

At the conclusion of this study, which aimed to determine the prevalence of anti-HBc antibodies among pregnant women at the Yaoundé University Teaching Hospital, we found that the presence of anti-HBc antibodies alone is observed in healthy individuals. However, when associated with HBV DNA, it becomes an important marker for the detection of occult Hepatitis B. Based on the results of this study, we recommend including HBV DNA testing in the routine screening of pregnant women in Cameroon and other highly endemic regions to reduce the risk of mother - to - child transmission. Our study demonstrates that occult Hepatitis B poses a significant public health issue among pregnant women. The detection of positive anti-HBc antibodies and HBV DNA in a woman during her pregnancy justifies the need for effective treatment. Systematically screening the family members of pregnant women with positive HBV DNA can help detect potential infected individuals early and take intervention measures. It is recommended that future research explore the molecular mechanisms of the isolated presence of anti-HBc antibodies and their relationship with viral reactivation.

Abbreviations

Ab Anti-HBc	Anti-HBc Antibodies
HBV	Hepatitis B Virus
HBsAg	HBV Surface Antigen
HIV	Human Immunodeficiency Virus
HCV	Hepatitis C Virus
YUTH	Yaoundé University Teaching Hospital

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Author Contributions

Riwom Essama Sara Honorine: Designed and set up the research project.

Mbongue-Mikangue Chris Andr é Conceptualization, Collected the samples, Writing - original draft, Project administration.

Dikabo-Mikangue Grace Emmanuelle: Formal Analysis, Investigation, Project administration, Writing - review & editing.

The analysis of the data and the writing of this article saw the collaboration of all authors.

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Data Availability Statement

The data supporting the results of this study are available on request from the corresponding author. The data is not publicly available because it contains information that could compromise the confidentiality of research participants.

Conflicts of Interest

The authors declare no conflicts of interest.

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