**Epidemiology of hepatitis B virus infection among Pregnant Women in Lubumbashi, Democratic Republic of Congo: Prevalence, risk factors, and Genotype Distribution**

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A.K. designed and performed all the experiments. A.K., A.L., B.K. wrote the manuscript in consultation with C.K., C.M., C.N., F.D., and G.D.

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**Abstract**

**Background**

Mother-to-child transmission (MTCT) of hepatitis B virus (HBV) is a significant public health problem. Most children under five years living with HBV in endemic areas like sub-Slivingan Africa. Vertical transmission is considered the main newborn's route of contamination, which leads in 90% of cases to the chronic stage of the disease.

**Objectives**

To determine the seroprevalence and identify risk factors of carrying hepatitis B surface Antigen (HBsAg) in pregnant women, assess biochemical parameters, and study the distribution of HBV genotypes among infected pregnant women in Lubumbashi.

**Methods**

This was a cross-sectional descriptive and experimental study in which 1711 pregnant women were recruited. The study took place in the hospital Jason Sendwe of Lubumbashi. A pre-established epidemiological survey form was used to collect data from the study population.

**Results**

The seroprevalence of HBV among pregnant women was 4.4%. Blood transfusion and unprotected sex have been associated with the risk of carrying HBsAg. Increased levels of bilirubin and transaminases were observed. The genotypes E (59.4%), A (40.6%), and a few drug resistance mutations were identified in the study population.

**Conclusion**

With an HBV seroprevalence of 4.4%, MTCT of HBV remains a public health concern in Lubumbashi. This result highlights the vital role of HBV screening in pregnant women and newborns and early HBV vaccination. In addition, the obtained HBV genotyping data could help better understand the local epidemiology of the disease, predict the outcome of the Antiviral therapy, and develop a mapping of HBV genotypes in Lubumbashi.

**Keywords**: *Epidemiology, Genotypes, Hepatitis B Virus, Pregnant women.*

**Introduction**

Hepatitis B infection is a significant public health concern. The disease is caused by hepatitis virus (HBV), a DNA virus belonging to the *Hepadnaviridae* family. Without treatment, the chronic infection may result in cirrhosis and hepatocellular carcinoma [1]. The world health organization (WHO) estimated in 2017 that 257 million people worldwide are chronic carriers of the virus [1-2]. Further, WHO reported that HBV is responsible for 1,34 million deaths annually [1]. HBV is subdivided into several genotypes (A-J), which might influence the emergence of mutations, the response to antiviral treatment, and possibly vaccination against the virus [3]. With a prevalence rate between 9% and

20% of sub-Saharan Africa is highly endemic to HBV [1]. To date, there is no national survey of HBV prevalence in DRC. Mother-to-child transmission (MTCT) of hepatitis B virus (HBV) is a significant public health problem. Most children under five years living with HBV [4]. Vertical transmission is considered the main newborn's route of contamination, which leads in 90% of cases to the chronic stage of the disease[5-6]. Data has shown that 10 to 20% of HBsAg-positive pregnant women transmit the virus to their newborns. Viral infections in pregnant women have been responsible for morbidity and mortality in both mothers and children [7]. In addition, HBV is believed to increase the threat of preterm delivery and spontaneous abortion [8]. In newborns, HBV may increase the frequency of low birthweight children, and fulminant hepatitis may occur. In addition, the risk of transition to chronicity is grown in 90 to 95% of perinatal infections when HBV persists for more than six months, exposing infected newborns to cirrhosis and liver cancer [9-10].

Even though different rates of HBV seroprevalence in pregnant women have been reported worldwide [7, 9, 11], in the DRC in general and in Lubumbashi in particular, limited studies have been done on the seroprevalence of HBV in pregnant women. Moreover, to the best of our knowledge, HBV genotypes distribution in infected pregnant women in Lubumbashi has not been studied yet. Thus, the objectives of this work were: (I) to determine the seroprevalence and identify risk factors of carrying HBsAg among pregnant women in Lubumbashi, (ii) to assess the biochemical parameters of pregnant women infected with HBV in Lubumbashi, and (iii) to study the distribution of HBV genotypes in the infected pregnant women in Lubumbashi.

**Materials and Methods**

**. The site, Type, and Study Period**

This was a cross-sectional descriptive study of the prevalence of HBsAg in pregnant women from June 2018 to November 2020 in the HôpitSendon Sendwe, a hospital of public interest in Lubumbashi.

**Population and study parameters**

The study focused on pregnant women aged 18 to 44 years who came to the prenatal consultation service (PCS) of Hôpital Jason Sendwe during the study period. Pregnant women under 18 years and those presenting co-morbidity were excluded from this study. The parameters studied were socio-demographic characteristics, risk factors of carrying HBsAg, biochemical parameters, and molecular characterization of HBV. A pre-established epidemiological survey identified and recorded the socio-demographic characteristics and risk factors associated with HBsAg.

The minimum sample size was determined using the single-population proportion formula, based on the previous prevalence of HBsAg among positive pregnant women in Maniema (DRC), estimated at 5.9% [7]. Accordingly, the calculated minimum sample size was 311. As the study took place over two years, 1711 pregnant women were recruited andscreened for HBV during this study period.

**Screening for viral hepatitis B**

Hepatitis B surface Antigen (HBsAg) detection was performed using a rapid diagnostic test (RDT), "One Step Hepatitis B Surface Antigen Test® Strip (Accurate, China)" [12]. The HBsAg positive samples

were then confirmed using the Liaison XL®Quant HBsAg kit (Diasorin, Italy). In addition, all the HBsAg positive samples were also screened for HCV using SD Bioline HCV (Diagnostics, Korea) as RDT. At the same time, HIV co-infection data were collected from the medical records.

**Biochemical analysis**

The evaluation of biochemical parameters involved 76 HBsAg positive pregnant women and a control group made up of healthy 76 HBsAg negative pregnant women randomly selected. These parameters were assessed using commercial kits (Cypress Diagnostics, Belgium) using the Cyan Smart automated analyzer (Cypress Diagnostics, Belgium).

**Molecular characterization of viral hepatitis B**

This analysis was performed according to the recently reported protocol [13]. Following the HBV DNA extraction, the HBV viral load was evaluated by real-time PCR (qPCR) using the Lightcycler® 96 automated analyzer (Roche Diagnostics, Germany). The evaluated detection limit of HBV DNA qPCR was 100 IU/mL, and the specificity of 100%. The sequencing reaction was performed on the Veriti™ Thermal Cycler (Applied Biosystems™, CA, USA) and achieved by capillary electrophoresis on an ABI PRISM 3500 DNA analyzer (Applied Biosystems, CA, USA).

Sequences were aligned using the software "Geneious version 11.1 (Biomatters, New Zealand). Genotyping was performed using a "Nucleotide Blast" of the consensus sequence using National Center for Biotechnology Information (NCBI) software. The online HBVseq (Stanford University, U.K.) and geno2pheno (Max Planck Institut Informatics, Germany) identified HBV drug resistance mutations.

The phylogenetic tree was done following the neighbor-joining method on Geneious software with genetic distances computed by the Tamora-Nei model. To confirm the reliability of phylogenetic trees, bootstrap resampling and reconstruction was carried out 100 times.

The sequencing analysis allowed genotyping with a minimum HBV-DNA viral load of 1000 IU/mL. All HBsAg positive samples, including those with viral loads below 1000 IU/mL, were used to estimate the prevalence of HBV in the study population.

**Data processing and analysis**

The collected data were encoded, processed, and analyzed using Microsoft Excel 2010 software and EPI INFOTM version 7.2.4.0 (USA). The mean and standard deviation were calculated. The Chi-square test or Fischer's Exact test, when necessary, was used to find the association between the socio-demographic characteristics and risk factors and the positivity of the HBsAg. We considered statistically significant differences for p ˂ 0.05.

**Ethical approval**

A documented free consent was obtained from each pregnant woman. This study had received the approval of the medical ethics committee of the University of Lubumbashi under the number: UNILU / CEM / 095/2017.

**Results**

**Socio-demographic characteristics, risk factors of carrying HBsAg, the seroprevalence of HBV, and biochemical disturbances among pregnant women.**

During the study period, 1711 pregnant women were recorded. The HBsAg seroprevalence rate in pregnant women in this study was 4.4% (76 HBsAg positive pregnant women out of 1711 tested). Of the 76 HBsAg-positive pregnant women, 11 were co-infected with HIV and 7 HCV. A diagram on HBV testing of pregnant women to prevent MTCT of HBV and refer eligible women to treatment is illustrated in figure 1.

|  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  | | | | HBV testing in pregnant women  using rdt n = 1711 examined for eligibility | | | |  | |
|  | | | | | | | | | |
|  | | | | | | | | | |
| HBsAg+ (n = 76 potentially eligible) | | | |  | | | | HBsAg- (n = 1635) | |
|  | | | | | | | | | |
|  | | | | | | | | | |
| HBV DNA ≥ 200,000 IU/ ml  + normal ALT | |  | | HBV DNA < 200,000 IU/ ml  + normal ALT | | |  | HBV DNA > 200,000 UI/ ml. increased ALT | |
|  | | | | | | | | | |
| (N = 5 confirmed eligible) |  | | | (N = 71) | | |  | (N = 27 confirmed eligible) | |
|  | | | | | | | | | |
| Start maternal Tenofovir prophylaxis  (from 28 weeks of pregnancy birth)  - Reassess for long-term maternal  Tenofovir treatment after delivery | | |  | | No Tenofovir prophylaxis  - No long-term maternal  Tenofovir treatment  (Monitor and Assess) |  | | | Start Long-term maternal  - Maternal Tenofovir treatment and Assess) | |
|  | | | | | | | | | |
| * Vaccination of the baby   HBIG for infants if HBeAg positive or HBV-DNA | | | | | | | | | |

**Fig 1.** The diagram on HBV testing of pregnant women to prevent MTCT of HBV and refer eligible women to treatment.

The socio-demographic characteristics such as gestation period, gravidity, and unvaccinated women were significantly associated with the risk of carrying HBsAg among the study pregnant women (p<0,05). On the other hand, the risk factors which appeared to be significantly associated with the seroprevalence of HBsAg were blood transfusion history and unprotected sex (p=0,03).

The socio-demographic characteristics and risk factors of carrying HBsAg among the study population are listed in (Table 1).

Table Seroprevalence of HBsAg in pregnant women according to their risk factors

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
|  |  | HBsAg | | OR (CI 95%) | P |
|  |  | Positive (%) | Negative (%) |  |  |
| Age (years) | < 20 | 14 (5.7) | 231 (94.3) | 1.3 (0.8 – 1.4) | 0.09 |
| [20 – 34] | 30 (3.8) | 755 (96.2) | 1.1 (0.9 – 1.4) |
| [35 – 44] | 32 (4.7) | 649 (95.3) | 1.7 (1.3 – 2.7) |
| Gestation period (trimesters) (weeks) | 1st (< 14) | 9 (2.3) | 387 (97.7) | 2.2 (1.4 – 2.7) | 0.02 |
| 2nd (14 – 28) | 28 (5.2) | 513 (94.8) | 1.4 (1.2 – 2.3) |
| 3rd (> 28) | 39 (5.0) | 735 (95.0) | 2.6 (1.1 – 3.7) |
| Gravidity | Primigravida | 29 (3.8) | 729 (96.2) | 2.2 (1.5 – 2.8) | 0.01 |
| Multigravida | 47 (4.9) | 906 (95.1) | 2.7 (1.6 – 3.9) |
| HBV vaccination | Vaccinated | 0 (0.0) | 188 (100.0) | 1.2 (0.8 – 2.2) | 0.01 |
| Unvaccinated | 76 (5.0) | 1447 (95.0) | 2.2 (1.6 – 2.7) |
| Marital status | Married | 51 (4.6) | 1051 (95.4) | 1.1 (0.6 – 2.1) | 0.17 |
| Single | 25 (4.1) | 584 (95.9) | 1.4 (0.9 – 1.9) |
| Occupation | Liberal profession | 41 (4.4) | 896 (95.6) | 1.3 (0.2 – 2 .7) | 0.32 |
| employees | 19 (4.0) | 462 (96.0) | 1.2 (0.4 – 3.2) |
| Students | 11 (5.8) | 179 (94.2) | 1 |
| Unemployed | 5 (4.9) | 98 (95.1) | 1 |
| history of jaundice | Yes | 17 (3.4) | 479 (96.6) | 1.3 (0.9 – 1.8) | 0.16 |
| No | 59 (4.9) | 1156 (95.1) |  |
| Family history of hepatitis B | Yes | 8 (5.2) | 146 (94.8) | 1.2 (0.9 – 1.5) | 0.08 |
| No | 68 (4.4) | 1489 (95.6) |  |
| Blood transfusion history | Yes | 19 (8.5) | 205 (91.5) | 3.3 (1.5 – 5.3) | 0.03 |
| No | 57 (3.8) | 1430 (96.2) |  |
| History of surgery | Yes | 0 (0.0) | 89 (100.0) | 0.0 (0.0 – 18.7) | 0.13 |
| No | 76 (4.7) | 1546 (95.3) |  |
| Tattoos or scarifications | Yes | 0 (0.0) | 12 (100.0) | 0.0 (0.0 – 13.6) | 0.15 |
| No | 76 (4.5) | 1623 (95.5) |  |
| Sharp injury | Yes | 5 (1.6) | 310 (98.4) | 1 | 0.06 |
| No | 71 (5.1) | 1325 (94.9) |  |
| Intravenous drug addiction | Yes | 0 (0.0) | 0 (0.0) | 0.0 (0.0 – 14.7) | 0.11 |
| No | 76 (4.4) | 1635 (95.6) |  |
| Unprotected sex | Yes | 13 (1.8) | 711 (98.2) | 2.7 (1.2 – 3.6) | 0.03 |
| No | 63 (6.4) | 924 (93.6) |  |
| Multiple sexual partners | Yes | 0 (0.0) | 129 (100.0) | 0.0 (0.0 – 19.7) | 0.14 |
| No | 76 (4.8) | 1506 (95.2) |  |

N: number, OR: odds ratio, %: percentage.

Pregnant women HBsAg positive group presented a significant increase in the levels of total bilirubin (p=0,001), direct bilirubin (p=0,003), and transaminases ALT (p=0,020), AST (p=0,031) (Table 2).

Table : Comparison between pregnant women in HBsAg positive group and HBsAg negative group according to the biochemical parameters.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Parameters | HBsAg + (n =76) | HBsAg - (n =76) | OR (CI95%) | P-value |
| T-C (< 200 mg/dL) | 193.2 ± 4.2 | 198.2 ± 4.1 | 1.8 (0.91 – 2.57) | 0.201 |
| HDL-C (> 45 mg/dL) | 39.1 ± 3.2 | 41.4 ± 3.3 | 2.1 (1.11 – 3.15) | 0.112 |
| LDL-C (< 130 mg/dL) | 141.3 ± 3.9 | 136.7 ± 4.1 | 1.3 (0.81 – 2.71) | 0.131 |
| TG (< 130 mg/dL) | 142.6 ± 3.6 | 139.2 ± 3.3 | 1.5 (1.17 –3.25) | 0.101 |
| T-Bil (˂ 12 mg/L) | 14.9 ± 3.1 | 12.9 ± 2.2 | 1.1 (0.47 –2.17) | 0.001 |
| D-Bil (˂ 2 mg/L) | 3.2 ± 1.8 | 1.8 ± 2.1 | 1.9 (1.12 – 2.21) | 0.003 |
| I-Bil (˂ 10 mg/L) | 11.3 ± 2.6 | 11.8 ± 2.2 | 1.3 (1.09 – 2.31) | 0.114 |
| ALT (8 – 35 UI/L) | 49.2 ± 5.2 | 27.9 ± 5.1 | 2.9 (1.11 – 3.17) | 0.020 |
| AST (8 – 30 UI/L) | 51.1 ± 3.8 | 29.3 ± 3.8 | 2.1 (1.13 – 2.79) | 0.031 |
| ALP (30 – 100 UI/L) | 88.8 ± 7.2 | 59.7 ± 9.1 | 2.3 (1.21 – 4.01) | 0.071 |
| γ GT (15 – 60 UI/L) | 54.7 ± 5.1 | 57.3 ± 4.4 | 1.7 (1.15 – 3.86) | 0.117 |
| LDH (190 – 400 UI/L) | 281.3 ± 5.3 | 271.6 ± 7.2 | 2.2 (1.15 – 3.19) | 0.089 |
| Alb (35 – 50 g/L) | 39.2 ± 4.6 | 43.9 ± 4.3 | 1.4 (0.96 – 2.57) | 0.121 |
| CRP (< 6 mg/L) | 6.6 ± 2.1 | 5.7 ± 3.6 | 1.7 (1.11 – 2.87) | 0.111 |

T-C: Total Cholesterol, HDL-C: HDL-Cholesterol, LDL-: LDL-Cholesterol, TG: triglycerides, T-Bil: Total bilirubin, D-Bil: Direct bilirubin, I-Bil: Indirect bilirubin, ALT: alanine aminotransferase, AST: aspartate aminotransferase, ALP: Alkaline phosphatase, gt: gamma-glutamyl transferase, LDH: Lactate dehydrogenase, Alb: Albumin, CRP: C reactive protein, OR: odds ratio, CI: confidence interval, ±: more or less, +: positive, -: negative.

**HBV molecular characterization among pregnant women in Lubumbashi**

Of the 76 HBsAg positive samples, 32 (42.1%) with a viral load greater than 1000 IU/mL were eligible for the study of HBV genotypes. The mean HBV viral load in HBsAg-positive pregnant women was 4.6 ± 1.6 log10 I.U./mL. Of the 32 analyzed samples, the distribution of the HBV identified genotypes was as follows (Table 3): 19 genotype E (59.4%), 13 genotype A (40.6%). Of the 13 infected pregnant women with genotype A, 11 were related to the A1 and 2 to the sub-genotype A3. The phylogenetic tree from alignments of different nucleotide sequences of HBV-positive pregnant women to the

reference sequences confirmed these genotypes (figure 2). In addition, the phylogenetic analysis showed that the identified HBV subgenotype A1 strains (N=11) formed a subgroup close to the

Rwanda strains. In contrast, the HBV genotype E was relative to the strains from the Ivory Coast and the Republic of South Africa. The mean viral load among pregnant women with genotypes E and A was 4.9 ± 1.7 log10 U.I./mL and 4.2 ± 1.6 log10 U.I./mL (Table 3).

Table Detected genotypes, sub-genotypes**,** mutations, and resistance to antiviral drugs

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| Genotypes | Number | Viral load | Mutants | Resistance mutations | Antiviral drugs | |
| N (%) | (Log10 IU/mL) | N (%) | Types | Resistance | Susceptibility |
| A1 | 11 (34.4) | 4.2 ± 1.6 | 1 (9.1) | 202T, 250L | ETV | LAM, ADV, TDF/TAF, LdT |
| A3 | 2 (6.2) | 5.0 ± 1.1 | 1 (50) | 181T, 202T, 180M, 204I, 184M, 173E, 173K, 173M, 202H, 202P, 202Q, 202N, 236H | LAM, ADV, ETV, TDF/TAF, LdT | - |
| E | 19 (59.4) | 4.9 ± 1.7 | 1 (5.3) | 173E, 173K, 173M | LAM | ADV, ETV, TDF/TAF, LdT |
| Total | 32 (100) | Mean : 4.6 ± 1.6 |  |  |  |  |

N: number, LAM: lamivudine, ETV: entecavir, ADV: adefovir, Ld: telbivudine, TDF/TAF: tenofovir

Few drug resistance mutations were found in 3 participants and are listed in Table 3. However, they resulted in resistance to HBV antivirals, including lamivudine, entecavir, or tenofovir.

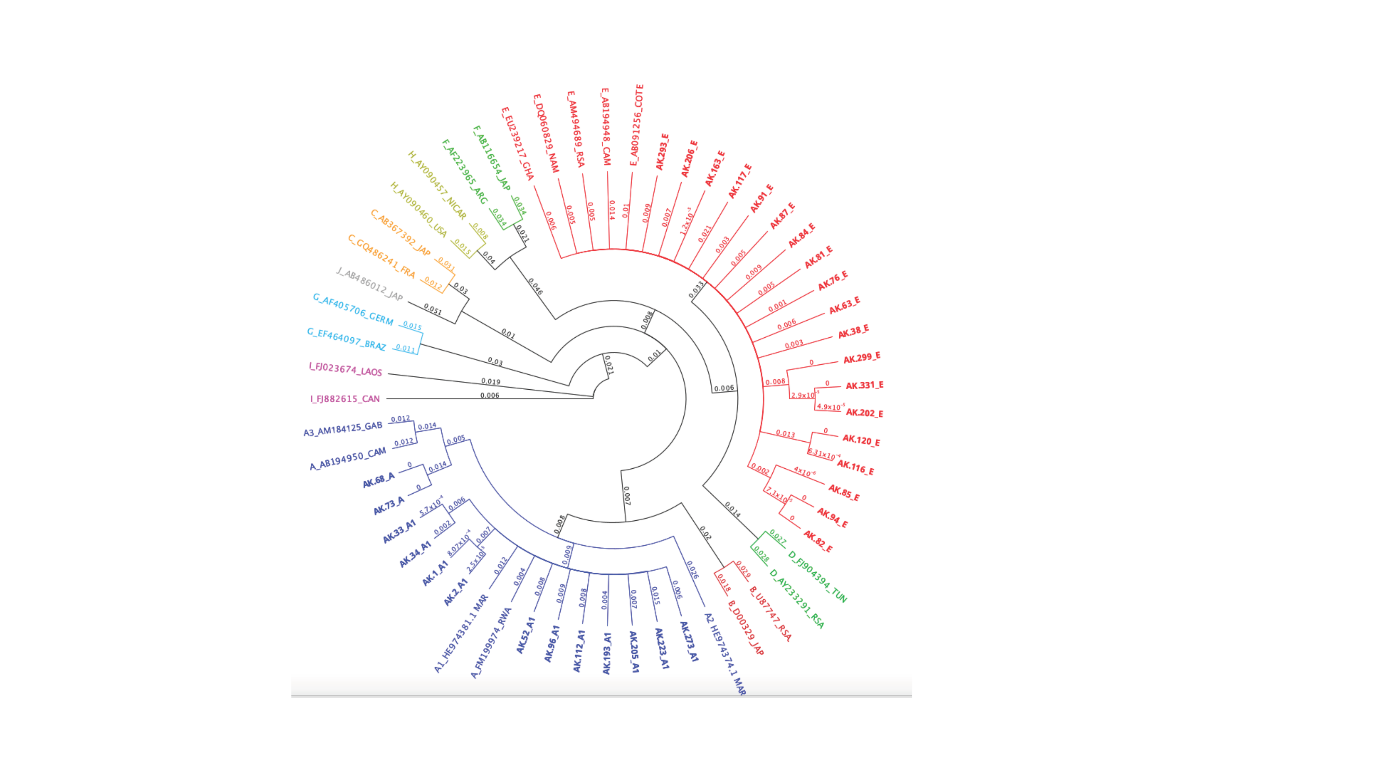


Fig2: Phylogenetic analysis of HBV genotypes A and E distribution among pregnant women in Lubumbashi positive HBV.This phylogenetic tree is derived from an alignment of 32 HBV DNA sequences and 25 reference sequences. Sequences from clinical samples are identified with the initials "A.K." (bold letters), while the reference sequences are identified by NCBI accession number. The tree branches are colored according to the HBV types, and each node's confidence above 70% is indicated.

**Discussion**

One thousand seven hundred eleven pregnant women were identified and included in the study during the study period. The overall prevalence observed was 4.4%. Similar results were reported from studies carried out among pregnant women in Tunisia [14] and Ethiopia [9], with 4% and 4.5%, respectively. In contrast, this HBV seroprevalence was lower than other rates of 15.5% reported in Bamako [15], 5.9% in DRC [7], and 11.4% in Ouagadougou [16]. However, it was higher than other rates of 1.7% reported in Japan [17], 2.2% in Nigeria [18], and 2.35% in Morocco [19]. Variations in HBV seroprevalence among pregnant women worldwide might be attributed to differences in sampling methods, geographical interpretation, cultural practices, sexual behavior, the quality of test methods used to detect HBsAg [9], and the vaccination coverage.

The socio-demographic characteristics of pregnant women, such as the gestation period (> 28 weeks), gravidity (multigravida), and unvaccinated women, were associated with the risk of carrying HBsAg. Our result is in line with the study of Oladeinde et al. [18]. Poor management, frequency of unprotected sex, and blood transfusions may explain the high risk of HBsAg carriage in multigravida women and pregnant women older than 28 weeks of pregnancy. Moreover, no pregnant woman among those vaccinated against HBV has been reactive for HBsAg. As some studies have indicated [20], vaccination against HBV is the most effective protection against infection with HBV. Therefore, WHO recommended incorporating HBV vaccination into the national immunization program of the endemic countries? DRC implemented this recommendation in 2007 [21], but the HBV vaccine is not compulsory in DRC.

Concerning the risk factors of carrying HBsAg, pregnant women with a history of blood transfusion and unsafe sex had a higher prevalence of HBV. This result is consistent with that reported in Nigeria [18]. Once again, these results highlight the need to elaborate strategies aiming to reduce HBV transmission by blood transfusion in the RDC since it has been demonstrated that the blood transfusion rate is higher in women than in other categories of individuals [22]. Further, awareness campaigns on different modes of transmission of HBV, particularly the sexual route, should be organized for pregnant women, as data has demonstrated that HBV infection is among the most widespread sexually transmitted diseases globally.

Significant modifications of total bilirubin, direct bilirubin, and transaminases (ALT and AST) were observed concerning the biochemical parameters in pregnant women. The obtained results concordance with Ashraf-Uz-Zaman et al. [23] and Atheer et al. [24]. In contrast, they did not corroborate those reported by Chen et al. [25] and Gao et al. [26]. Nevertheless, increases in transaminase activity are seen in liver disease, and several studies have shown a positive correlation between the rise in transaminase activity and viral infections [27].

Regarding the molecular characterization, the distribution of identified HBV genotypes was consistent with recent findings in blood donors in previous studies [13], with predominant genotypes E and A. Blood donors and pregnant women constitute populations at high risk of transmitting HBV. The observed results could be explained by the geographic location of the study population. In the DRC, few studies performed on HBV genotypes distribution reported a predominance of HBV genotype A strains in the eastern part of the DRC and HBV genotype E strains in the western part of the DRC [28-29]. These data might justify the presence of genotype A among pregnant women in Lubumbashi. However, the presence of genotype E may be attributed to the population's different migratory movements, including the people coming from Kinshasa and the Republic of South Africa. The identified HBV strains of genotype A (sub-genotype A1) are reported for the first time among pregnant women in Lubumbashi (the South-Eastern part of DRC). They were close to the strains found In Rwanda, a neighboring country to the eastern part of the DRC. African sub-genotype A1 has been described as a particularly aggressive genotype, conferring a higher risk of hepatocellular carcinoma among black African people [30].

The majority of the identified HBV genotype E was close to those reported in the Republic of South Africa and Ivory Coast, two neighboring countries of the DRC.

Unlike HCV genotyping, there are guidelines recommending HBV genotyping to personalize HBV treatment. However, the literature has shown that HBV genotyping could help predict the antiviral treatment outcome. Indeed, it has been reported that HBV genotypes A and C are associated with a lower rate of favorable response to interferon-alpha therapy than genotypes A and B. Further, the level of resistance to lamivudine is higher in patients with genotype A infection than in patients with genotype D infection. In addition, the virological response is worse during lamivudine therapy [13]. In addition, in the frame of this work, we did not study the aspect of HBV personalized treatment versus identified genotypes.

Quantifying the viral load during pregnancy could help assess the risk of HBV transmission to implement rapid and effective vaccination from birth and plan maternal antiviral treatment during the third trimester of pregnancy when the viremia is high [19]. Accordingly, an antenatal HBV DNA level > 6 log10 copies/ml (> 200000 IU/mL) was the most important predictor of MTCT [31].

Furthermore, the European Association for the Study of the Liver (EASL) and the Asian Pacific Association for the Study of the Liver (APASL) guidelines recommend treating pregnant women when HBV DNA levels are > 2x106 I.U./mL in the third trimester for the prevention of MTCT [31]. In the current study, the mean HBV viral load was 4.6 ± 1.6 log10 I U./mL.

Some drug resistance mutations have been identified in 3 HBsAg-positive pregnant women. They might be attributed to HBV antiviral treatment used in Lubumbashi with Lamivudine or Tenofovir. Previous combined antiretroviral therapy, including Lamivudine or Tenofovir in HIV coinfected pregnant women, could lead to HBV drug resistance. In DRC, tenofovir could be used in the case of resistance to Entecavir and Lamivudine, but the low income of the population limits access to antiviral treatment.

Our study population consisted only of pregnant women. Therefore, that could not generalize the obtained results to the general population. In addition, our study had limitations: first, the sequencing analysis allowed genotyping with a minimum HBV-DNA viral load of 1000 IU/mL. Second, limited risk factors associated with HBsAg among pregnant women were studied. Considering all of the above, there is a need to carry out a further large-scale study using more risk factors (such as abortion history, cesarean section history, tooth extraction, home delivery history, and so on) to highlight the association between the risk above factors and the HBV infection.

**Conclusion**

This study showed an HBV seroprevalence of 4.4% among the study pregnant women in Lubumbashi. Blood transfusion and unprotected sex have been identified as risk factors significantly associated with HBsAg carriage. Accordingly, efforts to reduce the risk of transmission and associated factors of HBV infection among pregnant women should be strongly encouraged. Such measures should include strengthening HBV blood and blood products screening, health education of pregnant women on the mode of contamination of HBV, and a systematic vaccination of pregnant women and newborns against HBV. In addition, the evaluation of changes in biochemical parameters could help assess the level of liver damage. Genotypes E and A (A1) and some HBV genome mutations causing resistance to antiviral drugs have been identified for the first time in Lubumbashi. These results could help better understand the local epidemiology of the disease, predict the outcome of the antiviral therapy, and develop a mapping of HBV genotypes in Lubumbashi.

**Footnotes.**

**Peer- Reviewers:** Hany Sadek, (professor of internal medicine), Mohamed Emara, (professor of hepatogastroenterology and infectious diseases), Mohamed Bassuny, (professor of internal medicine), Hanan Hamed Soliman (professor of tropical medicine), Hanan Hassan (professor of community medicine).

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**Declaration of competing interest**

There are no conflicts of interest related to this study.

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