Hepatitis B Surface Antibody Titer in Family Members of Patients with Chronic Hepatitis B Infection

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Abstract

Background: Hepatitis B virus (HBV) causes liver disease and death worldwide. It affects millions globally and may cause cancer. Chronic hepatitis B needs virological and serological testing, constant monitoring, and therapy. Females respond more to HBV vaccinations, making childhood vaccination essential.

Objectives: To assess the frequency of HBV infection in family members of chronic HBV patients and identified those at greatest risk based on kinship degrees.

Patients and methods: The study involved 100 family members of any age and sex of patients with chronic hepatitis B. The study was conducted at the outpatient clinic and inpatients sector of the Tropical Medicine & Gastroenterology department from 1st April 2022 to 30th April 2023. Complete history taking and clinical examination was done for all participants. Various laboratory tests, including HBsAg, HCV antibodies, HBs antibodies, and HBV DNA viral load, were conducted to assess HBV infection.

Results: The study showed that 55% of participants were males. Among females, 65.2%had HBsAb titers below 10, compared to 34.8% of males. There was no significant difference in HBsAb titers between rural and urban areas. Siblings had higher HBsAb titers compared to other family members. The study population demonstrated a wide range of HBsAb titers, from 8.50 to 1890, with vaccination significantly influencing titers.

Conclusion: The study highlighted the association between HBsAb titers and different factors such as age, family members, white blood cell count, serum creatinine, albumin level, and vaccination. Understanding these associations can aid in developing targeted prevention and management strategies for chronic hepatitis B.

Keywords: Hepatitis B; Chronic; Surface Antibody; Family Members.

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Introduction

HBV infection is a significant public health concern, causing high morbidity and mortality due to acute and chronic liver damage **(Elbahrawy et al., 2021).**

HBV, a member of the Hepadnaviridae family, is a small DNA virus with retrovirus-like characteristics. It affects over 300 million individuals worldwide, leading to liver diseases and cancer. Virological and serological testing are crucial for diagnosing and managing chronic hepatitis B infection, which can cause acute and chronic hepatic failure, cirrhosis, and hepatocellular carcinoma. Acute HBV infection may result in symptomatic or asymptomatic hepatitis, and while most infected adults recover, 5%-10% remain chronically infected. Chronic HBV infection can lead to mild liver disease or progress to severe outcomes, such as cirrhosis and liver cancer. Close monitoring and appropriate treatment are necessary for patients with chronic infections **(Van Damme et al., 2021).**

Perinatally infected children face a high risk of becoming chronic carriers of HBV, with approximately 90% developing chronic infection. The risk of carrier status decreases with age, dropping to 25-30% for children aged one to five and 5% for immunocompetent adults. Vaccination during childhood is essential to ensure adequate protection. Age and gender affect HBV vaccination efficacy measured by anti-HBs. According to a study, anti-HBs levels diminish with age and gender may affect viral vaccine responses. Female children and adults had stronger humoral and cell-mediated immune responses than men after immunization **(Schillie et al., 2018).** The study aims to study chronic hepatitis B infection in family members.

Patients and methods

The study was cross-sectional and conducted at the outpatient clinic and inpatient sector of the Tropical Medicine and Gastroenterology department from 1st April 2022 to 30 April 2023. Included 100 family members of chronic hepatitis B patients vaccinned with HBV vaccine.

Inclusion criteria: adult subjects > 18 year confirmed to be Family Members of Patients with Chronic Hepatitis B Infection. We excluded those already diagnosed, those who received vaccination, those with prior infection or exposure, healthcare workers, those with previous antiviral treatment, individuals with liver diseases other than chronic hepatitis B, immunosuppressive conditions, and those who refuse to participate or provide informed consent.

Methods

All the family members of chronic hepatitis B patients were subjected to:

1- Complete history taking, including associated health problem, receiving hepatitis B vaccine or not, and exposure to any risk factors (e.g.: injection drug use, healthcare workers, tattoos and body piercings, hemodialysis, occupational exposure, and comorbidity).

2- Full Clinical Examination: which includes (BMI), signs of any detectable disease.

3- Laboratory Investigations

- Blood Sampling: 7 ml venous was collected and divided into 4 tubes: 2 ml blood in an EDTA tube for complete blood count (CBC), 1.8 ml blood in a citrate tube for clotting assay, 1.5 ml blood in a 2 plain tube that left to clot. Citrated and plain tubes were centrifuged at 3000 x g for 15 minutes at room temperature to obtain plasma for prothrombin time and serum for liver functions, kidney functions, and serology for hepatitis B and C.
  - CBC (Complete Blood Count): Using Yumizen H550 analyzer by HORIBA ABX SAS, France.
  - Liver and Kidney Function Tests: for estimation of AST, ALT, albumin,

Total and direct bilirubin, and serum Creatinine (Beckman, Fullerton, California, USA).

- HCV Ab (Hepatitis C Virus Antibody): Using a two-step process. First, a Rapid Immunochromatography test (CTK/USA) was used for initial screening. Then, the Foresight-EIA-USA assay was used to test for anti-HCV antibodies (IgG).
- Hepatitis B Surface Antibodies (Anti-HBs and Anti-HBe Antibodies): using commercially available microparticle enzyme immunoassays (HBs Ag V2.0, HBe Ag 2.0 (AxSYM assay; Abbott, Rungis, France).
- Hepatitis B DNA Viral Load: HBV DNA viral load quantification was performed using the commercially available Hybrid Capture II Digene kit, Abbott, Rungis, France, and the HBV Monitor Cobas (Roche Diagnostics, Meylan, France).
- Patients were stratified into three subgroups based on their HBs Ab titres: a group achieving seroprotection following the standard hepatitis B vaccination protocol (anti-HBs ≥100 mIU/mL), a low-response group (anti-HBs 10-100 mIU/mL), and a nonresponse group (anti-HBs <10 mIU/mL) **(Wiedmann et al., 2000).**

4. Abdominal Ultrasonography (US): which includes: Liver size (diameter) and Liver echogenicity. Ultrasonography was done using (General Electric, Vivid 5S) and performed according to the hospital practice (**Griffin, 2019**).

Ultrasonographic criteria for diagnosing liver conditions encompass distinct features for fatty liver (hepatic steatosis), liver cirrhosis, and liver fibrosis. For fatty liver, these criteria include increased liver brightness (hyperechoic appearance), blurred vessel borders, and reduced diaphragm visibility **(Hernaez et al., 2011).**

In cases of liver cirrhosis, typical findings involve a nodular liver surface, splenomegaly, signs of portal hypertension such as dilated portal veins and portosystemic collaterals, ascites detection, potential hepatic nodules in advanced stages, and the use of Spectral Doppler to assess blood flow patterns in the liver and portal vein. Liver fibrosis, characterized by the buildup of scar tissue, is identified through findings such as altered liver texture and increased liver stiffness on elastography, a specialized ultrasound technique **(Aubé et al., 1999).**

Ethical Considerations: The study followed the ethical considerations of QUH under the code of ethics SVU-MED-GIT023-1-22-2-319.

Statistical analysis

IBM SPSS version 20.0 was used to input and analyze the data. Qualitative descriptive statistics used numbers and percentages. Shapiro-Wilk tested quantitative data distribution normality. Quantitative data included minimum, maximum, median, and interquartile range (IQR). Results were significant at <0.05. Statistical tests employed in the study include the Chi-Square Test, applied to categorical data presented as numbers and percentages; the One-Way ANOVA Test, used for continuous data denoted by mean and SD or median and Range; the Tukey Post-Hoc Test, which facilitates pairwise comparisons among continuous data following one-way ANOVA; and the Monte Carlo Test (MC), employed for targeted comparisons within categorical data.

Results

One hundred family members of chronic hepatitis B cases were included of them having positive liver comorbidity in

the form of hepatosplenomegaly, liver cirrhosis, positive HCV Ab, positive HBV.

HBs Ag titre in family members was 82.76 ± 62.42 mIU/mL. Based on HBs Ag titre we subdivided cases into three sub groups: those with <10 titre members (23) displaying a value of 6.43 ± 2.15 mIU/mL, a group of 10-99 titre members (36) with a value of 58.81 ± 21.9 mIU/mL, and a group with a titre more than 100 members (41) exhibiting a value of 146.61 ± 34.29 mIU/mL.

Demographics and HBs Ab titre (mIU/mL) values for 100 study participants are shown in this table. There were 55 men and 45 women. Females had insignificant higher HBs Ab titres >100 than men (p=0.085). Rural vs. urban residence did not impact HBs Ab titres (p=0.256). A strong correlation (p<0.001) was found between family members and HBs Ab titre levels, with brothers having the greatest (53.7%) proportion of HBs Ab titre >100. varied HBs Ab titre groups had significantly varied mean ages (p=0.003). Significant Age differences between <10 and 10-99 groups (p=0.006), 10-99 and >100 groups (p=0.013), but not between <10 and >100 groups (p=0.771).(**Table.1).**

Table 1. Demographic data Of all studied cases

Variables	HBs Ab titer (mIU/mL)						Test of Sig.	p
	<10 (n = 23)		10-99 (n = 36)		>100 (n = 41)			
	No.	%	No.	%	No.	%		
Sex								
Male	8	34.8	22	61.1	25	61	χ^2= 4.933	0.085
Female	15	65.2	14	38.9	16	39		
Residence								
Rural	15	65.2	22	61.1	19	46.3	χ^2= 2.727	0.256
Urban	8	34.8	14	38.9	22	53.7		
Family member							χ^2= 36.656*	^{MC}p <0.001*
Father	0	0	5	13.9	0	0		
Mother	0	0	8	22.2	6	14.6		
Wife	8	34.8	3	8.3	2	4.9		
Sister	6	26.1	0	0	4	9.8		
Son	1	4.3	5	13.9	3	7.3		
Brother	7	30.4	15	41.7	22	53.7		
Daughter	1	4.3	0	0	4	9.8		
Age (years)	32.87 ± 6.09		39.08 ± 9.64		34.20 ± 5.55		F= 6.307*	0.003*
Sig. bet. Grps. (Age)	p_1=0.006*, p_2=0.771, p_3=0.013*							

χ2: Chi square test, MC: Monte Carlo, F for One way ANOVA test, Pairwise comparison bet. each 2 groups was done using Post Hoc Test (Tukey). p: p value for comparing between the three studied groups, p1: p value for comparing between <10 and 10 – 99, p2: p value for comparing between <10 and >100, p3: p value for comparing between 10 – 99 and >100.

41.0% of the patients had HBs Ab titre larger than 100 (mIU/mL), 36.0% were between 10 and 99 (mIU/mL), and 23.0% were below 10 (mIU/mL). HBs Ab titre varies from 8.50 to 1890 (mIU/mL), demonstrating substantial variation in responses, (**Table.2).**

Table 2. HBs Ab titre in all studied cases (n = 100)

HBs Ab titre (mIU/mL)	No.	%
<10	23	23
10-99	36	36
>100	41	41
Min. – Max.	8.50 – 1890.0	
Mean ± SD.	527.9 ± 633.4	
Median (IQR)	11.35 (10.20 – 1260.0)	

IQR: Inter quartile rangeSD: Standard deviation

The observed insignificant difference in hemoglobin levels between these groups was not statistically significant (p = 0.186). and no observed correlation with HBs Ab titre (mIU/mL).

HBs Ab titre groups showed insignificant difference in the mean PLT counts (p = 0.235).

The mean white blood cell (WBC) counts differed significantly across these groups (p = 0.004). The 10-99 (mIU/mL) and >100 (mIU/mL) groups had significant higher WBC counts while <10 (mIU/mL) and 10-99 (mIU/mL) groups were similar (p1=0.976).

However, ALT and AST levels did not significantly differ between these groups (p=0.155, p=0.753).

Mean albumin (ALB) levels did vary significantly among the three groups. The >100 (mIU/mL) HBs Ab titre group had lower ALB levels compared to the 10-99 (mIU/mL) and <10 (mIU/mL) groups (p1=0.832, p2=0.037).

Total bilirubin and direct bilirubin levels did not show significant differences among the three groups (p=0.68, p=0.129, respectively).

Regarding prothrombin time (PT), there was insignificant difference among the groups (F=1.625, p=0.202). The INR values were comparable among the three groups (F=0.485, p=0.617).

WBC (White Blood Cell) count exhibited significant differences across the groups (p=0.004). Specifically, the >100 (mIU/mL) group had a significantly higher WBC count compared to the 10-99 (mIU/mL) group (p2=0.018) and the <10 (mIU/mL) group (p3=0.012). ALB (Albumin) levels also showed significant differences (p=0.002). The >100 (mIU/mL) group had a decreased ALB level compared to the 10-99 (mIU/mL) group (p2=0.037) and the <10 (mIU/mL) group (p3=0.002). Serum creatinine levels had significant differences (p=0.003). The <10 (mIU/mL) group had increased serum creatinine levels compared to both the 10-99 (mIU/mL) group (p2=0.037) and the >100 (mIU/mL) group (p3=0.002). No significant differences were observed in other measured parameters among the three groups, (**Table. 3).**

Table 3.Comparison between the three studied groups according to laboratory data

Variables	HBs Ab titre (mIU/mL)			F	p
	<10 (n = 23)	10-99 (n = 36)	>100 (n = 41)		
CBC					
Hb (g/dL) (Mean ± SD)	11.72 ± 0.96	11.45 ± 1.35	11.17 ± 1.07	1.71	0.186
PLT (x10^3/µL) (Mean ± SD)	234.8 ± 24.76	227.8 ± 31.55	222.7 ± 24.08	1.47	0 .235
WBC (x10^3/µL) (Mean ± SD)	5592.9±1170.0	5661.6±1246.2	6472.1±1223.9	5.718*	0.004*
Sig. bet. Grps. (WBC)	**p_1=0.976, p_2=0.018*, p_3=0.012***				
Liver Function Test					
ALT (U/l) (Mean ± SD)	30.48 ± 9.15	35.03 ± 7.80	32.41 ± 9.84	F=1.901	0.155
AST (U/l) (Mean ± SD)	33.91 ± 10.61	35.83 ± 7.58	34.95 ± 10.51	F=0.284	0.753
ALB (g/dl) (Mean ± SD)	4.52 ± 0.52	4.61 ± 0.70	4.12 ± 0.56	F=6.877*	0.002*
Sig. bet. Grps.	**p_1=0.832, p_2=0.037*, p_3=0.002***				
Total Bil (mg/dl) (Mean ± SD)	0.79 ± 0.26	0.82 ± 0.27	0.86 ± 0.25	H=0.771	0.68
Direct Bil (mg/dl) (Mean ± SD)	0.32 ± 0.19	0.31 ± 0.17	0.33 ± 0.13	H=4.096	0.129
Coagulation Profile					
PT (seconds)	12.67 ± 0.58	12.63 ± 0.70	12.36 ± 0.93	F=1.625	0.202
INR	1.08 ± 0.12	1.04 ± 0.11	1.06 ± 0.15	F=0.485	0.617
Serum Creatinine (Mean ± SD)	1.09 ± 0.14	1.03 ± 0.14	0.91 ± 0.30	F=6.110*	0.003*
Sig. bet. Grps. (Serum Creatinine)	**p_1=0.832, p_2=0.037*, p_3=0.002***				

Prothrombin Time (PT), International Normalized Ratio (INR), Albumin (ALB), Hemoglobin (Hb), Platelet count (PLT), White Blood Cell count (WBC). SD: Standard deviation, F for One way ANOVA test, Pairwise comparison bet. each 2 groups was done using Post Hoc Test (Tukey). p: p value for comparing between the three studied groups, p1: p value for comparing between <10 and 10 – 99, p2: p value for comparing between <10 and >100, p3: p value for comparing between 10 – 99 and >100.

The distribution of HBs Ag status and the presence or absence of HCV Ab did not significantly differ across the three HBs Ab titre groups (p = 0.651 and p = 0.187, respectively). In both cases, HBs Ag and HCV Ab did not appear to significantly affect HBs Ab titre levels (mIU/mL) in this research group, (**Table. 4).**

Table 4. HBs-Ab titre in concern with HBs Ag and HCV Ab

Variables	HBs Ab titer (mIU/mL)						χ^2	^{MC}p
	<10 (n = 23)		10-99 (n = 36)		>100 (n = 41)			
	No.	%	No.	%	No.	%		
HBs Ag								
Negative	18	78.3	27	75	28	68.3	0.857	0.651
Positive	5	21.7	9	25	13	31.7		
HCV Ab								
Negative	23	100	31	86.1	36	87.8	3.543	0.187
Positive	0	0	5	3.9	5	2.2		

Hepatitis B surface antigen (HBs Ag), Hepatitis C virus antibodies (HCV Ab) χ2: Chi square test
MC: Monte Carlo

The normal ultrasonography was highest in the <10 (mIU/mL) HBs Ab titre group (87.0%) and lowest in the >100 (mIU/mL) titer group (58.3%). Fatty Liver was observed in 13%, 25%, and 12.2% of individuals in the three groups, respectively, with the 10-99 (mIU/mL) HBs Ab titre group having the highest frequency. Cirrhosis was found in 13.9% of patients with 10-99 (mIU/mL) HBs Ab titre and 12.2% of patients with >100 (mIU/mL) HBs Ab titre. Hepatomegaly was observed in 2.8% and 7.3% of those with 10-99 (mIU/mL) and >100 (mIU/mL) HBs Ab titre, respectively. The ultrasound results across the three HBs Ab titre groups did not show a significant difference (p = 0.170). (**Table.5).**

Table 5. HBs-Ab titre in concern with abdominal ultrasongraphy findings

Variables	HBs Ab titre (mIU/mL)						χ^2	^{MC}p
	<10 (n = 23)		10-99 (n = 36)		>100 (n = 41)			
	No.	%	No.	%	No.	%		
Abdominal ultrasound								
Normal	20	87	21	58.3	28	68.3	8.328	^{MC}p= 0.170
Fatty liver	3	13	9	25	5	12.2		
Cirrhosis	0	0	5	13.9	5	12.2		
Hepatomegaly	0	0	1	2.8	3	7.3		

χ2: Chi square test; MC: Monte

The majority of participants (65%) in the study were not vaccinated against hepatitis B. Among the unvaccinated individuals, all those with HBs Ab titre (mIU/mL) below 10 (100%) and those with titers between 10 and 99 (100%) lacked protective immunity against the virus. In contrast, a small proportion of the unvaccinated group (14.6%) had HBs Ab titre (mIU/mL)s above 100, indicating

potential exposure to the virus. A statistically significant difference was observed when comparing the three groups ($p < 0.001$). (**Table.6).**

Table 6.Comparison between the three studied groups according to HB

Variables	HBs Ab titre (mIU/mL)						χ^2	^{MC}p
	<10 (n = 23)		10-99 (n = 36)		>100 (n = 41)			
	No.	%	No.	%	No.	%		
Vaccinated or not								
No	23	100	36	100	6	14.6	77.486*	<0.001*
Yes	0	0	0	0	35	85.4		

χ2: Chi square test; MC: Monte Carlo

Discussion

HBV affects 350 million individuals (5% of the world's population) as chronic carriers. Chronic HBV infection is characterized by HBs Ag persistence for more than 6 months**.** Anti-HBs antibodies help the humoral and cellular immune systems eliminate viruses **(Anugwom et al., 2021).** From blood donors, as they represent a sample of the whole population, In Egypt, the prevalence of HBsAg seropositivity among blood donors decreased from 1.1% in 2015 to 0.91% in 2018 **(Elbahrawy et al., 2021).** HBV prevalence varies by country, although protective devices and other efforts have reduced transmission hazards. HBV vaccinations are still the most important step in avoiding HBV infection **(Nguyen et al., 2020).**

We analyzed 100 family members of chronic hepatitis B cases, with an average HBs Ag titre of 82.76 ± 62.42 mIU/mL. They were grouped into <10 (23 members), 10-99 (36 members), and >100 titre (41 members) groups. Demographics and HBs Ab titre values were assessed for 100 participants, revealing minor gender-based differences and insignificant rural-urban distinctions. Strong correlations between family members and HBs Ab titre levels were observed, particularly among brothers. Age variations were significant between certain groups, particularly those with HBs Ab titres of <10 and 10-99 and between 10-99 and >100, but not between <10 and >100. Further analysis showed distinctions in HBs Ab titre distribution among the groups, with significant variations in white blood cell counts and albumin levels. Additional laboratory parameters and the presence of HCV Ab did not significantly impact HBs Ab titre levels. Ultrasonography results exhibited varying rates of fatty liver, cirrhosis, and hepatomegaly among the groups, though not significantly different. Notably, a large portion of the participants were unvaccinated, with no protective immunity in those with HBs Ab titre <10 or between 10-99, while a fraction exhibited high titres (>100). These findings held statistical significance.

In our study, the mean age of family members was notably higher at 35.65 years, and we examined diverse levels of HBs Ab titres. We observed age-related variations in seropositivity rates among family members and found correlations between HBs Ab titres, age, gender, and family relationships. On the other hand, **Ragheb et al. (2012)** study reported a mean age of 20.6 years

among family members and primarily explored HBsAg seropositivity at a rate of 12.2%. They categorized individuals into vaccinated and unvaccinated groups, investigating vaccination's impact on serological markers. Additionally, they addressed HCV co-infection and conducted a molecular evolutionary analysis of HBV isolates.

Along with our study, **Nemr et al. (2022)** conducted a study on intense intra-familial transmission of HBV in rural Egypt. Our study and their study both focused on chronic hepatitis B, albeit with differing sample sizes and age demographics. While our study involved 100 family members with a mean age of 35.65 ± 7.78 years, they examined 96 individuals with a mean age of 17.8 ± 13.1 years. Both investigations revealed age-related relationships with HBsAb levels, albeit in different age groups. Brothers in our study and family members in this study showed higher HBsAb titers >100. Gender-wise, both studies found lower prevalence in males. Variations in HBsAb titers were observed in both investigations, with similar trends in white blood cell (WBC) counts in the >100 titer group. Neither study found significant differences in ALT and AST levels across titer groups. Comorbidity analysis showed no significant differences in HBsAg or HCV Ab status across titer groups in both studies. Both identified unvaccinated individuals with HBsAb titers >100, signifying potential virus exposure. Ultrasound results did not significantly differ. Collectively, these studies deepen our understanding of chronic hepatitis B, offering insights into demographics, laboratory findings, and comorbidities while highlighting variations in age and specific correlations.

According to current vaccination recommendations, antibody concentration declines over time are the most probable cause of antibody negativity in vaccinated people. Immunological memory allows one revaccination to substantially raise antibody titer **(Kim et al., 2016).**

Conclusion

Our study showed that HBsAb titre was significantly differing according to HCV Ab, age and family member, WBC, serum creatinine, albumin level, and vaccination.

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