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Original Article

Prevalence of occult hepatitis B infection in Diyala province, Iraq

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Abstract

Background: Occult HBV infection (OBI) is the absence of hepatitis surface antigens (HBsAg) that is not apparent during detection by serological tests despite the presence of virus DNA. This study aimed to explore the prevalence of OBI infection among various populations in Diyala province, Iraq.

Methods: A prospective cross-sectional study was conducted from 1st January to 30th September, 2021, at Ibn Sina Dialysis Center, Baquba Teaching Hospital, Iraq. Three hundred and sixty participants were equally involved (90 individuals for each) from the dialysis department, the thalassemia department, blood bank donation Centre, and the control group. Study populations were screened for HBV Ag, HBV c IgG, HBV c IgM, abusing the enzyme-linked immunosorbent assay (ELISA) test, and detecting HB core gene. Demographic data of the study group were recorded. Descriptive analysis was done using SPSS Version 25, and the P-value was considered significant wherever it was below 0.05.

Results: The positivity rate of serological markers of OBI among the study population was (6.7%) of the participants were HBs Ag positive. Whereas 22 (6.1%) were anti-HBc IgG positive and 3 (0.8%) were anti-HBc IgM positive. The detection rates of the PCR products of 76 participants after amplification using specific primers for (core-gene) have been presented to the gel electrophoresis, which showed none of the 76 participants were positive for the HBc gene.

Conclusion: The current study showed a medium percentage of anti-HBc IgG in the serum of the study groups without the presence of HBs Ag, which indicates the presence of a previous infection that was resolved or the occurrence of occult hepatitis B infection. The current study results also showed that the serum of any of the study groups was not positive for the core gene, which confirms the possibility of infection with OBI.

Keywords: Occult Hepatitis B virus, HBc IgM, HBc IgG, Core Gene, Diyala, Iraq

Background

Occult HBV infection (OBI) is the absence of hepatitis surface antigens (HBsAg) that is not apparent during detection by serological tests despite the presence of virus DNA [1,2]. Anti-hepatitis B virus in the serum of the sick person is an important factor for detection and tracking of OBI cases. The prevalence of infection with occult hepatitis is affected by several factors such as geographical differences, the presence of co-morbidities such as chronic hepatitis C infection as well as the sensitivity of different diagnostic methods [3,4]. The prevalence of OBI in Iraq, especially in Diyala province, was recorded in 3.9% of blood donors in 2012 [5], and 2014 [6], however the prevalence come up to 5.12% in 2018 [7]. Findings from Erbil city (North of Iraq) showed that the seropositivity of OBI was 39.1% (108/276) [8]. In Basra city (South of Iraq), the prevalence was 14.0% in HBc Abs positive donors [9]. In nearby and abroad countries, the frequency of OBI was reported at 0.0% in Turkey and Iran [10, 11] and 1.25 % in Saudi Arabia [12]. This study aimed to explore the prevalence of OBI infection among various populations in Diyala province, Iraq.

Methods

Study Design and Sample

In Diyala province, east of Iraq, a prospective cross-sectional study was conducted from 1st January to 30th September 2021. Four study populations were included in the current study:

a. Ninety patients with renal failure who are regularly attending the Ibn-Sina Dialysis Center-Diyala Directorate of Health for hemodialysis.

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b. Ninety patients with beta-thalassemia who are regularly attending the Blood Specialist Center-Diyala Directorate of Health for treatment including blood transfusion.
c. Ninety blood donor individuals who are attending Blood Bank Donation Centre, Diyala Directorate of Health.
d. Ninety healthy volunteers collected from the outpatient clinics of Baquba teaching hospital as control.
The participants were interviewed with a semi-structured questionnaire to collect information about their age, gender, place of residency, level of education, and HBV vaccination.

Detection of serological marker HBs Ag (serum), Anti-HBc IgG, and Anti-HBc IgM

This test was performed using a commercially available kit (Dia.PRO, Italy HBs Ag ELISA). Reactive results were indicated by the absorbance reading of 1.1 and above, while the non-reactive results were indicated by the absorbance reading of less than 0.9.

Nucleic Acid Extraction

Genomic DNA was isolated from serum samples according to the protocol of the QIAamp® MinElute® Virus Spin Kit.

Primer

Sets of Polymerase Chain Reaction (PCR) primers for the core Hepatitis B virus gene have been used in the conventional PCR amplification to get PCR products used in the sequencing method for genotyping the virus and phylogenetic tree analysis. These primers were used for positive samples detected by the ELISA test for detected HBVs Ag, Anti-HBc IgG, and Anti-HBc IgM. Primers were provided by Macrogen/ Korea and sequences (Table 1).

Table 1: Primers used for detection of HBV-core gene

Type of virus	Target gene	Primer	Oligo sequence [5'-3']	Annealing Temperature [°C]	Product size [bp]	Reference
HBV	*core*	Forward 1	**5`-CAG GTC TTG CCC AAC GTC TTA-3. `**	**56**	**976**	**Farooq *et al.*, [40]**
HBV	*core*	R 1	**5`-CTG TCA GAG GGC CCA CAT ATT -3. `**			
HBV	*Core*	Forward 2	**5`-GAC CGA CCT TGA GGC ATA TTT-3. `**	**65**	**790**	
HBV	*Core*	Reverse 2	**5`-TCC CAC CTT ATG AGT CCA AGG-3.`**			

Assay Optimization

Following the optimization process of primer concentration, Polymerase Chain Reaction detection HBV core gene was carried out. In order to detect "the optimum annealing temperature, gradient PCR was set at 56°C". The best condition for the HBV was obtained, and then samples along with negative (water) and positive controls (previously known PCR-positive samples of HBV) were amplified.

HBV core gene PCR detection

Procedures of molecular detection of HBV DNA were carried out as follows:
a. "An initial activation was set at 95°C/5 minutes, 40 cycles at 94°C/30 seconds, 56°C/30 seconds, and 72°C/30 seconds".
b. "The final extension step was of 72°C/10 minutes".
c. "Semi-nest PCR amplifications were carried out which are similar to the first step using different reverse primers".
d. "Hepatitis B virus genotyping was performed under the same conditions but using other primer pairs which targeted the S gene on HBV DNA-positive samples". All reactions were performed in duplicate and the presence of negative and positive controls.

The final products were detected by electrophoresis on 2.0% agarose gel, and the size of the PCR

Statistical analysis

Statistical analysis was carried out using the Statistical Packages for Social Sciences (SPSS) version 25. Description of data presented as frequency, percentage, mean, standard deviation, and range (minimum-maximum values). An independent student t-test, paired t-test and the ANOVA test were recruited to compare between different means. The significance of the difference among different percentages was tested using the Pearson Chi-square test with the application of Yate's correction or Fisher Exact test. Statistical significance was considered whenever the P-value was equal to or less than 0.05.

Results

Serological markers

The positivity rate of serological markers of HBV among the study population is shown in the table 2. Twenty-four (6.7%) of the participants were HBs Ag positive, 22 (6.1%) were anti-HBc IgG positive and 3 (0.8%) were anti-HBc IgM positive.

Table 2: Positivity rate of HBV and HCV serological markers

Marker	Status	No. (%)
HBsAg	Positive	24 (6.7)
	Negative	336 (93.3)
Anti-HB core IgG	Positive	22 (6.1)
	Negative	338 (93.9)
Anti-HB core IgM	Positive	3 (0.8)
	Negative	357 (99.2)

Distribution of serological markers according to study groups

Table 3 revealed that the HBs Ag positivity rate among each group of participants (renal dialysis, thalassemia patients, blood donors, and healthy individuals) were 4.4%, 6.7%, 7.8%, and 7.8%, respectively. However, the difference among the study groups was statistically insignificant (P= 0.748). Regarding the anti-HBc IgG antibody, the results showed that the anti-HBc IgG Ab was positive among 16 (17.8%) of renal dialysis patients, and 6 (6.7%) of thalassemia patients. The difference was statistically significant (P= 0.0001). However, all individuals in the blood donors and healthy individuals were negative for this marker, and there was no statistical analysis could be applied. Two (2.2%) of renal dialysis patients were positive of the anti-HBc IgM Ab and one (1.1%) of thalassemia patients. The difference was statistically insignificant (P= 0.296). Again, none of the individuals in the blood donors and health groups were positive for anti-HBc IgM Ab, so statistical analysis could not apply.

Table 3: Distribution of study groups according to serological markers (n=360)

Serological Markers	Renal Dialysis N=90	Thalassemia N=90	Blood donors N=90	Control individuals N=90	*p* value
HBsAg	N (%)	N (%)	N (%)	N (%)	
Positive	4 (4.4)	6 (6.7)	7 (7.8)	7 (7.8)	0.784
Negative	86 (95.6)	84 (93.3)	83 (92.2)	83(92.2)	
Anti-HBV core IgG					
Positive	16 (17.8)	6 (6.7)	-	-	0.0001*
Negative	74 (82.2)	84 (93.3)	90 (100)	90 (100)	
Anti-HBV core IgM					
Positive	2 (2.2)	1(1.1)	-	-	0.296
Negative	88 (97.8)	89 (98.9)	90 (100)	90 (100)	

Relationship between HBs Ag positivity rate and serological markers

Table 4 shows that out of 24 (6.7%) HBs Ag positive participants 2 (8.3%) of them were also positive for anti-HBc IgG Ab compared to 22 (91.7%) of HBs Ag positive participants were anti-HBc IgG negative. Moreover, 20(6.0%) of HBs Ag negative participants were positive for anti-HBc IgG Ab, and 316 (94.0%) were negative for anti-HBc IgG Ab. The difference was statistically insignificant (*P*= 0.638). Furthermore, none (0.0%) of the HBs Ag positive participants were positive for anti-HBc IgM Ab, but all of them (24; 100%) were negative for anti-HBc IgM Ab. Additionally, 3(0.9%) of HBs Ag negative participants were anti-HBc IgM positive compared to 333(99.1%) were negative for anti-HBc IgM Ab. The difference was statistically insignificant (*p*= 0.642).

Table 4: Relationship between HBs Ag positivity rate and serological markers (n=360)

Serological marker	HBs Ag positive N=24 (6.7%)	HBs Ag negative N=336(93.3%)	*p*-value
	N%	N%	
Anti-HBc IgG			
Positive	2(8.3)	20(6.0)	0.638
Negative	22(91.7)	316(94.0)	
Anti-HBc IgM			
Positive	-	3(0.9)	0.642
Negative	24(100)	333(99.1)	

*Significant difference between proportions using Pearson Chi-square test at 0.05 level.

Relationship between anti-HBc IgG positivity rate and serological markers

Results in table 5 found that out of 22 (6.1%) of anti-HBc IgG positive participants, 2 (9.1%) were HBs Ag positive, while 20 (90.9%) were HBs Ag negative. At the same time, among the 338(93.9%) of anti-HBc IgG negative participants, 22(6.5%) were HBs Ag positive, while 316 (93.5%) were HBs Ag negative. The difference was statistically insignificant (P= 0.638). Similarly, table 5 found that out of 22 (6.1%) of anti-HBc IgG positive participants, 2 (9.1%) were anti-HBc IgM positive, while 20 (90.9%) were anti-HBc IgM negative. However, among the 338(93.9%) of anti-HBc IgG negative participants, 1(0.3%) was anti-HBc IgM positive, while 337 (99.7%) were anti-HBc IgM negative. The difference was statistically significant (*P*= 0.0001).

Table 5: Relationship between anti-HBc IgG positivity rate and serological markers (n=360)

Serological marker	Anti-HBc IgG positive N=22 (6.1%)	Anti-HBc IgG negative N=338(93.9%)	*P*-value
	N%	N%	
HBs Ag			
Positive	2(9.1)	22(6.5)	0.638
Negative	20(90.9)	316(93.5)	
Anti-HBc IgM			
Positive	2(9.1)	1(0.3)	0.0001*
Negative	20(90.9)	337(99.7)	

*Significant difference between proportions using Pearson Chi-square test at 0.05 level.

Relationship between anti-HBc IgM positivity rate and serological markers

Results in table 6 found that none (0.0%) of the 3 positive participants for anti-HBc IgM (0.8%) was positive for HBs Ag, and all of them were negative for anti-HBc IgM. However, 24 (6.7%) of anti-HBc IgM negative participants were positive for HBs Ag compared to 333(93.3%) were negative for HBs Ag. Thus, the statistical comparison was inapplicable. The results also found that out of 3 (0.8%) of anti-HBc IgM positive participants, 2 (66.7%) were positive for HBs Ag, while 1 (33.3%) were HBs Ag negative. However, among the 357(99.2%) of anti-HBc IgM negative participants, 20(5.6%) were anti-HBc IgG positive, while 337 (94.4%) were anti-HBc IgG negative. The difference was statistically significant (P= 0.0001).

Table 6: Relationship between anti-HBc IgM positivity rate and serological markers (n=360)

Serological marker	Anti-HBc IgM positive N=3 (0.8%)	Anti-HBc IgM Negative N=357(99.2%)	*P*-value
	N (%)	N (%)	
HBs Ag			
Positive	-	24(6.7)	-
Negative	3(100.0)	333(93.3)	
Anti-HBc IgG			
Positive	2(66.7)	20 (5.6)	0.0001*
Negative	1(33.3)	337(94.4)	

Molecular detection

Amplifying of HBV [core] gene by conventional PCR

Table 7 revealed the detection rates of the PCR products 76 participants after amplification using specific primers for (core-gene) have been presented to the gel electrophoresis, which showed none of the 76 participants were positive for HBc gene.

Association positivity of anti-HBc IgG with molecular marker

Table 8 illustrates the anti-HBc IgG positivity rate distribution according to the molecular marker included in this study. About the HBc gene, the results found that the gene was undetectable neither among participants with positive anti-HBc IgG nor those with anti-HBc IgG negative; thus, no statistical analysis was applicable.

Association positivity of anti-HBc IgM with molecular marker

Regarding the HB core gene and HCV core SC2 gene, the results found that neither anti-HBc IgM positive nor anti-HBc IgM negative participants had detected the HBV core gene and HCV core SC2 gene. Thus, statistical comparisons were inapplicable. All details are shown in the table 9.

Table 7: Detection rate of HBV and HCV genes among participants (n=360)

Genes	Status	N (%)
HBV core gene at 791bp	Detected	-
	Not-detected	76 (21.1)
	Not done	284 (78.9)

Table 8: Association positivity of anti-HBc IgG with a molecular marker (n=360)

Molecule marker	Anti-HBc IgG positive N=22	Anti-HBc IgG Negative N=338	*p*-value
HB core gene at 791 bp	N (%)	N (%)	
Detected	-	-	-
Not-detected	20 (90.9)	56(16.6)	-
Not done	2(9.1)	282(83.4)	-

Table 9: Association positivity of anti-HBc IgM with a molecular marker (n=360)

Molecule marker	Anti-HBc IgM positive N=3	Anti-HBc IgM negative N=357	*p*-value
HB core gene at 791 bp	N (%)	N (%)	
Detected	-	-	
Not-detected	3(100.0)	73 (20.4)	-
Not done	-	284 (79.6)	-

Discussion

In this study the positivity rate of HBs Ag was 6.7% which is higher than previous studies conducted in Iraq that reported a positivity rate of 0.4%, 3.0%, 1.3%, 3.0%, and 0.7% respectively [7,14,15,16]. Also, higher than the rates reported in Iran (3.8%), Saudi Arabia (3.24%), and Turkey (0.3%), respectively [17,18,19]. In our study the positivity rate of anti-HBc IgG was 6.1% which is lower than earlier local studies conducted in Diyala province (9.65%) and Mosul province (8.3%) but higher than that rate reported in Duhok province (3.49%) respectively [7,20,21]. Moreover, the positivity rate of anti-HBc IgG was lower than that reported in Iran (11.6%), but higher than that reported in Saudi Arabia (0.28%) and Turkey (1.2%), respectively [22,18,19].

Like to rate reported in Mosul (1.0 %) [23], the positivity rate for anti-HBc IgM was 0.8%, but was lower than the rate reported among blood donors between 2011-2012 in Diyala province (3.2%) [5]. Also, our finding was lower than rates reported in Iran (8.5%) [22], Egypt (2.25%) [24], Nigeria (5.8%) [25], and Turkey (7.5%) [26], respectively.

In the current study, HBs Ag was detected in 4.4% of renal dialysis patients which is higher than the rates reported in a previous studies conducted in Baghdad city (1.3%) [15] and in the Duhok province by Zana et al (3.49%) [21] and by Ibrahim et al. (3.2%) [27]. However, the situation was completely different in Mosul province; Al-Taan and Khalid [20] found anti-HBS Ag positive among 66.0% of examined hemodialysis (HD) patients. Concerning the neighboring countries our result was higher than that reported by Rastegarvand et al. [28] in Iran and that reported by Kizilates et al. [29] in Turkey.

The prevalence of HBsAg among the thalassemia patients was 6.7%, which is higher than earlier local findings from Mosul province (0.55%) [30], and Babylon province (3.0%) [16]. Also, the finding was higher than that reported by Dumaidi et al. [31] among Palestinian thalassemia patients (0.7%). Additionally, out of ninety blood donors, 7 (7.8%) were positive for HBs Ag, which was higher than previously

published studies in different provinces of Iraq including Diyala (0.4%) [7], Duhok (0.24%) [32], and Erbil (3.0 %) [8], respectively. Moreover, our finding was higher than results from Saudi Arabia (3.24%) [18], and Kuwait (3.5%) [33]. The current study found that renal dialysis patients' HBc IgM and HBc IgG positivity rates were 2.2% and 17.8%, respectively. Similar local findings were found by Al-Taan GS, Khalid MD in Mosul province [20] and by Zana et al. [21] in Duhok province of Iraq. However, Samadi et al. [34] reported an anti-HBc Ab rate of 23.5% in Iran.

The rates of HBc IgM and IgG in our study were 1.1% and 6.7% among thalassemia patients, respectively. Such findings were lower than the rates reported worldwide such as Palestine (19.0%), Nigeria (2.49%), and Iran (4.33%), respectively [31,35,36]. However, local studies conducted by Salim and Abdullah [30] reported similar finding of 1.1% positivity rate of HBc Ab among thalassemia patients in Mosul province. While Al-Sharifi et al. [16] reported that 12.0% of thalassemia patients had a positive anti-HBc Ab in Babylon province of Iraq. As for blood donor individuals, the current result was 0.0% for anti-HBc Ab, however, other Iraqi studies conducted in Diyala province [7] and Erbil province [8], reported 5.01% and 2.27% of anti-HBc Ab positive rate, respectively. Bahrami et al. [22] reported 11.6% of anti-HBc Ab positive among blood donors in Iran, while Alzahrani et al. [18] reported 0.28% in Saudi Arabia.

Table (4) showed that among HBs Ag positive participants (8.3%) and (0.0%) were positive for anti-HBc IgG and anti-HBc IgM, respectively, against (6.0%), (100%) of HBs Ag negative participants were positive for anti-HBc IgG Ab and anti-HBc IgM respectively. Similarly, Hassan et al. [7] reported 5.01% positive to anti-HBc IgG and anti-HBc IgM respectively among HBs Ag positive participants in Diyala province, east of Iraq. However, our findings were different from study conducted by Abdulla and Goreal [37] in Duhok city, north of Iraq. Authors reported 100% and 0.0% positive for anti-HBc IgG and anti-HBc IgM, respectively, among HBs Ag positive participants [37]. Also, our results differed from the study conducted by Al-Zubaidi et al. [38] in Al-Diwaniya, south of Iraq. Authors found that 12.0% and 10.0% positive for anti-HBc IgG and anti-HBc IgM, respectively, among HBs Ag positive participants. Additionally, our findings were consistent with some global studies in terms of the distribution of HBs Ag positivity rate by anti-HBc IgG and anti-HBc IgM [24,39,40]. Generally, the several agreed that the anti-HBc Ab test increased the detection rate of HBV positive in the community. The reason might because uncovered the occult HBV infection, which gave HBsAg negative, but they were positive for anti-HBc antibodies [41,42]. Aiming to minimize the transfusion transmission risk of HBV, the current study and others are concordant that the addition of anti-HBc Ab (IgM or IgG) test for screening policy of Blood gives marvelous results [7,43]. Actually, in Diyala province, this decision was implemented in the local Blood Bank in 2014. Among the fascinating results of this study is that out of 24 HBs Ag positive participants 2 (8.3%) were positive for both HBs Ag and anti-HBc IgG, while 22(91.7%) were positive for HBs Ag but negative for anti-HBc IgG. Additionally, none (0.0%) were positive for both HBsAg and anti-HBc IgM, but all the positive HBs Ag (24, 100%) were negative for anti-HBc IgM. So, there was a problem in Diyala province called "occult HBV infection," and probably this problem became more prominent in high-risk groups. Among such risky groups, this phenomenon may arise due to the pressure of HBV vaccination or anti-viral therapy. Further serological or molecular studies are recommended in this respect.

Indeed, anti-HBc in the serum without serological HBs Ag indicates occult HBV infection, as suggested by several studies [21,23,38,44]. The results in table (5) were consistent with other Iraqi studies [21,23,38] that reported 50.0%, 10.0%, and 3.49% of participants with anti-HBc IgG positive were HBs Ag positive, respectively. Moreover, other studies from Poland [44] and Saudi Arabia [45] also reported similar results. It is well documented that participants presented with anti-HBc IgG positive only indicating a previous infection with HBV. Whereas HBs Ag testing used to review acute and chronic HBV infection [38]. The presence of anti-HBc in the serum without serological HBsAg is indicative of a resolved HBV infection. However, it is a sign of occult HBV infection, as suggested by several researchers [21,23,38,44,46].

Findings in table 5 and 6 showed statistically significant difference in the anti-HBc IgG positivity rate distribution according to anti-HBc IgM. The above-mentioned results agreed with other results reported by local studies [23,24,38].
The IgM class of the anti-HBc is the first to appear even late in the incubation period and indicates a recent infection. So anti-HBc IgM is an excellent marker for HBV infection in HBsAg negative participants. While the IgG class of anti-HBc appears later and indicates a past infection. Individuals with anti-HBc IgG may not be infectious, and their Blood is suitable for blood transfusion as they may have anti- HBs, which are protective in nature [24]. The current study confirms previous findings reported by other local studies [23,38] where none of those positive participants for anti-HBc IgM was positive for HBs Ag. However, several international studies reported different results. Abdou et al. [24] reported 2.25% of samples [HBs Ag negative] were positive for anti-HBc IgM. Ogunfemi et al. [25] found that the prevalence of anti-HBc IgM was positive at 5.7% among Nigerian patients. Ayatollahi et al. [47] reported that 2.4% anti-HBc IgM was positive among HBs Ag negative.

The IgM class of the anti-HBc is the first to appear even late in the incubation period and indicates a recent infection. Ogunfemi et al. [25] indicated that “the finding of anti-HBc IgM alone may result from the presence of anti-HBc IgM during the window period following acute HBV infection” [25]. So anti-HBc IgM is an excellent marker for HBV infection in HBsAg negative individuals. The IgG class of anti-HBc appears later of anti-HBc IgM and indicates a past infection. Individuals with anti- HBc IgG may not be infected, and their Blood is suitable for blood transfusion as they may have sufficiently high titers of anti- HBs, which are protective [48]. So, the results of our study agreed with other studies that reported significant differences in the distribution of anti-HBc IgM positivity rate among anti- HBc IgG positive individuals [23,24,38,47].

Part of our results showed that none of the participants were positive for the HBV core gene at 791bp. Similarly, Rios-Ocampo et al. [49] reported the absence of positive PCR results for the core region in patients. However, different findings reported by Hassan and Hussain [8] in north of Iraq. The author found that 37.0% of the study population was positive for HBV

core gene. Additionally, Rastegarvand et al. [28] revealed that two (0.98%) were positive for pre-core regions among all HBsAg-negative samples.

Concerning the HBc gene, the gene was undetectable neither among participants with positive anti-HBc IgG nor those with anti-HBc IgG negative. However, Ayatollahi et al. [47] detected 2.4% HBc gene among participants with positive anti-HBc IgG. The difference in the results might be due to the sensitivity and specificity of the ELISA and PCR technique for the detection, primers used, and technical conditions.

Unlike to previous international studies [23,24,38,47,50], none of the HB core genes tested subjects were positive among anti- HBc IgM positive participants. The disparity among results may be due to clearance of HBV infection, ELISA test, or HBV PCR kits, rarely that a person has intermittent or low-level viremia.

Conclusion

The overall OBI among study participants (hemodialysis patients, thalassemia patients, blood donors, and the control group) indicates that Diyala province is still in the intermediate zone of endemicity. The HBC gene at 791 bp was not detected among any included specimens since the HBc genes are restricted to the hepatocytes. Further molecular studies on detecting these genes in liver biopsies, especially for the risky groups, are recommended.

Abbreviation

OBI: Occult Hepatitis B Infection; HBs Ag: Hepatitis B surface antigen; HBc IgG: Hepatitis B core immunoglobulin G; HBc IgM: Hepatitis B core immunoglobulin M; ELISA: Enzyme-Linked Immunosorbent Assay; PCR: Polymerase Chain Reaction.

Declaration

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Availability of data and materials

Data will be available by emailing asmaa.haseeb@ymail.com

Authors' contributions

Ansam Dawod Salman (ADS) designed the experiments and wrote and reviewed the manuscript. Iman Abass Ali (IAA) reviewed, revised, and edited the manuscript. Ansam Dawod Salman, Iman Abass Ali, and Asmaa Haseeb Hwaid (AHH) participated in the study design, performed the experiments, and collected and analyzed the data. All authors contributed to the article and approved the submitted version.

Ethics approval and consent to participate

We conducted the research following the Declaration of Helsinki. Ethical permission was granted by the Ethics Committee (Department of Higher Education), College of Education for Pure Sciences, University of Diyala, Iraq (Ref No. 5103/12-13 November 2019) and Ethical Approval granted by Centre of Training and Human Development, Knowledge and Research Management of the Institution of Health directorate in Diyala Province (Ref No. 45024/ on 18 November 2019). All patients gave written informed consent.

Consent for publication

Not applicable

Competing interest

The authors declare that they have no competing interests.

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References

1. Pak K, Ordway S, Torres D. Occult HBV infection: a case series at a military treatment facility. Mil Med. 2020 Jun 8;185(5-6): e795-e798. doi: 10.1093/milmed/usz379.
2. Makvandi M. Update on occult hepatitis B virus infection. World J Gastroenterol. 2016 Oct 21;22(39):8720-8734. doi: 10.3748/wjg. v22.i39.8720.
3. Allain JP. Occult hepatitis B virus infection. Transfus Clin Biol. 2004 Feb;11(1):18-25. doi: 10.1016/j.tracli.2003.11.007.
4. Kwak MS, Kim YJ. Occult hepatitis B virus infection. World J Hepatol. 2014;6(12):860-869. doi:10.4254/wjh. v6.i12.860.
5. Hasan AS. Prevalence of antibodies to Hepatitis B virus antigens and occult Hepatitis B virus infection in blood donors. Diyala Journal of Medicine. 2012;3(1):57-61.
6. Abdu AN, Hasan AR, Al-Azi IH. Detection of occult Hepatitis B virus infection in Diyala Province. Diyala Journal for Pure Science. 2014;10(4): 69-81.
7. Hasan AS, Fayyadh HM, Al-Taie WS. Prevalence of Hepatitis B Surface Antigen and Anti-Hepatitis B Core Antibodies among Blood Donors in Diyala, Iraq. Asian Journal of Nursing Education and Research. 2018;8(4):489-92. doi : 10.5958/2349-2996.2018.00100.3.

8. Hassan RN, Hussain AH. Hepatitis B virus DNA in blood donors positive of anti-hepatitis B core antibodies and negative for surface antigen in Hawler major blood bank, Kurdistan Region, Iraq. Journal of the Faculty of Medicine Baghdad. 2018 Apr 1;60[1]:57-61.
9. Hasan AS, Al-Aboudi SA, Al-Hmudi HA. Some of immunogenetic status of HBsAg negative, HBcAb positive Blood donors in Basra province-Iraq. Annals of Biological Research. 2015;6(4):47-54.
10. Findik D, Arslan U, Baykan M. Determination of hepatitis B virus DNA incidence, viral load, and mutations in blood donors with HBsAg and anti-HBs-negative serology and antibodies to hepatitis B core antigen. Eur J Intern Med. 2007 Dec;18(8):571-5. doi: 10.1016/j.ejim.2007.07.001.
11. Karimi G, Zadsar M, Vafaei N, Sharifi Z, FalahTafti M. Prevalence of antibody to Hepatitis B core antigen and Hepatitis B virus DNA in HBsAg negative healthy blood donors. Virol J. 2016 Mar 5; 13:36. doi: 10.1186/s12985-016-0492-8.
12. Zekri AR, Awlia AA, Mahalawi HE, Ismail EF, Mabrouk GM. Evaluation of blood units with isolated anti HBC for the presence of HBV DNA. Disease markers. 2002 Jan 1;18(3):107-10. https://doi.org/10.1155/2002/156359.
13. Farooq A, Waheed U, Saba N, Kaleem M, Majeed N, Wazeer A, Cheema NA, Ahmed S, Arshad M. Molecular and genetic characterization of hepatitis B virus among multitransfused thalassaemia patients in Islamabad, Pakistan. J Family Med Prim Care. 2021 Feb;10(2):998-1002. doi: 10.4103/jfmpc.jfmpc_1880_20. Epub 2021 Feb 27..
14. Aboud RS, Aboud AS, Saleh GM, Fadil HY, Abid SG. Seroprevalence of Hepatitis B markers in Iraqi patients with chronic Hepatitis B virus. IOSR Journal of Pharmacy and Biological Sciences. 2018;13(1):01-04. DOI: 10.9790/3008-1301050104.
15. Nasir NA, Abdilazeem MA. Prevalence of Viral Hepatitis B, C, and human immunodeficiency virus among end stage renal disease patients on hemodialysis. Iraqi Postgraduate Medical Journal. 2019;18(2):79-86.
16. Al-Sharifi LM, Murtadha J, Shahad A, Mohammed Y, Sura J, Waleed Z, Raheeq M, Sura A, Ehab H, Shahad M, Abbas Q. Prevalence of hepatitis B and C in thalassemic patients and its relation with type of thalassemia, frequency of blood transfusion, and spleen status. Medical Journal of Babylon. 2019 Apr 1;16)2):108-111. Doi: 10.4103/MJBL.MJBL_6_19.
17. Hoseini H. Seroprevalence of Hepatitis B, Hepatitis C and human immunodeficiency virus in patients with thalassemia major in Zahedan, Southeast of Iran. jcbr. 2019; 3 (1): 29-33.doi: 10.29252/jcbr.3.1.29.
18. Alzahrani FM, Muzaheed, Shaikh SS, Alomar AI, Acharya S, Elhadi N. Prevalence of Hepatitis B Virus (HBV) Among Blood Donors in Eastern Saudi Arabia: Results From a Five-Year Retrospective Study of HBV Seromarkers. Ann Lab Med. 2019 Jan;39(1):81-85. doi: 10.3343/alm.2019.39.1.81.
19. Acikgoz A, Cimrin D, Kizildag S, Esen N, Balci P, Sayiner AA. Hepatitis A, B and C seropositivity among first-year healthcare students in western Turkey: a seroprevalence study. BMC Infect Dis. 2020 Jul 22;20(1):529. doi: 10.1186/s12879-020-05247-5.
20. Al-Taan GS, Khalid MD. Prevalence of hepatitis B virus in hemodialysis patients infected with hepatitis C virus in Mosul District/Iraq. American Academic Scientific Research Journal for Engineering, Technology, and Sciences. 2020 Jan 8;63(1):1-6.
21. Zana SM, Ibrahim AN, Nawfal RH, Mohammad SJ, Noaman JS, Haji RS, et al. The prevalence of Hepatitis B and C virus in patients with end-stage kidney disease on regular hemodialysis in Duhok, Iraq: A Brief Report. Avicenna J Clin Microbiol Infect. 2020;7: 31-33. doi: 10.34172/ajcmi.2020.06.
22. Bahrami A, Sharifi Z, Parsania M, Haghighat S. Prevalence of anti-HBc in HBsAg negative blood donors using two enzyme immunoassays kits. Sci J Iran Blood Transfus Organ 2020, 17(2): 83-90.
23. Khalid MD. Detection of Anti-HBc and HBV-DNA in blood donors negative for hepatitis B virus surface antigen in Mosul central blood bank-Iraq. Iraqi journal of biotechnology. 2014;13(2-2):119-127.
24. Abdou A, Hasssan M, El Gamal S, El Dewi D. The role of detection of anti-HBc IgM in HBs Ag negative blood donors. Journal of Recent Advances in Medicine. 2020 Jul 1;1(2):67-74. Doi: 10.21608/JRAM.2020.19827.1020.
25. Ogunfemi MK, Olawumi HO, Olokoba AB, Kagu MB, Biliaminu SA, Durowade KA, Durotoye IA, Shittu AO. Prevalence of antibody to hepatitis B core antigen among hepatitis B surface antigen-negative blood donors in Ilorin, Nigeria: A cross-sectional study. Malawi Med J. 2017 Mar;29(1):32-36. doi: 10.4314/mmj. v29i1.7.
26. Ercaliskan A, Eskazan AE, Yurttas NO, Kucukyurt S, Salihoglu A, Keskin D, Ozmen D, Elverdi T, Ar MC, Soysal T. The real-life data on the outcome of hepatitis B Infection in patients with B-cell lymphoproliferative neoplasms treated with ibrutinib. Blood. 2019 Nov 13; 134:4316. https://doi.org/10.1182/blood-2019-127794.
27. Ibrahim NM, Saleem ZS, Hussein NR. The Prevalence of HIV, HCV, and HBV Among hemodialysis patients attending Duhok hemodialysis center. Int J Infect. 2018;5(1): e63246. doi: 10.5812/iji.63246.
28. Rastegarvand N, Makvandi M, Samarbafzadeh A, Rasti M, Neisi N, Pouremamali A, Teimoori A, Shabani A. Molecular characterization of pre-core/core and S region of hepatitis B virus in hemodialysis patients with occult hepatitis B infection. Jundishapur J Microbiol. 2015 Oct 26;8(10): e23686. doi: 10.5812/jjm.23686.
29. Kizilates F, Berk H, Coban M, Seyman D, Sarikaya M, Sari F, Oztoprak N. Seroprevalence of hepatitis B and C virus in patients who undergo hemodialysis in Antalya province, Turkey. Asian Biomedicine. 2016 Aug 1;10(4):339-44. https://doi.org/10.5372/1905-7415.1004.496.
30. Salim RW, Abdullah BA. The prevalence of hepatitis B virus in high risk groups in Nineveh governorate/Iraq. Baghdad Science Journal. June 2014;11(2):888-893.
31. Dumaidi K, Al-Jawabreh A, Samarah F, Rabayaa M. Prevalence of Sero-Molecular Markers of Hepatitis C and B Viruses among Patients with β-Thalassemia Major in Northern West Bank, Palestine. Can J Infect Dis Med Microbiol. 2018 Sep 5;2018: 1039423. doi: 10.1155/2018/1039423.
32. Hussein NR, Haj SM, Almizori LA, Taha AA. The prevalence of hepatitis B and C viruses among blood donors attending blood bank in Duhok, Kurdistan region, Iraq. Int J Infect. 2017;4(1): e39008. doi: 10.17795/iji-39008.
33. Ameen R, Sanad N, Al-Shemmari S, Siddique I, Chowdhury RI, Al-Hamdan S, Al-Bashir A. Prevalence of viral markers among first-time Arab blood donors in

Kuwait. Transfusion. 2005 Dec;45(12):1973-80. https://doi.org/10.1111/j.1537-2995.2005.00635.x.
34. Samadi E, Mirshahabi H, Motamed N, Sadeghi H. Prevalence of occult hepatitis B Virus infection in hemodialysis patients using nested PCR. Rep Biochem Mol Biol. 2020 Apr;9(1):82-88. doi: 10.29252/rbmb.9.1.82.
35. Mohammed N, Ashafa AM, Aminu M. Seroprevalence of hepatitis B virus e antigen among blood donors in Kaduna State, Nigeria. International Journal of Science for Global Sustainability. 2020 Oct 8;6(3):89.
36. Ramezani A, Aghasadeghi MR, Ahmadi F, Razeghi E, Eslamifar A, Banifazl M, et al. Isolated anti-hbc and occult HBV infection in dialysis patients. Nephrourol Mon. 2014 Nov 30;7(1): e22674. doi: 10.5812/numonthly.22674.
37. Abdulla IM, Goreal AA. Detection of Hepatitis-B virus genotypes among chronic carriers in Duhok-Iraq. Journal of the Faculty of Medicine Baghdad. 2016 Jul 3;58(2):170-175.
38. Al-Zubaidi MA, Bahreini M, Al-Rubaye WS. Detection and determination of hepatitis B using molecular and serological methods in patients with hepatitis B in Al-Diwaniya Iraq. AL-Qadisiyah Medical Journal. 2019 Aug 26;15(1):87-97.
39. Alabdallat NG, Bin Dukhyil AAA. Significance of HBV NAT Among HBs Antigen-Negative Blood Donors in Saudi Arabia. Lab Med. 2018 Oct 11;49(4):342-346. doi: 10.1093/labmed/lmy023..
40. Mansour-Ghanaei F, Joukar F, Naghipour M, Hassanipour S, Yeganeh S, Sepehrimanesh M, Fathalipour M. Epidemiologic profile of viral hepatitis B and C in North of Iran: results from PERSIAN Guilan Cohort Study (PGCS). BMC Res Notes. 2021 Feb 10;14(1):59. doi: 10.1186/s13104-021-05474-2.
41. Makroo RN, Chowdhry M, Bhatia A, Arora B, Rosamma NL. Hepatitis B core antibody testing in Indian blood donors: A double-edged sword! Asian J Transfus Sci. 2012 Jan;6(1):10-3. doi: 10.4103/0973-6247.95043.
42. Allain JP. Global epidemiology of occult HBV infection. Annals of Blood. 2017; 2(7):1-13. http://dx.doi.org/10.21037/aob.2017.06.01.
43. Al-Rubaye A, Tariq Z, Alrubaiy L. Prevalence of hepatitis B seromarkers and hepatitis C antibodies in blood donors in Basra, Iraq. BMJ Open Gastroenterol. 2016 Jan 4;3(1):e000067. doi: 10.1136/bmjgast-2015-000067.
44. Ganczak M, Topczewska K, Budnik-Szymoniuk M, Korzeń M. Seroprevalence of anti-HBc, risk factors of occupationally acquired HBV infection and HBV vaccination among hospital staff in Poland: a multicenter study. BMC Public Health. 2019 Mar 12;19(1):298. doi: 10.1186/s12889-019-6628-1.
45. Al-Momen AK, Alghamdi E, Al-Humaidan H, Al-Mishari M, Suhaibani O, Alnowaiser A. Current Management of Blood Donors with Positive Anti-Hbc in Saudi Arabia. Blood.2016; 128(22): 5041. https://doi.org/10.1182/blood.V128.22.5041.5041.
46. Coppola N, Gentile I, Pasquale G, Buonomo AR, Capoluongo N, D'Armiento M, Borgia G, Sagnelli E. Anti-HBc positivity was associated with histological cirrhosis in patients with chronic hepatitis C. Ann Hepatol. 2013 Jan-2014 Feb;13(1):20-6.
47. Ayatollahi J, Jahanabadi S, Sharifyazdi M, Hemayati R, Vakili M, Shahcheraghi SH. The Prevalence of Occult Hepatitis B Virus in the Hemodialysis Patients in Yazd, Iran. Acta Med Iran. 2016 Dec;54(12):784-787.
48. Esposito A, Sabia C, Iannone C, Nicoletti GF, Sommese L, Napoli C. Occult Hepatitis Infection in Transfusion Medicine: Screening Policy and Assessment of Current Use of Anti-HBc Testing. Transfus Med Hemother. 2017 Aug;44(4):263-272. doi: 10.1159/000460301.
49. Rios-Ocampo WA, Cortes-Mancera F, Olarte JC, Soto A, Navas MC. Occult hepatitis B virus infection among blood donors in Colombia. Virol J. 2014; 11:206. doi: 10.1186/s12985-014-0206-z.
50. Hiva S, Negar K, Mohammad-Reza P, Gholam-Reza G, Mohsen A, Ali-Asghar NG, Mohammed-Jafar S. High level of vaccination and protection against hepatitis B with low rate of HCV infection markers among hospital health care personnel in north of Iran: a cross-sectional study. BMC Public Health. 2020 Jun 12;20(1):920. doi: 10.1186/s12889-020-09032-6.