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

Rasha N. Hassan* **MBChB**
Ali H. Hussain** **MBChB, MSc, PhD (Microbiology)**

Abstract:

Background: Occult Hepatitis B virus infection (OBI) among blood donors is an important medical concern.

Objectives: This study was done to detect the presence of occult hepatitis B virus infections among blood donors with negative hepatitis B surface antigen and positive total anti-hepatitis B core antibodies in Hawler Major Blood Bank in Hawler city/Kurdistan Region of Iraq.

Methods: A total number of 12,185 blood donors in Hawler Major Blood Bank were screened for HBsAg and total anti-HBcAb using ELISA technique, and then positive results were retested by confirmatory technique by Chemiluminescence assay. All HBsAg-/HBcAb+ were selected as the study group; HBV DNA was tested among HBsAg-/HBcAb+ by conventional PCR and Real time-PCR. Clinical and demographic data of study group were recorded.

Results: Among the 12,185 blood donors, HBsAg was positive in 27 (0.22%) donors using Chemiluminescence assay, the frequency of HBs Ag -/ HBc Ab+ was 276 (2.27%), and then the total prevalence of HBV infection in all blood donors was 2.49%. Among the 276 HBs Ag-/HBcAb+, occult hepatitis B virus infection (OBI) was positive in 39.1% (108/276) using conventional PCR and Real time-PCR techniques, while the prevalence among all blood donors (n=12,185) was 0.09%. Testing of HBV-DNA in HBs Ag -/ HBc Ab+ group for OBI was done by qualitative PCR (positive HBV-DNA=102/276) or by quantitative Real time-PCR (positive HBV-DNA=108/276).

Conclusions: The OBI is frequently detected among blood donors in Hawler city especially those have HBsAg-/HBcAb+, and the total anti-HBcAb is an essential serological marker for screening HBV among blood donors. The risk factors for developing OBI among blood donors should be elucidated.

Keywords: Blood donors, Occult HBV, Hawler city, Blood Bank.

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Introduction:

The safety of blood in blood bank is an important global issue, as blood transfusion is a vital therapeutic procedure (1); the prevention of pathogen transmission through blood transfusion is a challenging subject. The presence of new cases of hepatitis B virus infections is continue to occur despite screening testing of HBV using HBs Ag with highly sensitive and specific techniques (2). Many blood banks have added antibodies specific to Hepatitis B core antigen (anti-HBc Abs) as a second HBV marker for detection HBV infections (3). The presence of anti-HBc Abs in absence of HBs Ag positivity has two scenarios: either blood donor was infected and now cured from infection or the donor is still infected and the infection can be elucidated by viral nucleic acid amplification technique (4). The Blood donor with serological evidence of negative HBs Ag, positive or negative HBc Abs, and positive HBV nucleic acid is known as occult HBV infection (5, 6). This infection is a hidden source of HBV transmission, not only through blood in blood bank, but also to families of infected patients. This study was conducted to detect the prevalence of occult hepatitis B virus infections among blood donors negative for HBsAg and positive for total anti-HBc antibodies in Hawler Major Blood Bank.

**Microbiology/Pathology, Kurdistan Board of Medical Specialties/ Kurdistan region.*
*** Dept. of Community Health, Technical College of Health, University of Sulaimani Polytechnic.*
Email: Ali.hussain@spu.edu.iq,

Methodology:

During a period of three months (from January till March 2017), volunteer blood donors in Hawler Major Blood Bank were routinely tested for HBs Ag by ELISA technique (HBsAg/Biorad/France) and total anti-HBc Abs using ELISA technique (Total Anti-HBc Abs/Biorad/ France); donors with negative HBs Ag and positive total anti-HBc Abs were our target study population and blood samples were aspirated from two hundred and seventy six (n=276) donors with HBsAg-/HBcAb+. Written informed consent was obtained from blood donors with HBsAg-/HBcAb+ who accepted participation in the study. The aspirated blood from each donor in the study group was centrifuged and each serum sample was divided into four aliquots of 1.5 ml tubes then stored in deep freeze (-70ºC) until examined. Rechecking of HBsAg and total anti-HBcAb for the all 276 samples using Chemiluminescence kits (LIAISON® HBs, LIAISON® Anti-HBc kits/Diasorin/Italy) was done, and then polymerase chain reaction (PCR) technique was performed on all 276 sera of the study group to detect HBV. Both qualitative and quantitative Real time-PCR techniques were done. Qualitative PCR was done by extraction of viral DNA with Sinaclon

DNP extraction kit (Sinaclon Company/Iran then amplification with Hepatitis B Virus PCR Detection Kit (Sinaclon Company/Iran), while quantitative PCR was achieved by extraction with Exprep Plus viral DNA/RNA kit (Bioneer Company/ Republic of Korea) and amplification with Accupower HBV Quantitative PCR kit (Bioneer Company/ Republic of Korea). The purity of DNA yield by extraction was quantified by NanoDrop 2000 spectrophotometer (ThermoFisher Scientific Company/USA). Demographic and clinical data were gathered from each participant in the study. The results were analyzed using SPSS program.

Results:

A total number of 12,185 blood donations were screened for hepatitis B virus infection using two serological markers: HBsAg and total anti-HBcAb. The combination of positive HBsAg and anti-HBc Abs were found in 27 donors, while 276 donors were HBsAg-/anti-HBcAb+. The prevalence of HBV infection among blood donors during the period of sample collection was 2.49%, and the prevalence of HBs Ag -/ HBc Ab+ was 2.27%, while the prevalence of positive HBsAg was 0.22%. Two molecular techniques, quantitative and qualitative PCR techniques, were used for detecting HBV nucleic acid in HBs Ag-/HBc Abs+ blood donors and the results showed that 39.1% (108/276) of them were positive for HBV in real time-PCR, while 37% were positive in qualitative (conventional) PCR, the differences were statistically not significant (table 1). Depending on RT-PCR results of HBV load, the prevalence of OBI among blood donors in Hawler Major Blood Bank was 0.09%, and the prevalence of OBI among HBs Ag -/HBc Ab + was 39.1%.

Table (1): HBV nucleic acid positivity in HBsAg negative/HBc Abs positive blood donors

Molecular Technique	Positive (%)	Negative (%)	Total (%)
Real time PCR	108 (39.1%)	168 (60.9%)	276 (100%)
Qualitative PCR	102 (37%)	174 (63%)	276 (100%)

The chi-square: 0.2767. The *p*-value is .598878 at $p < 0.05$.

For the 108 positive HBV load by Real time-PCR, the mean of the HBV PCR viral load is 11996 copies/ml, the median is 89.5copies/ml, and the range is 19-308000 copies/ml. The mean age of OBI was 42.72 ±10.988; most of blood donors in study group were males 273 (98.91%), while only 3 (1.09%) were females, and all OBI were males. Most of HBsAg-/HBcAb+ blood donors were having blood group O+, while the other blood groups in descending sequence were B+, A+, O-, A-, AB+, and AB- (table 2). None of the OBI donors was B-, or AB-, and most of them were O+ and the overall differences in blood groups between OBI donors and other donors with negative HBV nucleic acid was statistically not significant $p > 0.05$, figure (1).

Table (2): Blood groups of blood donors with HBs Ag negative /HBc Ab positive

Blood group	Frequency	Percent
O+	117	42.4
B+	66	23.9
A+	48	17.4
O-	24	8.7
A-	6	2.2
AB+	12	4.3
AB-	3	1.1
Total	276	100.0

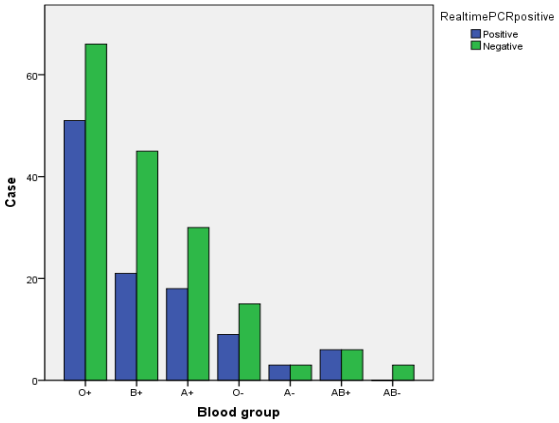

Figure (1): frequency of blood groups among positive HBV nucleic acid (OBI) and negative HBV nucleic acid groups.

Concerning the occupation of blood donors of both groups, OBI group (n=108) and negative HBV DNA group (n=168), most of them were working in free business and employees, while few of them retired or unemployed, and no significant statistical differences were present in professions of all HBsAg-/HBcAb+, figure (2).

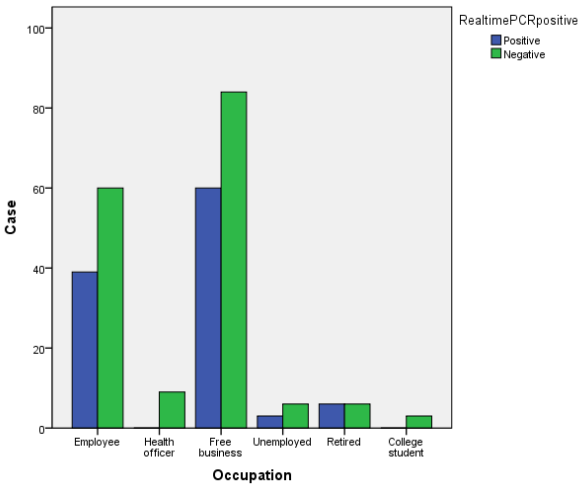

Figure (2); the professions of HBs Ag negative / HBc Abs positive blood donors.

The residency of blood donors with HBs Ag-/HBc Ab revealed that most of them are living in city center districts while few of the Real time-PCR

negative group was living in villages but none of RT-PCR positive group was living in a village, figure (3).

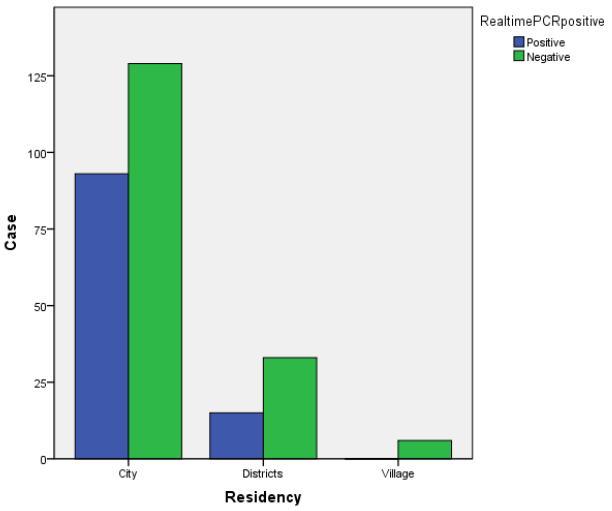

Figure (3): The residency of blood donors with negative HBs Ag/positive HBcAb.

The significance of differences in clinical data between OBI group (n=108) and negative HBV DNA (n=168) showed that donation and receiving of blood, history of contact with HBV infected persons, history of surgical operations, and history of jaundice were not statistically significant while history of chronic diseases and History of tattooing or skin piercing were statistically significant, table (3). The history of HBV vaccination was positive in only 5 donors of OBI group and in only 16 donors of HBV DNA negative group, while all the remaining were not vaccinated against HBV. The difference in history of HBV vaccination was statistically not significant (p= 0.0816), figure (4). The correlation between total DNA yield concentration for each sample extracted by viral DNA/RNA kit, and measured by NanoDrop spectrophotometer, with the viral load of HBV measured by RT-PCR showed no linear correlation between the two results (r = 0.042).

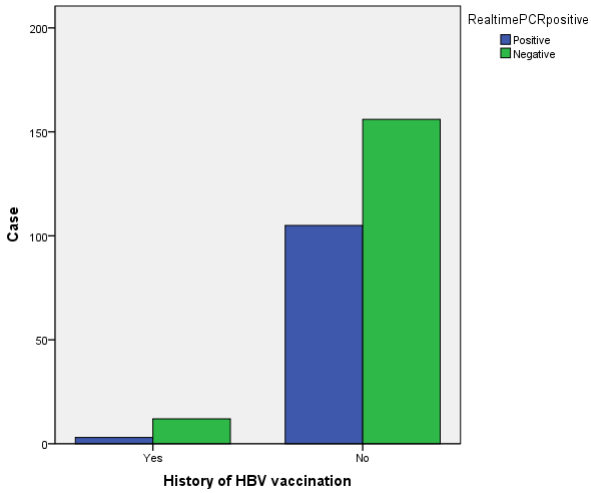

Figure (4): History of vaccination among OBI and those with negative HBV load.

Table (3): Clinical data of positive HBV nucleic acid (OBI) compared to negative OBI blood donors, both having negative HBs Ag/positive anti-HBcAb.

Parameter	Outcome	Real time-PCR positivity		P value (Significance at p < 0.05)
		Positive (OBI)	Negative	
		Count	Count	
History of blood donation	Yes	108	168	0.7537
	No	0	0	
History of receiving blood	Yes	3	9	0.3051
	No	105	159	
History of contact with HBV infected persons	Yes	18	42	0.1014
	No	90	126	
History of chronic disease	Yes	21	18	0.0421
	No	87	150	
History of surgical operation	Yes	18	33	0.5341
	No	90	135	
History of Jaundice	Yes	3	0	0.0593
	No	105	168	
History of tattooing or skin piercing	Yes	0	9	0.0368
	No	108	159	

Discussion:

The occult HBV infected blood donors can transmit infection to others. The absence of HBs Ag and the persistence of HBV nucleic acid in the blood or in hepatocytes is the hallmark of occult HBV. The prevalence of positive HBs Ag among blood donors in this study is much lower than the corresponding HBV marker in most regions of Iraq, for example in Duhok it is 0.78% (7), in Baghdad it is 0.6% (8), in Babylon governorate 0.7% (9), in Najaf governorate 0.66%, and 3.5%in Karbala (10), the prevalence of HBs Ag was higher than the that recorded in Basra, 0.02% (11). The HBs Ag was also screened in neighboring countries, in Kuwait, in 2002, it was 1.1% among Kuwaiti blood donors (12); in Jordan, it was 1.4% (13), while in Denizli, Turkey it was 0.97% (14). The prevalence of HBV infection in the current study was 2.49%, which is close to that recorded among blood donors (2.3%) in Basra in2013 (11), but less than a study in Erode District, India which recorded a prevalence of 10.9% (15). The present study measured the prevalence of HBs Ag -/ HBc Ab+ among blood donors in Hawler Major Blood Bank and it was 2.27%. A Previous national survey in Iraq tested HBs Ag -/ HBc Ab+ in all over Iraqi governorate and it was 8.1% (16), while two studies in Basra recorded 2.1%, 18.58%, (11, 17). OBI was detected in 0.09% of all blood donors during the study period and it was 39.1% among HBs Ag -/HBc Ab +. Absence of HBsAg may be due to mutated HBsAg, low level expression of HBsAg or entrapment of antigen in the circulatory immune complexes (18). The prevalence of OBI in Iraq recorded in 3.9% of blood donors in Diyala (19), while in Basra it was 14% in HBc Abs positive donors (17). In nearby and abroad countries, the frequency of OBI was reported 0 % in Turkey, Iran, and Greece (20–22), 1.25 % in Saudi Arabia

(23), 1.59 % in Germany (24), 4.86 % in Italy (25), 7.5 % in India (26), 17.2 % in Egypt (27), 38 % in Japan (28) and 90.5 % in Sudan (29). In the current study the mean and median of HBV DNA load among OBI were low, 11996 copies/ml and 89.5 copies/ml respectively, these results are in accordance to results of Ye X, et al (30) and to the general impression that OBI is characterized by very low HBV DNA load (31). In this study, most of OBI are in their 5th decade of age and are male gender, and most of clinical and demographic data, with the exception of history of chronic diseases, are not significant risk factors for the development of OBI and HBsAg-/HBcAb+. While a study by Said ZN et al demonstrated that age of OBI is mostly below thirty (32). The Concentration of DNA yield from viral DNA extraction has no correlation with the HBV load, this is an interesting finding which might be due to other nonspecific viral nucleic acids extracted which are not amplified by HBV amplification PCR kit.

Conclusions:

HBV infection among blood donors in Hawler Major Blood Bank is 2.49%, and the prevalence of HBs Ag -/ HBc Ab+ is 2.27%. The OBI is frequently detected among blood donors in Hawler city especially those have HBsAg-/HBcAb+, and the total anti-HBcAb is an essential serological marker for screening HBV among blood donors. The introduction of Real time-PCR in screening of HBV in blood banks in Iraq is a vital screening tool. The risk factors for developing OBI among blood donors should be elucidated.

Authors' contributions:

Ali Hattem Bayati: designed the aim of the study and methodology.

Rasha Nazar Hassan: performed the laboratory tests and data collection. Both researchers analyzed data and wrote the manuscript.

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