Prevalence of Antibodies to Hepatitis B Virus Antigens and Occult Hepatitis B Virus Infection in Blood Donors

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Abstract

Background: Occult hepatitis B virus (HBV) is the presence of HBV DNA in liver tissue and/or in serum in the absence of detectable hepatitis B surface antigen (HBsAg). It is a world-wide entity that is considered as a potential risk for transmission of HBV infection.
Objectives: This study was aimed to determine the rate of occult HBV infection among unpaid blood donors in Diyala province-Iraq.
Subjects and methods: This study was conducted for the period from 1st. May 2011 to 1st. April 2012**.** A total of 186 unpaid blood donors were chosen by simple random selection from those attending the Central Blood Bank in the Public Health Laboratory in Diyala province. 171 (91.9%) were male and 15 (8.1%) were female. The age range was 19-60 years. Sera of blood donors were subjected for HBsAg screening test, anti-HBs antibody, anti-HBc IgM antibody by enzyme linked immunosorbant assay (ELISA), as well as detection of HBV DNA by conventional polymerase chain reaction (PCR) technique. Data were statistically analyzed.
Results: The positivity rate of HBsAg, anti-HBc IgM and HBV DNA were 4.3%, 3.2% and 8.1%. Among the HBsAg negative blood donors, the HBc IgM positivity rate were 3.4% and the HBV DNA was detected in 3.9% (occult HBV).
Conclusion: The relatively high rate of occult hepatitis B viral infection among blood donors may be responsible, at least in part, for the perpetuation of the infection in the community.
Keywords: Occult HBV, Anti-HBc IgM, HBV DNA, blood donors.

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الخلاصة

خلفية: التهاب الكبد B الغامض فيروس (HBV) هو وجود DNA في أنسجة الكبد HBV و / أو في مصل الدم في حالة عدم وجود مستضد التهاب الكبد كشف السطح B (HBsAg و). بل هو كيان في جميع أنحاء العالم التي تعتبر خطرا محتملا لانتقال عدوى فيروس التهاب الكبد نوع B.
الأهداف: تهدف هذه الدراسة إلى تحديد معدل الإصابة HBV غامض بين المتبرعين بالدم غير المدفوعة في محافظة ديالى العراقية.
المواضيع والأساليب: قد أجريت هذه الدراسة للفترة من ١. مايو ٢٠١١ إلى ١st. أبريل ٢٠١٢. وقد تم اختيار ما مجموعه ١٨٦ المتبرعين بالدم غير المدفوعة عن طريق الانتقاء العشوائي البسيط من أولئك الذين يحضرون بنك الدم المركزي في مختبر الصحة العامة في محافظة ديالى. وكانت ١٧١ (٩١.٩٪) من الذكور و ١٥ (٨.١٪) من الإناث. كان الفئة العمرية ١٩-٦٠ عاما. وتعرض سيرا من المتبرعين بالدم ل HBsAg اختبار الفرز، ومكافحة هبس الأجسام المضادة، ومكافحة فيروس الكبد الضد IgM بواسطة الاليزا (ELISA)، وكذلك الكشف عن الحمض النووي HBV بواسطة تفاعل البلمرة سلسلة التقليدية (PCR) التقنية. وقد تم تحليل البيانات إحصائيا.
النتائج: كانت نسبة إيجابية من HBsAg و، الغلوبولين المناعي المضادة للمستضد اللب وHBV DNA ٤.٣٪، ٣.٢٪ و ٨.١٪. بين المتبرعين بالدم HBsAg سلبي، كانت إيجابية الغلوبولين المناعي مستضد اللب معدل ٣.٤٪ وتم الكشف عن الحمض النووي HBV في ٣.٩٪ (غامض HBV).
الاستنتاج: قد يكون معدل عال نسبيا من الإصابة بالتهاب الكبد الفيروسي B غامض بين المتبرعين بالدم تكون مسؤولة، جزئيا على الأقل، من أجل بقاء العدوى في المجتمع.
كلمات البحث: الغيبيات HBV، نظام مضاد للمستضد اللب الغلوبولين المناعي، DNA HBV، المتبرعين بالدم.

Introduction

Occult hepatitis B virus infection is defined as the persistence of HBV DNA without HBsAg with or without the presence of HBV antibodies outside the acute phase window period. Occult HBV infection, in addition to its risk of transmission through the transfusion of infected blood, reactivation of hepatitis B in occult HBV infected patients and recipients of their blood can lead to cirrhosis, hepatic cancer, and reactivation of viral replication in the carrier [1,2]. It was described in hepatocellular carcinoma, chronic hepatitis B, healthy HBV carriers and recovered infection, chronic hepatitis C and individuals without serological markers of HBV [3]. The frequency of the diagnosis depends on the relative sensitivity of both HBsAg and HBV DNA assays and on the prevalence of HBV infection in the population [4].

Occult HBV in blood donors has a wide range of potential origins within the natural history of the infection. Among Korean blood donors the prevalence of occult HBV infection was 0.016%, and the HBV nucleic acid test yield was 0.12% [5]. In Italian blood donors 4.86% were confirmed to have circulating HBV-DNA, estimating that the risk of an HBV-DNA positive unit from an occult carrier being released into the blood supply is more than 100 times higher than the estimated residual risk related to the window phase of HBV infection [6]. The occult HBV infection was detected in 1.26% of the Egyptian accepted blood donations, suggesting that implementing anti-HBc test to the routine assay is beneficial to decrease the risk of HBV transmission with its potential consequences particularly in immunocompromised recipients [7]. In Pakistan, it has been reported that 0.5% blood donors have occult HBV infection, with likelihood of transmission of hepatitis B in recipients of blood components [8]. In China, the rate of occult HBV infection among blood donors was 0.13 % as detected by nested PCR [9].However, among Colombian blood donors, no occult HBV infection was detected [10].

Subjects and Method

This study was conducted for the period from 1st. May 2011 to 1st. April 2012. A total of 186 unpaid blood donors were chosen by simple random selection from those attending the Central Blood Bank in the Public Health Laboratory in Diyala province. 171 (91.9%) were male and 15 (8.1%) were female. The age range was 19-60 years. Sera of blood donors were subjected for HBsAg screening test, anti-HBs antibody, anti-HBc IgM antibody by enzyme linked immunosorbant assay (DRG-Germany), as well as detection of HBV DNA by conventional polymerase chain reaction (PCR) technique (Sacace-Italy). Data were translated into a computerized database structure. An expert statistical advice was sought using SPSS version 20 computer software (Statistical Package for Social Sciences). The statistical significance of association between 2 categorical variables is assessed by Chi-square test. The level of statistical significance was assumed at $P < 0.05$. To measure the strength of association between 2 categorical variables, the odds ratio (OR) was used. The statistical significance of the measured OR is assessed by a special $\chi 2$ formula.

Results

The results revealed that the positivity rate of HBsAg,anti-HBs Ab, anti-HBc IgM, HBV DNA among blood donors were 4.3%, 9.1%, 3.2%, 8.1% respectively, table (1).

Table (1): Rate of positive viral markers among blood donors.

Viral marker	No. positive	%	95% Confidence interval
Serum HBsAg	8	4.3	(1.4-7.2)
Serum anti-HBs antibody	17	9.1	(5-13.2)
Serum antiHBc IgM	6	3.2	(0.7-5.7)
Viral DNA (PCR)	15	8.1	(4.2-12)
Any positive marker	30	16.1	(10.8-21.4)

n = 186

Results in table (2) showed that the positivity rate of anti-HBs Ab, anti-HBc IgM, HBV DNA in HBsAg negative blood donors were 9.0%, 3.4%, and 3.9% respectively. Accordingly, the rate of occult HBV infection was 3.9%.

Table (2): Rates of positive markers among HBs Ag negative blood donors.

Viral marker	No. positive	%	95% confidence interval
Serum anti-HBs antibody	16	9.0	(4.8-13.2)
Serum anti-HBc IgM	6	3.4	(0.7-6.1)
Viral DNA (PCR)	7	3.9	(1.1-6.7)
Any positive marker	22	12.4	(7.6- 17.2)

n = 178

The results also revealed that all the blood donors who were HBsAg positive or anti-HBsAb positive were negative to HBcIgM, indicated that there was no association among these markers, table (3).

Table (3): Rate of positive HBs Ag and anti-HBs Ab against HBc IgM.

Positive viral marker	Serum anti-HBc IgM				P value
	Negative (n=180)		Positive (n=6)		
	No.	%	No.	%	
Serum HBsAg	8	4.4	0	0.0	1[NS]
Serum anti-HBs Ab	17	9.4	0	0.0	1 [NS]

Results in table (4) showed that all the blood donors who were ant-HBcIgM were also negative for HBsAb. Furthermore, all except one blood donor who were positive for HBsAg were negative for HBsAb. In both conditions there was no statistically significant association among these markers.

Table (4): Rates of positive HBs Ag and HBc IgM against anti-HBs Ab.

Positive viral marker	Serum anti-HBs Ab				P value
	Negative (n=169)		Positive (n=17)		
	No.	%	No.	%	
Serum HBsAg	7	4.1	1	5.9	0.54[NS]
Serum anti-HBc IgM	6	3.6	0	0	1 [NS]

The rate of HBV RNA detection does not influenced significantly by age, gender, and residence of the blood donors although the risk factor in female was 3.3 times more than that in male, table (5).

Table (5): Detection rate of HBV DNA by gender, age and residence.

Variables	Total No.	viral DNA Positive No. (%)	P value	Odd ratio	95% CI for OR
Gender					
Female	15	3 (20.0)	0.1 [NS]	3.31	(0.82- 13.36)
Male	171	12 (7.0)		Reference	
Residence					
Urban	91	9 (9.9)	0.37 [NS]	1.63	(0.56- 4.77)
Rural	95	6 (6.3)		Reference	
Age group (ys)					
< 30	68	5 (7.4)	0.95 [NS]	**	**
30-39	80	7 (8.8)			
40 +	38	3 (7.9)			

Discussion

Iraq is still rank in the intermediate zone of endemicity of HBV infection although screening of blood donors and other risky groups for HBV infection was started since 1989 [11]. It is worth to mention here that the screening test is still based on the serological detection of HBsAg only as implemented by Iraqi Ministry of Health.

In the present study, the rate of occult HBV infection among unpaid blood donors was 3.9% based on the detection of HBV DNA in HBsAg negative blood donors, i.e. each one hundred blood units screened by HBsAg test, about 4 units may pass undetectable and may carry the risk of transmission of HBV infection to recipients. Unfortunately, this rate is relatively higher that that reported by other studies [5,7-9]. It was well documented that the prevalence of occult HBV infection is quite variable depending on the level of endemic disease in different parts of the world, the relative sensitivity of both HBsAg and HBV DNA assays utilized in the studies, and the different populations studied [3,4,12,13]. The most accepted explanation of the occult HBV infection is that it may originate from recovered infections with anti-HBs and persistent, low-level, viral replication; escape mutants undetected by the HBsAg assays or healthy chronic carriage [3,14-16].

The results obtained in this study affirm the existence of occult HBV infection in some donors that cannot be detected by the HBsAg routine serological assays. The question is to what extent that occult HBV is transmissible by transfusion?. Several studies have reported that the potential infectivity of these units is debated, but their use cannot be considered safe at least in immunocompromised recipients [3,5,7,12]. In our country, probably one of the reasons that were behind the maintenance of HBV infection in Iraqi community is the inability of the screening test employed to detect the occult HBV infection, a fact that was documented by many other workers [12,15,17].

Therefore, a new strategy needs to be implemented for blood screening based on HBV endemicity; yields of infectious units detected by different serologic/HBV DNA assays, and cost effectiveness of test methods in ensuring blood safety.

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