

Detection of anti-Hepatitis B core antibodies among Hepatitis B surface antigen negative blood donors in Sana'a city, Yemen

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Abstract

Hepatitis B is still a serious global infectious disease that remains a high risk for patients requiring blood transfusions, despite the introduction of appropriate methods for diagnosis of the causative virus in blood banks, thus this cross-sectional study aimed to estimate anti-HBc (IgM and IgG) antibodies in blood donors who were HBsAg negative, to determine HBV-DNA among the positive anti-HBc blood donors and finally to study the association of positive anti-HBc Abs donors with the predisposing factors contributing for HBV transmission in Sana'a city, Yemen.

This study included 700 Yemeni male blood donors who came for blood donation to the National Center for Blood Transfusion and Research in Sana'a city during a one year period (January - December 2010). Their age ranged from < 20 years to \geq 40 years old with a mean age of 38 years and a standard deviation (SD) of 6.9 years. Blood samples and information data were collected from each subject recruited in the study. Serum was separated and tested for anti-HBc antibodies and HBV-DNA by an automated ELISA and RT-PCR method. Data were analyzed by SPSS (version 15) for statistical significance.

In this study, the prevalence of anti-HBc-IgG among the studied subjects was 9%, whereas the prevalence of anti-HBc-IgM was zero. HBV-DNA was detected in 4.8% of these positive anti-HBc-IgG subjects. This study showed that blood donors with positive anti-HBc-IgG had a significant association with increased age. The main predisposing factors that had a significant association with positive anti-HBc-IgG subjects were blood transfusion, history of jaundice and family history for hepatitis.

It can be concluded from this study that there was a relatively high positive percentage for both anti-HBc-IgG and HBV-DNA among subjects who were HBs Ag negative. Increased age, previous blood transfusions, history of jaundice and family history of hepatitis were the main predisposing factors associated with positive anti-HBc-IgG subjects.

Introduction

Hepatitis B virus (HBV) is the most common cause of serious liver infection in the world.^{1,2} It is estimated that worldwide more than two billion people have been infected by HBV and 350 million people have chronic infection.^{1,3} Transfusion transmitted HBV has always been a dreaded disease, which has an unholy reputation of being transmitted fairly often among donated blood.^{4,5} The safety of blood and blood products are one of the major issues in the area of transfusion medicine.^{3,5}

It has been demonstrated that some hepatitis B surface antigen (HBs Ag) negative individuals and those positive for hepatitis B core antibody (anti-HBc) continue to replicate HBV.^{6,7} Thus the absence of HBs Ag in the blood of apparently healthy individuals may not be enough to ensure lack of circulating HBV and blood that containing anti-HBc with or without detectable presence of HBs antibody might be infectious; therefore, routine blood donor screening for anti-HBc has been implemented in some countries, resulting in a decrease in the risk of post-transfusion HBV infection.⁸

HBV infection is one of the major health problems in Yemen, thus to increase the blood safety screening for anti-HBc antibodies must be introduced among blood donor to reduce HBV transmission.

In a previous study, the prevalence of HBs Ag among Yemeni blood donors was 6.7%, whereas the prevalence of anti-HBc antibody was 17.4%.⁹

In blood donors the incidence of transfusion-related HBV has significantly decreased due to routine screening for HBs Ag.¹⁰ Post transfusion HBV depends on several factors like prevalence and donor testing strategies. In low prevalence areas it is estimated to be one to four per million blood components transfused.^{10,11} In Yemen, the prevalence of anti-HBc-IgG among blood donors who were HBs Ag negative was 16.5%.¹²

In Saudi Arabia, Iran and the most parts of Middle East region, the prevalence of anti-HBc-IgG was documented with varies percentages among blood donors who were HBs Ag negative. HBV-DNA was also detected in positive anti-HBc blood donors who were HBs Ag negative as reported in Egypt, India and Iran, but with different percentages.¹³⁻¹⁵

There are no published data about this issue, although its importance in Yemen, therefore the aims of this study was to estimate anti-HBc (IgM and IgG) in blood donors who were HBs Ag negative, to determine HBV-DNA among the positive anti-HBc blood donors and finally to study the association of positive anti-HBc donors with the predisposing factors contracting for HBV transmission in Sana'a city, Yemen.

Materials and Methods

This cross sectional study was carried out during a period of one year (January to December 2010). The study group consists of 700 male blood donors who donated blood at the National Center for Blood Transfusion and Research (NCBTR) in Sana'a city.

Blood samples were collected from these blood donors in plane tubes (3 ml) from each donor. Blood in plane tube allowed to clotting then the sera were separated and kept in a deep freeze at -70o C. All collected samples were tested for HBs Ag, anti-HBc total, anti-HBc-IgM and HBV-DNA at the NCBTR in Sana'a city. The sera were tested for HBs Ag, anti-HBc-total and anti-HBc-IgM by an automated ELISA method using the Elecsys device system (Roche Diagnostics, Germany). All subjects who had negative result for HBs Ag as confirmatory test were selected and tested for anti-HBc-total and anti-HBc-IgM. The positive anti-HBc samples were then analyzed for HBV-DNA by a RT-PCR technique using the COBAS TaqMan 48 analyzer (Roche Diagnostics, Germany) for automated amplification and detection of HBV-DNA in human serum by the high pure system viral nucleic acid kit for manual specimen preparation.

The collected data from each subject were analyzed using a statistical package of social science program (SPSS, version 15) and the probability value (p) of <0.05 was considered as statistically significant.

Results:

This study included 700 male blood donors who were HBs Ag negative by an ELISA technique performed at the NCBTR in Sana'a city. Their age ranged from < 20 years to ≥ 40 years old with a mean age of 38 years and a standard deviation (SD) of 6.9 years. Out of the total studied subjects, 63 subjects were only positive for anti-HBc-IgG with a percentage of 9% and the rest 637 subjects were negative for these antibodies. HBV-DNA was detected in 3 of the 63 positive anti-HBc-IgG subjects with a percentage of 4.8%. The detailed results of this study are presented in the following tables:

Table (1): Distribution of the total studied subjects according to the different serological markers

Serological Markers	Positive		Negative		Total	
	No.	%	No.	%	No.	%
Total anti-HBc	63	9	637	91	700	100
Anti-HBc-IgG	63	9	637	91	700	100
Anti-HBc-IgM	0	0	700	100	700	100

Table 1 shows the distribution of the total studied subjects according to the different serological markers. Out of the total 700 studied subjects, all were negative for anti-HBc-IgM, only 63 subjects were positive for anti-HBc-IgG and at the same time for the total anti-HBc with a percentage of 9% for each.

Table (2): Distribution of HBV-DNA level among positive anti-HBc-IgG subjects

HBV-DNA	Positive anti-HBc-IgG	
	No.	%
Detected	3	4.8
Undetected	60	95.2
Total	63	100

Table 2 shows the distribution of HBV-DNA level among positive anti-HBc-IgG subjects. HBV-DNA was detected among 3 of 63 positive anti-HBc-IgG subjects with a percentage of 4.8%.

Table (3): Distribution of positive anti-HBc-IgG subjects according to their age

Age in years	Anti-HBc-IgG (n=63)		Total (n=700)		χ^2	p
	No.	%	No.	%		
< 20	4	6.2	65	9.3	8.85	0.031
20-29	25	6.7	375	53.6		
30-39	25	12.5	201	28.7		
≥ 40	9	15.3	59	8.4		
$\chi^2 \geq 3.84, p < 0.05$ (significant)						

Table 3 shows the distribution of positive anti-HBc-IgG subjects according to the age groups. The highest prevalence of positive anti-HBc-IgG occurred in the age group ≥ 40 years which was 15.3% and the lowest prevalence of anti-HBc-IgG was in the age group < 20 with 6.2%. This result was statistically significant with values of $\chi^2 = 8.85, p < 0.031$.

Table (4): The prevalence and relative risk of positive anti-HBc-IgG subjects according to the

Predisposing Factors	Anti-HBc-IgG (n=63)		RR	CI	χ^2	p
	No.	%				
Surgical Operation (n=34)	3	8.8	0.98	0.32-2.96	0.001	0.971
Dental/Clinic Visit (n=116)	10	8.6	0.95	0.50-1.81	0.024	0.876
Cupping(n=17)	2	11.8	1.32	0.35-4.95	0.163	0.687
History of jaundice (n=21)	7	33.3	4.04	2.10-7.78	15.66	0.0001
Blood transfusion (n=30)	11	36.7	4.72	2.76-8.10	29.29	0.0001
Accidence (n=14)	1	7.1	0.79	0.12-5.30	0.060	0.806
Shared Shaving bald (n=49)	6	12.2	1.40	0.64-3.08	0.677	0.411
Traveling Abroad (n=25)	2	8.0	0.89	0.23-3.42	0.032	0.859
Intravenous drug abuse (n=32)	4	12.5	1.42	0.55-3.65	0.502	0.479
Family history for hepatitis (n=53)	10	31.3	2.30	1.24-4.26	6.82	0.0001
$\chi^2 \geq 3.84, p < 0.05$ (significant), RR > 1 (at risk).						

Table 4 summarizes the prevalence and relative risk of positive anti-HBc-IgG subjects according to the predisposing factors. The main risk factors associated with positive anti-HBc-IgG were blood transfusion, history of jaundice and family history for hepatitis with percentages of 36.7%, 33.3%

and 31.1% respectively. These results were statistically significant with values of $\chi^2 = 29.29$, $p < 0.0001$ for blood transfusion, $\chi^2 = 15.67$, $p < 0.0001$ and for history of jaundice and finally $\chi^2 = 20.27$, $p < 0.0001$ for family history for hepatitis. As regard the relative risk (RR), blood transfusion was nearly 5 times, history of jaundice was 4 times and finally family history for hepatitis was nearly 2 times more at risk for each than other predisposing factors.

Discussion

Hepatitis B is still a serious global infectious disease that remains a high risk for patients requiring blood transfusions, despite the introduction of appropriate methods for diagnosis of the causative virus in blood banks.

In this study, out of the 700 enrolled subjects who were negative for HBs Ag, anti-HBc-IgG was detected in 9% of them as shown in tables (1). This result was nearly similar to other studies performed in Brazil (8.7%)¹⁶, India (8.4%)¹⁷, Egypt (7.8%)¹⁴ and finally Iran (8%)¹³. On the other hand, higher results were reported in Saudi Arabia (16.4%)¹⁸, Oman (20.5%)¹⁹, Pakistan (17.3%)²⁰, Turkey (21.4%)²¹, Yemen (16.5%)¹² and finally India (19.8%)²².

In addition, the highest prevalence at all was reported 43.4 % in Indonesia (23). Lower results were reported in Iran (2.1%), Italy (4.8%), and finally in China (4.9%)²⁴⁻²⁶. These disagreeable findings could be mainly explained by the result of regional differences in the prevalence of HBV infection.

In the present study, out of the 700 enrolled subjects who were negative for HBs Ag, all were anti-HBc-IgM negative as shown in tables (1). Result of this study was in agreement with a study conducted in Yemen previously.¹² In contrast, the present study result was lower than that reported in India and Nigeria, which showed a percentage for anti-HBc-IgM of 0.4% and 5.4% respectively.^{5,27} This difference could be explained by the difference in sample size, geographical distribution and/or personal behavior.

In this study, HBV-DNA was detected among 3 of 63 (4.8%) positive anti-HBc subjects as shown in table (2). This result was to somehow corroborated with other studies reported in Italy (4.9%)²⁵, Egypt (6.3%)¹⁴, India (7.5%)²² and finally in Indonesia (8.1%)²³. However, our result was higher than that of another study reported by Allain and co-workers (2003) in Ghana (0.5%)²⁸. In contrast, this same result was lower than that reported in Iran (30%) and in India (30%)^{13,15}. Other studies performed in UK and Greece mentioned undetected HBV-DNA among positive anti-HBc blood donors.^{29,30} This dissimilarity in HBV-DNA detection among positive anti-HBc blood donors could be attributed by the difference in the endemicity

area of hepatitis B infection and the difference in the sensitivity and accuracy of PCR techniques.

The prevalence of positive anti-HBc-IgG showed a statistical significance with increased age ($\chi^2 = 8.85$, $p = 0.031$), where the highest prevalence was found among the subjects aged ≥ 40 years as shown in tables (3), this similar observation was reported by Panhotra and co-workers (2005)³¹ and El-Beltagy and co-workers (2007) in Saudi Arabia³², who reported that the highest prevalent rate was found among the donor population aged ≥ 40 years. In addition, Mudawi and co-workers (2007) in Sudan also mentioned that the highest prevalent rate was noted among the donor population aged ≥ 50 years.³³ Moreover, additional studies in Ghana and Iran reported that also the highest prevalence of HBV infection in general was found in the age groups older than 40 years.^{28,34} The reason for this increased prevalent rate in the age group ≥ 40 years may be related to a past infection before introducing of national vaccination program or due to that older persons probably have a longer disease exposure, duration and may practicing many inappropriate and unacceptable habits such as non-hygienic shared shaving tool or out marriage intercourse.^{31,35,36}

The main predisposing factors for positive anti-HBc-IgG among studied subjects were blood transfusion, history of jaundice and family history for hepatitis, which showed a high statistical significance as seen in table (4). Anti-HBc-IgG were positive in 36.7% of subjects with pervious blood transfusion comparing with those without pervious blood transfusion. This result was higher than that reported in Sudan and Brazil, in which the prevalence of anti-HBc-IgG were 5.1% and 26.7% respectively.^{33,36} On the other hand, the above mentioned result agreed with a pervious study conducted in Yemen by Al-Kyal¹², which was also statistically significant. In contrast, the present result disagreed with a study performed in Iran by Merat and co-workers (2009), in which the prevalence of anti-HBc-IgG was 17% in pervious blood transfusion subjects and this result was not statistically significant.³⁷ This difference could be attributed to the lack of specific and/or inadequate diagnostic tests in blood banks for screening Yemeni donors. Positive anti-HBc-IgG in subjects with family history for hepatitis showed 31.3% and was statistically significant as shown in table (4). This result agreed with two studies conducted in Saudi Arabia and Iran, which reported also a positive result for anti-HBc-IgG among donors with family history of hepatitis.^{32, 37} Positive anti-HBc-IgG among donors with history of jaundice was 33.3% as mentioned in table (4). Most studies exclude the donors with history of jaundice as a part of the international guideline of blood donation.³⁸

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تحديد أضداد المُستضدِّ اللَّبِّيِّ بين متبرعي الدم السالبيين للمستضد السطحي لفيروس التهاب الكبد البائي بمدينة صنعاء-اليمن

الملخص العربي :

لا يزال التهاب الكبد الفيروسي البائي مرضاً معدياً عالمياً خطيراً ومصدر عدوى رئيسياً للمرضى الذين يعتمدون على نقل الدم بشكل دوري بالرغم من تقدم الطرق المعملية الملائمة لتشخيص الفيروس المسبب .

هدفت هذه الدراسة إلى تحديد أضداد المستضد اللَّبِّيِّ (IgG + IgM) لفيروس التهاب الكبد البائي بين متبرعي الدم الذين كانوا سالبين للمستضد السطحي لذلك الفيروس وكذا تحديد الحمض النووي DNA للفيروس بين الحالات الإيجابية لأضداد المستضد اللَّبِّيِّ.

تضمنت هذه الدراسة المقطعية ٧٠٠ متبرع بالدم من الذكور والمتريدين على المركز الوطني لنقل الدم وأبحاثه بمدينة صنعاء خلال الفترة من يناير إلى ديسمبر ٢٠١٠. و تراوحت أعمارهم من أقل من ٢٠ سنة إلى ما يساوي أو أكثر من ٤٠ سنة مع متوسط عمري ٣٨ سنة وانحراف معياري ± ٦.٩ . تم جمع بيانات وعينة دم من كل متبرع مدرج في الدراسة، ثم فصل المصل منها واختباره لأضداد المستضد اللَّبِّيِّ بواسطة طريقة مقياسية الممتز المناعي المرئيط بالإنزيم ومن ثم الحمض النووي للحالات الإيجابية لأضداد فقط باستخدام طريقة تفاعل سلسلة البوليميريز. أما البيانات فقد تم تحليلها إحصائياً باستخدام برنامج الحزمة الإحصائية (SPSS).

تم تحديد أضداد المستضد اللَّبِّيِّ IgG في ٩٤٪ من إجمالي الحالات بينما كانت أضداد المستضد اللَّبِّيِّ IgM سالبة. أما ما يخص الحمض النووي الفيروسي DNA فقد تم تحديده في ٣ عينات من إجمالي ٦٣ عينة الإيجابية لأضداد المستضد اللَّبِّيِّ بنسبة ٤.٨٪. كما بينت هذه الدراسة أن هناك علاقة ذات دلالة إحصائية بين متبرعي الدم الإيجابيين لأضداد المستضد اللَّبِّيِّ وتقدم العمر لديهم، وكانت عوامل الاختطار الرئيسية ذات دلالة إحصائية عند المتبرعين الإيجابيين لتلك الأضداد هي نقل الدم سابقاً والتاريخ المرضي لليرقان والتاريخ العائلي لالتهاب الكبد البائي.

يستنتج من هذه الدراسة أن هناك نسبة أضداد عالية للمستضد اللَّبِّيِّ IgG عند الذكور اليمنيين المتبرعين بالدم وكذلك نسبة عالية للحمض النووي الفيروسي DNA في المتبرعين الإيجابيين لتلك الأضداد والسالبيين للمستضد السطحي لفيروس التهاب الكبد البائي مما يؤكد عدم سلامة دم هؤلاء المتبرعين وانتقال الفيروس إلى المستقبلين لهذا الدم مباشرة. كذلك أوضحت هذه الدراسة أن عوامل الاختطار الرئيسية المرتبطة بمتبرعي الدم الإيجابيين لهذه الأضداد هي تقدم العمر ونقل الدم سابقاً والتاريخ المرضي لليرقان والتاريخ العائلي لالتهاب الكبد البائي.