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Detection of Hepatitis B Virus by HBsAg and Total HBc Antibody among Blood Donors at National Blood Transfusion and Research Center in Taiz City, Yemen

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الكشف عن فيروس الكبد البائي (HBV) بواسطة المستضد (HbsAg) والأجسام المضادة الكلية للب الفيروس (total HbcAb) بين المتبرعين بالدم في المركز الوطني لنقل الدم وأبحاثه في مدينة تعز، اليمن

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الملخص

فيروس التهاب الكبد البائي (HBV) يصيب تقريبا 350 مليون شخص سنويا، ونسبة الإصابة به قد تؤدي إلى تحطم خلايا الكبد خلال الإصابة الحادة أو المزمنة، حيث قد تنتج مضاعفات تليف الكبد أو الإصابة بسرطان الكبد. نقل الدم يمثل إحدى الطرق المعتادة لانتقال فيروس الكبد البائي. في الآونة الأخيرة زاد الاحتياج إلى نقل الدم ومشتقاته في اليمن نتيجة لكثرة جرحى الحرب، فقر الدم وسوء التغذية. هدفت هذه الدراسة إلى تحديد انتشار فيروس التهاب الكبد البائي عن طريق فحص مستضد سطح فيروس الكبد (HBsAg) ومضاد لب فيروس الكبد البائي الكلي (total HBcAb) بين المتبرعين بالدم في المركز الوطني لنقل الدم – فرع تعز (NBTRC- TB).

تم جمع عينات الدم من المتبرعين في المركز الوطني لنقل الدم وأبحاثه فرع تعز خلال الفترة من أبريل إلى ديسمبر 2021م. بلغ عدد المتبرعين بالدم 2129 متبرعا. كل عينات الدم المأخوذة من المتبرعين تم فحصها بطريقة الوميض الكهربي باستخدام جهاز كوبس E411. تم تحليل نتائج فحوصات HBsAg وHBcAb الكلي إحصائيًا باستخدام برنامج SPSS إصدار 26. إجمالي عدد المتبرعين بالدم الذين تم فحص عينات دمهم كان 2129، منهم 2106 (98.9%) ذكور و23 (1.1%) إناث. أظهرت النتائج أن 1.22٪ من العينات كانت إيجابية لفحص مستضد سطح فيروس الكبد البائي (HBsAg) وجميعها كانت لمتبرعين ذكور فقط. في حين أن 10.38% من العينات كانت إيجابية لمضاد لب فيروس الكبد البائي الكلي (HBcAb)، حيث كانت نسبة إصابة المتبرعين الذكور (10%) والإناث (0.38%).

وبتحديد نتيجة كلا من مستضد سطح فيروس الكبد البائي (HBsAg) ومضاد لب فيروس الكبد البائي الكلي (HBcAb) معا كانت النتيجة (HBsAg سلبية/HBcAb إيجابية) بنسبة 9.16%، بينما (HBsAg إيجابية/HBcAb إيجابية) ظهرت بنسبة 1.22% أما المجموعة الثالثة (HBsAg سلبية/HBcAb سلبية) فظهرت بنسبة 89.62%؛ وبفارق معنوي ذو دلالة إحصائية ($P < 0.001$). تشير النسبة العالية لظهور المجموعة (HBsAg سلبية/HBcAb إيجابية) إلى الإصابة السابقة للمتبرعين بفيروس الكبد البائي.

الكلمات المفتاحية: فيروس الكبد البائي، المركز الوطني لنقل الدم، المستضد السطحي لفيروس الكبد البائي، الأجسام المضادة الكلية للب الفيروس البائي، تعز، اليمن.

Detection of Hepatitis B Virus by HBsAg and Total HBc Antibody among Blood Donors at National Blood Transfusion and Research Center in Taiz City, Yemen

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Abstract

Hepatitis B virus (HBV) infects almost 350 million infected persons universally. HBV can cause hepatocyte damage resulting from acute or chronic hepatitis which complicated to liver cirrhosis and hepatocellular carcinoma (HCC). Blood transfusion represent one of commonly route transmission of HBV. Recently, blood transfusion in Yemen are increasing due to high demands for blood and blood products resulting from war injury, anemia, and malnutrition. This study aimed to determine hepatitis B virus prevalence by investigate surface HBsAg and total HBcAb among blood donors at National Blood Transfusion Center -Taiz branch (NBTRC-TB). All blood samples were collected from blood donors at NBTRC-TB during the period of April to December 2021. A total of 2129 blood donors donated blood at the center, all information were registered at the reception department, and the blood was screened for both markers by ECLIA (Cobas E411). Data of HBsAg and total HBcAb were analyzed statistically by using program SPSS version 26. Among 2129 of blood donors, 98.9% of them were males and 1.1%) were females. All blood donor samples were tested and the result appeared as 1.22% were seropositive to HBsAg, which all positive samples were only for male donors. Whereas, the seroprevalence of total HBcAb was 10.38%; the males have a higher seroprevalence rate (10%) compared to 0.38% in females. For investigation of both markers (HBsAg and HbcAb together) the result appears as three groups (HBsAg negative/HBcAb positive) was 9.16%, while (HBsAg positive/HBcAb positive) was 1.22% and third group (HBsAg negative/ HBcAb negative) was 89.62%; there was statistically significant difference ($P<0.001$). The high result seropositivity of (HBsAg negative/HBcAb positive) indicates to donors usually with post HBV infection.

Keywords: HBV, blood donors, NBTRC-TB, HBsAg, HBcAb, ECLIA, Taiz, Yemen.

Introduction

Hepatitis B virus (HBV) is an enveloped DNA virus belong to family *hepadnaviridae* and a small, circular, partial double stranded DNA virus (Al-Sadeq *et al.*, 2019; Fopa *et al.*, 2019). HBV represents one of the most common health problems which can cause mortality and morbidity that more than 350 million infected persons universally (Sallam *et al.*, 2012; Thabit *et al.*, 2012; Jayalakshmi *et al.*, 2013; Babanejad *et al.*, 2016). HBV Infection can cause liver damage that appear in both acute and chronic hepatitis diseases, which chronic hepatitis infection may be complicated to liver cirrhosis and hepatocellular carcinoma (HCC) (Al-Sadeq *et al.*, 2019).

The world health Organization (WHO) has define three level endemicity of HBV with HBsAg carrier rate; low level less than 2%, intermediate 2 – 8% and high level more than 8% (Akinbami *et al.*, 2012; Sallam *et al.*, 2012). One of the commonly route of HBV transmission is blood transfusion and /or its products that increase the HBV infection around the world (Olotu *et al.*, 2016; AlZubiery *et al.*, 2017). Furthermore, HBV transmission from asymptomatic donors which at low HBV or occult HBV infection which HBsAg cannot be detectable in blood donor serum that may be cause of residual infections like transfusion-transmitted infections, thus an additional sero-marker antibody should be investigated (Alabdallat & Bin Dukhyil, 2018). Accordingly, detection of HBV infection by both (HBs Ag and total HBcAb) before blood transfusion is very important to minimize the risk of HBV infection among recipients (Akinbami *et al.*, 2012; AlZubiery *et al.*, 2017).

Blood transfusion in Yemen was increased due to urgent need of blood donation for people that injured during war or suffered from anemia and malnutrition; in addition the blood transfusion risk is relatively increased (AlZubiery *et al.*, 2017).

There are some different methods are help for diagnostic of HBV antigens and antibodies that varies greatly from low sensitivity and specificity like chromatographic device (rapid test), to high sensitive and specific Enzyme linked immunosorbent assays (ELISA), Electrochemiluminescent immunoassay (ECLIA) method and molecular assays (PCR) (AlZubiery *et al.*, 2017).

Definitively, the National Blood Transfusion and Research Center in Taiz city and other centers in Sana'a and Aden are examined donors by both HBsAg and total HBcAb to detected HBV infection, blood units are rejected

when HBsAg is negative and total HBcAb is positive (AlZubiery *et al.*, 2017). So, this study aimed to determine the seroprevalence of HBV by investigate HBsAg and total HBcAb among blood donors at National Blood Transfusion Center in Taiz city.

Material and Methods

Study design

A descriptive cross-sectional study was conducted among blood donors to detection surface of HBsAg and total HbcAb.

Sample collection

Data were collected from National Blood Transfusion and Research Center - Taiz branch (NBTRC-TB) during the period from April to December 2021. A total of 2129 blood samples were collected from blood donors attending NBTRC-TB. Blood donor information was registered at the reception department. Five milliliters of blood was drawn from each donor in Vacuum glass tube, during blood donation. Blood samples were separated serum from packed cell volume (PCV) by centrifugation.

Detection of HBsAg and HBcAb

All Blood samples were investigated for HBsAg and total HBcAb by using Cobas E411 method (Elecsys HBsAgII) REF 0468778190, (Elecsys Anti- HBc) REF. according to manufacture protocol.

Statistical analysis

Data of HBsAg and total HBcAb were entered and statistically analyzed by using SPSS program (Version 26). The frequencies and percents were computed for all variables and Chi-Square was used to know the relationship between the variables, while tabulations were carried out by Microsoft Word. P-values less than 0.05 were considered statistically significant.

Results

The present study included blood samples from 2129 donors who attended the National Blood Transfusion and Research Center in Taiz city for blood donation, among of them 2106 (98.9 %) were males and only 23 (1.1 %) were females. Out of 2129 blood samples were screened to detection surface HBsAg and total HBcAb by ECLIA method, it showed that 26

(1.22%) of total blood donors were seropositive to HbsAg and all of them were males; there was no statistically significant deference (P > 0.05). Whereas, the seropositivity of total HbcAb was 10.38%. From total blood donors, the males have a higher seroprevalence rate (10%) of HbcAb than (0.38%) in females, with high significant difference (P<0.001), as shown in table (1).

Table (1). Seroprevalence of surface HBsAg and total HBcAb among blood donors, according to gender.

Gender	No. examined	HBsAg Positive		P- value	Total HBcAb Positive		P-value
		No	%		No	%	
Male	2106	26	1.22	0.861	213	10	0.001*
Female	23	0	0		8	0.38	
Total	2129	26	1.22		221	10.38	

*** Statistically significant (P < 0.001)**

The blood donors in recent study can be divided into two main groups of donations, with more frequency blood donors 1194 (56.1%) were voluntary blood donors and 905 (42.5%) were relative replacement blood donors, but the third group of donations is called therapeutic group which represent only 30 (1.4%) which visit the center to donate blood as a therapeutic blood donation and them blood bags not transfused to patients. Moreover, the seroprevalence rate of HBsAg among voluntary blood donors was 1.51% compared to 0.88% among replacement blood donors; there was no significant difference (P>0.05). Whereas, the difference in the seroprevalence rates of HBcAb among voluntary (10.89%) and replacement donors (8.62%) was statistically high significant (P <0.001), as shown in table (2).

Table (2). Seroprevalence rate of HBsAg and HbcAb among blood donors, according to type of donation.

Type of donation	No. donors	HbsAg					HbcAb				
		Positive		Negative		P-value	Positive		Negative		P-value
		No	%	No	%		No	%	No	%	
Voluntary	1194	18	1.51	1176	98.49	0.496	130	10.89	1064	89.11	0.000*
Replacement	905	8	0.88	897	99.12		78	8.62	827	91.38	
Therapeutic	30	0	0	30	100		13	43.33	17	56.67	
Total	2129	26	1.22	2103	98.78		221	10.38	1908	89.62	

*** Statistically significant (P < 0.001)**

Regarding to age, the blood donors in this study were aged from 18 to >57 years old, and also divided into 5 groups interval (Table 3). The seroprevalence rate of HBsAg were higher to be 42.3% in the age group 28 – 37 years followed by 30.8% in the age group 18 – 27 years and 23.1% in the age group 38 – 47% years. The least seroprevalence rate (3.8%) was found in the age group 48 – 57 years, and no infection in donors aged more than 57 years. There were no significant differences between HBsAg and age groups (P > 0.05), as shown in table (3). Furthermore, the same table also shows the seroprevalence rate of HBcAb in relation to age. The highest rate 32.1% was recorded in the age group 28 – 37 years old, followed by 28.5%, 19.5%, 12,7% and 7.2% in the age groups 38 – 47, 18 – 27, 48 – 57 and more than 57 years, respectively. The association between age groups of donors and HBcAb seropositivity was statistically high significant (P< 0.001).

Table (3). Frequency and seropositivity distribution of HBsAg and HbcAb among blood donors, according to age groups.

Age group (years)	Frequency		HbsAg		P-value	HbcAb		P-value
			Positive			Positive		
	No	%	No	%		No	%	
18 – 27	1004	47.15	8	30.8	0.627	43	19.5	0.000*
28 - 37	662	31.1	11	42.3		71	32.1	
38 – 47	332	15.6	6	23.1		63	28.5	
48 - 57	97	4.56	1	3.8		28	12.7	
> 57	34	1.59	0	0		16	7.2	
Total	2129	100	26	100		221	100	

*** Statistically significant. (P < 0.001)**

On the other hand, the result of this study was showed three groups when investigate HBsAg and total HbcAb markers together, which the first group (HBsAg negative/ HBcAb positive) was 195 (9.16%), while the second group (HBsAg positive/HBcAb positive) was 26 (1.22%) and the third group (HBsAg negative/HBcAb negative) was 1908 (89.62%), the difference in the result was statistically significant (P< 0.001), as shown in table (4).

Table (4). Distribution results of both HBV markers together (HBsAg/HBcAb) among 2129 blood donors tested by ECLIA method.

HBV marker	No. frequency	Percentage	P-value
HBsAg Negative / HBcAb Positive	195	9.16	0.000*
HBsAg Positive / HBc Ab Positive	26	1.22	
HBsAg Negative / HBcAb Negative	1908	89.62	
Total	2129	100	

*** Statistically significant (P < 0.001).**

Discussion

In this study, a total of 2129 blood donors, 2106 (98.9%) were males and 23 (1.1%) were females investigated for HBV (HBsAg & HBcAb). The overall seroprevalence of hepatitis B surface antigen (HBsAg) among the blood donors at NBTR–TB was 26/2129 (1.22%), which was lower than that reported in other previous studies in Yemen such as 7.4% in Sana'a (Al-Nassiri & Raja'a, 2001), 9.8% in Hajjah (Haidar, 2002), 6.7% in Aden & 15% in Sana'a (Sallam *et al.*, 2003), 5.1% in Aden city (Al-Waleedi & Khader, 2012), 2.4% in Hodeida (Saghir *et al.*, 2012), 4.1% in Sana'a (AlZubiery *et al.*, 2017) and recently in Sana'a 2.6% and 2.4% (Al-Nwany *et al.*, 2021; AlZubiery *et al.*, 2022), respectively. The low seroprevalence in this study may be due to successful vaccine program, increased awareness of HBV transmission and safe blood transfusion, geographic deference between studies and the technique used (Al-Waleedi & Khader, 2012).

On the other hand, when comparing the HBV seroprevalence in the present study with some studies conducted in Arabic neighboring countries such as Saudi Arabia, the seroprevalence of HBV in this study was lower than that recorded in Tabuk, Saudi Arabia which the seropositivity of HBsAg was 3% among blood donors (El Beltagy *et al.*, 2008). Like wise, the other studies of HBV seropositive among blood donors in Saudi Arabia which conducted in Jazan region and in Eastern Saudi Arabia that showed high seroprevalence 3.8% and 3.24% respectively (Mohammed, 2013; Alzahrani *et al.*, 2019). Whereas, the highest seroprevalence of HBsAg among blood donors (9.02%) was recorded in AL Madinah AL Munawara (Mohiadeen *et al.*, 2014).

Furthermore, the seroprevalence of HBsAg in this study was showed lower than other studies in deferent countries such as Republic of Djibouti it was 10.4% (Dray *et al.*, 2005), Pakistan 6.2% (Mujeeb & Pearce, 2008), Egypt 1.65% (Khattab *et al.*, 2010), India 3.5% (Lavanya *et al.*, 2012), 1.32% in Rajasthan, India (Jadeja *et al.*, 2014) and Pakistan 1.65% (Abdullah *et al.*, 2019). On the other hand, the lower seroprevalence rates of HBsAg were recorded as 0.56% in Syria (Hamdane & Harfouch, 2021), and 0.7% in central Saudi Arabia (Alshayea *et al.*, 2016) and 0.19% in the Northern Region of Riyadh Province, Saudi Arabia (Alqahtani *et al.*, 2021). Finally, the seroprevalence rate of HBsAg among blood donors in the present study was analogous to the study carried out among blood donors (1.18%) in Apollo Hospitals, New Delhi, India (Makroo *et al.*, 2015).

The seroprevalence of total HBcAb in the current study was 10.38%. This is consistent with the studies carried out in Tamil, India (10.9%) by Lavanya *et al*. (2012), Syria (10.3%) by Muselmani *et al*. (2014), New Delhi, India (9.87%) by Makroo *et al.* (2015) and Sana'a, Yemen (10.8%) by AlZubiery *et al.* (2022). A higher prevalence rate of HBcAb was found in Sana'a (14%), Yemen (AlZubiery *et al.*, 2017), 18.5%, 17.4% that was recorded in Sana'a and Aden, respectively (Sallam *et al.*, 2003), Kuwait (17%) in Kuwaiti national (Ameen *et al.*, 2005), India (18.9%) by Asim *et al.* (2010), Ibadan (16.9%), Iran (Ebenezer *et al*., 2017) and Abuja (17.7%), Nigeria (Ikerionwu, 2017). Furthermore, the highest seroprevalence rate of HBcAb was reported in Kuwait, it was 33.3% in non-Kuwaiti Arab (Ameen *et al.*, 2005), in Nigeria 32.5% (Ogunfemi *et al.*, 2017) and in Cameroon 48.7% (Fopa *et al.*, 2019).

Contrarily, the lower rates of HBcAb were recorded in various locations such as 5.7% in Jazan Region, Saudi Arabia (Abdullah, 2013), 2.3% in Basra, Iraq (Al-Rubaye *et al.*, 2016), 4.9% in Iran (Karimi *et al.*, 2016), 9.0% in Saudi Arabia (Alabdallat & Bin Dukhyil, 2018), 8.2% in Duhok city, Kurdistan region, Iraq (Hussein, 2018), 6.96% in Northeran region of Riyadh province, Saudi Arabia (Alqahtani *et al*., 2021) and 2.0% – 4.1% in Jordan (Souan *et al*., 2021). The variations between the results of this study and other studies might be attributed to difference of geographical location and technique used. In the present study, the males have a higher seroprevalence rate (10%) of HbcAb as compared to (0.38%) in female donors. This difference may be due to unequal number of male and female donors. Another reason for higher seroprevalence in males may also be attributed to males are more proactive in deciding to donate blood.

The present study indicated that 1.51% of the voluntary blood donors were positive for HBsAg. The higher positivity rate of HBsAg among voluntary blood donors was reported in some previous studies, such as 3.0% in Sana'a, Yemen (AlZubiery *et al*., 2022), 2.5% in Saudi Arabia (Abdullah, 2013) and 2.4% in Ethiopia (Habte *et al*., 2016). Also, there was a lower positivity rate of HBsAg (0.88%) among replacement donors in this study than those reported rates of 2.0% in Sana'a (AlZubiery *et al*., 2022), 4.2% in Saudi Arabia (Abdullah, 2013) and 4.1% in Ethiopia (Habte *et al*., 2016). Whereas, the seroprevalence rates of HBcAb among voluntary and replacement blood donors were 10.89% and 8.62% respectively, which are lower than in the

previous study conducted in Sana'a, with 11.6% and 10.2% in voluntary and replacement donors, respectively (AlZubiery *et al*., 2022). The current study revealed that HBsAg and HBcAb were more prevalent among voluntary blood donors compared to replacement donors, which might be due to the greater awareness of HBV among replacement blood donors.

In relation to age, all blood donors were aged 18 to >57 years old, most of them 1004 (47.2 %) were between 18 and 27 years old, which is similar to the figures published by the WHO that reported 45% of donors were aged 25 or less (Teo *et al*., 2011). Furthermore, this study was appeared reverse relationship between age of blood donors and donation rate, while an increasing in blood donors age, the frequency of donation rate was decreased as the following order age groups, (28 to 37) was 31.1%, age group (38 to 47) was 15.6%, age group (48 to 57) was 4.6% and less frequency of donation rate was only 1.6 % among donors aged above 57 years. The difference between age groups for donation was significant ($P < 0.05$), which we interpret this result as the donors aged 18 – 27 years were young adults and good healthy with more hemoglobin concentration whereas the donors aged more than 57 years considered old adults, poor healthy with less hemoglobin concentration (Banafa & Al-Rabeei, 2018). The present study reported a higher seropositivity rate (42.3%) for HbsAg and (32.1%) for HbcAb in the age group 28-37 years of age than younger and older age groups. Additionally, the seropositivity rate observed to be decreased with increasing of age. This finding is in agreement with the results reported among blood donors in Pakistan (Zorob *et al*., 2023). So, this finding may also be explained by the increased awareness encountered with age increasing.

On the other hand, an investigation of both HBsAg and HBcAb together was appeared as three groups, this study focuses on the result of (HBsAg negative/ HBcAb positive) which have the highest rate (9.16%) of seropositivity result. The result of HBsAg negative and HBcAb positive indicate to the blood donors with post HBV infection or chronic infection which the quantity of HBsAg very less and not detectable by ECLIA method. So, blood bag should not transfused to patient because when transfused to patient who was suffering from immunity depression, the virus can be re-replicated and can cause HBV infection (Saghir *et al.*, 2012). Then, this result was showed low rate than result reported by Alzubiery *et al*., 2017 at NBTRC-SB in Yemen which his report appeared 12.1% (AlZubiery *et al.*, 2017).

Whereas, the seropositivity rate in the current study was higher than that recorded in neighboring Saudi Arabia, the seropositivity rate was reported as 5.7% (Abdullah, 2013), 8.8% (Alabdallat & Bin Dukhyil, 2018) and result from a five years was showed that 2.91% of blood donors were positive for HBcAb among the HBsAg-negative (Alzahrani *et al.*, 2019).

On the other hand, the blood donors with HBsAg positive and HBcAb positive represent only 1.22% among all blood donors. Which this result indicates the donors have HBV infection and they should be permanent deferral.

Conclusion

This study is proven the high prevalence of post HBV infection through increase percentage of (HBsAg negative/HBcAb positive). So, investigation of both markers (HBsAg and HBcAb) for all donors before blood transfusion is very important to reduce risk transmitted by blood transfusion. Investigation HBsAg is very obligatory for all donors to exclude HBV infection. Investigation HBcAb alone does not enough to exclude donors HBV infection.

Recommendation

This study recommended for investigation HBV by detect HBsAg and total HbcAb for all blood donors who visit NBTCs and small blood bank in hospitals. Moreover, addition nucleic acid techniques (NAT) by used real time-PCR method which more sensitivity and specificity method to enhance safety and at the same time reduce the blood units rejecting in our country.

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