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Analysis of factors related to ELISA-HBsAg negative and HBV DNA positive blood donors

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ABSTRACT

This report analyzed factors relating to ELISA-HBsAg and Hepatitis B virus (HBV) DNA in blood donors. To provide a reference for an accurate screening model for HBV infection in donated blood, we collected relevant information from 124 blood donors testing ELISA-HBsAg negative and HBV DNA positive in 2017, including ELISA-HBsAg Max s/co, gender, age, residence, education level, blood donation record and alanine aminotransferase(ALT) value. Meanwhile, 99 blood donors with the results ELISA-HBsAg negative and HBV DNA negative were randomly selected as control. Univariate logistic analysis was conducted for possible correlation factors, then multivariate logistic regression analysis was performed for statistically significant observation indicators. The results showed that the *Ct* value of HBV DNA mixed test in the observation group donor was higher than that of HBV DNA single test ($P<0.05$). If one of the two ELISA-HBsAg s/co values was within the range of 0.259 to 0.304, the chance of HBV DNA positive was increased. Univariate logistic regression analysis showed that ELISA-HBsAg Max s/co, gender, age, blood donation history and ALT value were all risk factors in the observation group. Multivariate logistic regression analysis showed that ELISA-HBsAg Max s/co, (OR=5352.448, $P<0.05$), age (OR= 4.527, $P<0.05$) and blood donation history (OR=0.441) were risk factors. The study concluded that ELISA-HBsAg Max s/co, age and number of blood donations are risk factors for ELISA-HBsAg negative and HBV DNA positive blood donors, women or donors under 26 years of age had the lowest risk.

Keywords: nucleic acid technology, ELISA, risk factor, blood donor

INTRODUCTION

Hepatitis B virus (HBV) infection is a serious global problem and threatens a vast amount of people´s health: 18% of all HBV carriers are found in Asian . This study mainly used an enzyme linked immunosorbent assay (ELISA) to detect HBsAg in blood donors´ samples. The existence of HBV infection and the occult HBV infection (OBU) is caused by a "window period." Thus it is possible to screen for concealed HBV infection (OBI) in HBsAg negative HBV DNA positive donors. However HBV DNA is complicated and has certain epidemiological characteristics. In order to understand these characteristics, serology, models of HBsAg negative and HBV DNA positive donors were constructed and analyzed to provide a reference for the screening model of HBV infection in donated blood.

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MATERIALS AND METHODS

Specimen source

A total of 85,422 samples were tested for ELISA-HBsAg and HBV DNA in 2017. From these, 124

ELISA-HBsAg negative samples testing HBV DNA positive,which were included in the observation group, were selected with the following criteria: HBsAg, anti-HCV, HIV Ab/Ag, anti-TP negative and alanine aminotransferase(ALT)<50U/mL. Ninty-nine cases of ELISA-HBsAg negative with HBV DNA testing negative were served as the control group, selected randomly from qualified samples (also in 2017) at an average of 8 to 9 samples per month, which use the same ELISA plate as above. The blood donor´s health examination according to national health examination standard(GB18467–2011).

Three tubes were retained after each sample was collected and transported. A 5 mL EDTA•K_2 vacuum anticoagulant tube was used for ELISA detection. In the second tube, a similarly specified tube with separation adhesive was retained for nucleic acid technology(NAT). In the third tube, 5 mL was retained in a gel-promoting tube for two-year preservation. The sample was placed in a frozen storage tank, at a temperature of 2~10 °C, centrifuged within 4 h and testing within 72 h.

Reagents and instruments

ELISA reagents: HBsAg, anti-HCV, HIV-Ag/Ab and anti-TP reagents were provided by Zhuhailizhu and Beijing Wantai biotechnology co., Ltd., all of which were approved and were used within the validity period. Roche Cobas TaqScreen MPX version 2.0 (MPX v2.0), Roche Cobas s201 (Roche Diagnostics, Switzerland), Micro lab STAR IVD (Hamilton, Switzerland), Sorvall ST 40R Centrifuge (Thermo Fisher Scientific, USA), HBV DNA standard serum (lot NO. 201509001, concentration: 50 U/mL), HCV RNA standard serum (lot NO. 201509001, concentration: 200 U/mL), HIV-1 RNA (lot NO. 201509001, concentration: 1,000 U/mL) for viruses by NAT, were provided by Beijing Control & Standards Biotechnology Co., Ltd. All reagents were used within the validity period. All instruments had been verified or calibrated and were used in normal states. All sample detection operation procedures were performed in accordance with the professional standards set out by our blood center.

Methods of ELISA and NAT and screening models

The samples in our laboratory were firstly tested with two different ELISA reagents, and ABO/RhD groups were identified; the samples which were reactive in ELISA tests were retested on double samples from the original tube and blood-sampling bag, if they were reactive in retests, NAT was not performed. All samples with non-reactive in ELISA, were NAT tested with the Roche Cobas s201.6 system and mixed into one pool. If the mixed pools were reactive, all mixed samples were separated for a NAT single test; if the single test was reactive, the sample was deemed as reactive, and if the single test was negative, the sample was deemed as negative; if the pool was negative, the final results of all 6 mixed samples were all accepted as negative. The donor´s information (including: ELISA-HBsAg Max s/co, gender, age, level of education, native place, marriage, blood donors, ALT) was obtained from all blood donation files with 124 cases (positive observation group) and 99 cases (negative control group). ELISA-HBsAg Max s/co represents the greater value of s/co after detection of two ELISA reagents.

Statistical analysis

SPSS 18.0 was used for statistical analysis. The χ^2 test was used to determine the related various factors in observation group. Firstly, basic univariate logistic regression analysis was carried out, and then the multivariate logistic regression analysis was performed for the statistically significant factors. On the basis of single factor analysis, the risk factor with significant difference was the independent variable (Xi), and multivariate logistic regression analysis was conducted. $P<0.05$ was set of standards statistical significance.

RESULTS

Comparison of positive rate of NAT in single test and mixed test

In 2017, a total of 85,422 blood donation samples tested with ELISA and NAT. And 124 (0.145%) cases were found ELISA-HBsAg negative combined with HBV DNA positive (with the HBV infection), including 100 males (0.12%)and 24 females(0.03%). The observation group´s *Ct* value which tested positive in the NAT mixed test, was significantly higher than that obtained by the NAT single test ($t=673.77$, $P<0.01$).

Single factor correlation analysis on ELISA-HBs Ag negative and HBV DNA positive samples

In the observation group, positive related factors such as ELISA-HBsAg Max s/co were obviously higher than those of the control group, when analyzed by the χ^2 test and *t* test ($P<0.05$). If one of the two ELISA reagents obtained as/co value within the range of 0.259 to 0.304, the chances of HBV DNA positive result were higher. The results of male donors´observation group were significantly higher

than the female (P<0.05).The rate of observation was significantly higher in individuals > 26 years/old than in those ≤ 26 years/old (P<0.05); ELISA-HBsAg negative and HBV DNA positive rate of the first group donors were higher than repeated groups (P<0.05); ALT (taken by chemistry rate method) was higher in the observation group than control group (P<0.05,***Table 1***).

Table 1 **Single factor correlation analysis on ELISA-HBsAg negative and HBV DNA positive**

Item	Indicators	Observation group(n=124)	Control group(n=99)	Statistic value	P value
ELISA–HbsAg Max s/co		0.224±0.080	0.154±0.105	5.649	<0.05
Gender	Male	100	61	9.93	<0.01
	female	24	38		
Age	>26 years old	116	81	7.35	<0.01
	≤ 26 years old	8	18		
Level of education	High school and below	99	68	3.64	>0.05
	Above University	25	31		
Native place	Guangdong	39	41	2.37	>0.05
	Non-Guangdong	85	58		
Marriage	married	98	70	2.05	>0.05
	unmarried	26	29		
Blood donors	First donor	80	49	5.09	<0.05
	Repeat donors	44	50		
ALT(U/ml)		24.35±9.84	21.13±10.90	2.31	<0.05

Univariate and multivariate logistic regression analysis of ELISA-HBsAg negative and HBV DNA positive donors

Univariate logistic regression analysis showed that ELISA-HBsAg Max s/co, age and number of blood donations were risk factors for the observation group (***Table 2***). Multivariate logistic regression was used to analyze the risk factors for ELISA-HBsAg Max s/co, age and donation history (donation times have already given) in the observation group, from which the logistic regression equation was obtained: Logistic (P)= −2.231+ 8.585X1+1.510 X3−0.821 X7. The OR values from large to small are ELISA-HBsAg Max s/co (X1)> age (X3)> donation times (X7), indicating that ELISA-HBsAg Max s/co has higher risk indications than other factors (***Table 3***).

Table 2 **Univariate logistic regression analysis of ELISA-HBsAg negative and HBV DNA positive donors**

Indicators	B	Std. Error	Wald	P value	Exp(B)
X1 (ELISA Max s/co)	8.580	1.784	23.145	<0.001	<0.001
X2 (Gender)	−0.684	0.373	3.357	0.067	0.505
X3 (Age)	−1.325	0.575	5.299	0.021	0.266
X4 (Level of education)	−0.160	0.379	0.178	0.673	0.852
X5 (Native place)	0.004	0.346	0.000	0.990	1.004
X6 (Marriage)	0.142	0.419	0.115	0.735	1.152
X7 (Blood donors)	0.822	0.315	6.796	0.009	2.275
X8 (ALT)	0.016	0.016	1.005	0.316	1.016

B: regression coefficient; SE: standard error; Walds: χ^2 value; OR: advantage ratio

Table 3 **Multivariate logistic regression analysis of ELISA-HBsAg negative and HBV DNA positive donors**

Indicators	B	SE.	Wald	df	P value	OR
X1 (ELISA Max s/co)	8.585	1.771	23.500	1	<0.001	5,352.448
X3 (age)	1.510	0.495	9.308	1	0.002	4.527
X7 (Blood donors)	−0.821	0.306	7.185	1	0.007	0.440
(Constant)	−2.231	0.751	8.825	1	0.003	0.107

B: regression coefficient; SE: standard error; Walds: χ^2 value; OR: advantage ratio

DISCUSSION

The screening of blood donors for infectious factors is a mandatory requirement set out by Chinese technical operational guidelines for blood stations (2015 edition). The results of this screening can be divided into: ELISA-HBsAg negative and HBV DNA negative, twice ELISA-HBsAg positive, ELISA-HBsAg positive and HBV DNA positive, and ELISA-HBsAg negative and HBV DNA positive. Wang *et al*. [1] reported 144 HBV DNA positive samples detected in 259,329 ELISA-HBsAg negative samples at a positive rate of 0.056%, the majority of them with OBI. While

the HBV infection may be caused by OBI blood transfusion[2], research also shows that HBV infection is the most important factor leading to HBV spread and seeding risk in donors. A study by Zhou *et al.* [3] speculated that the risk of HBV infection after blood transfusion caused by OBI can reach 9.16 times higher than that of the "window stage" infection. It was reported that twice ELISA-HBsAg was negative, and the positive rate of HBV DNA was 0.072% [4]. Therefore it is very necessary for ELISA negative results to be confirmed by NAT again. Only in this way can the incidence of hemostasis infection be minimized.

There were many reports on the characteristics of ELISA-HBsAg positive blood donors in the past, but there were few reports on the characteristics of ELISA-HBsAg negative and HBV DNA positive donors. Although it is believed the probability of positive blood donors is extremely low,with the development of HBV DNA, more and more people have been found to be ELISA-HBsAg negative and HBV DNA positive, which researchers are subsequently investigated. In this study, the results show that there is a significant difference in rates of ELISA-HBsAg negative and HBV DNA positive between males and females and age (above or below 26). Male donors had a positive rate (0.12%) which was significantly higher than females (0.03%), which are both in accord with a Chinese report by Ou *et al.* [5] and Murokawa et al. [6] in Japan. This may increase opportunities for males to engage in social intercourse more than females. At the same time, we also found that rates of ELISA-HBsAg negative and HBV DNA positive were significantly higher than those over 26 as opposed to under. This may be due to efforts in China, since 1992, infant children to inoculate against hepatitis B, with blood donors aged less than 26 being the beneficiaries. In areas with high HBV prevalence, such as China and Japan, studies suggest that HBV DNA testing should be carried out, particularly for HBsAg negative donors older than 26. Li *et al*.[7] also reported that the HBV DNA positive rate of male donors in Zheng Zhou district was higher than that of female donors, and the HBV DNA positive rate was the highest among the 36~45 age group. This is consistent with the results of this study.

The recruitment of blood donors from low-risk populations is the key to ensuring safe blood. Therefore, the world health organization(WHO) advocates volunteers, fixed and repeated blood donors to donate regularly throughout the world[8]. Zhang *et al.* [9] found that the unqualified rate of ELISA and NAT in primary donors was higher than that of repeat donors. In this study, single factor and multiple factor analysis showed that the occurrence rate in the observation group in repeat blood donors was lower than that of first time donors.

In the single factor χ^2 test, the gender factor had statistical significance, but in the single factor logistic analysis, the gender factor showed no statistical difference. This may have been that other factors neutralized the gender factor, due to the differences of statistical methods´ efficiency.

In conclusion, this test found that female donors, aged from l8~25, and repeated donors had low rate of ELISA-HBsAg negative and HBV DNA positive. So it is better to recruit more female, young and repeated donors, to take measures to encourage more donors repeated.

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