Comparative Analysis of Hepatitis B Virus Infections in Blood Donors Born Before and After the Implementation of Universal HBV Vaccination in Southern China

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Comparative analysis of hepatitis B virus infections in blood donors born before and after the implementation of universal HBV vaccination in Southern China

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ABSTRACT

Background: In China, the vaccinated blood donors have rapidly increased by recent years, which may impact blood safety. The true prevalence of HBV between vaccinated blood donors and non-vaccinated blood donors should be explored.

Study design and methods: The samples of blood donors were collected and detected for serologic markers of HBV in the Shenzhen blood center(SZBC) between Feb 2016 and Jun 2016. The discrepant results were tested with commercial electrochemiluminescence immunoassay (ELCI), alternative MPX ID NAT, nested PCR, and a quantitative real-time polymerase chain reaction (qPCR) assay. HBsAg and anti-HBs were quantified. The serological and molecular characteristics of HBV infected blood donors were analyzed, and the effects on blood safety for donors born before and after the implementation of universal HBV vaccination were compared.

Results: Total of 242 reactive by NAT and/or HBsAg ELISA samples from 26318 candidate donors, 192 (0.73%, [95%CI, 0.63-0.84]) HBV+, 131 (0.49%, [95%CI, 0.43-0.59]) HBsAg+, 58 (0.22%, [95%CI,0.17-0.28]) occult hepatitis B infections (OBI) were confirmed respectively. The HBV+ rate in vaccinated donors is lower than in non-vaccinated donors ($P<0.05$). The HBsAg titers of vaccinated infected blood donors are much higher than non vaccinated infected blood donors. The OBI yield rates in the vaccinated blood donors were 0.11% (7/6422), and significantly lower than the non-vaccinated blood donors (0.26% , 51/19898, $P<0.05$). There 102/124 (82.3%) samples were genotype B, 22/124 (17.7%) were genotype C respectively. There was no significant difference in the distribution of genotype between non-vaccinated blood donors (B/C,86/17) and vaccinated blood donors (B/C,23/6) ($P>0.05$). High frequency of vaccine escape mutations M133L (32.4%) and E164G in S region of genotype B strains and substitution L175S (40.9%) related to vaccine escape in S region of genotype C strains were identified.

Conclusion: The universal HBV vaccination program markedly reduces the risk of HBV infection in blood donors, and provides a significant guarantee for the safety of blood transfusion. Several important mutations detected related vaccine escape and notable mutations needed further investigated.

Keywords: Hepatitis B virus; The universal HBV vaccination program; Blood donors; Mutations

Background

HBV infection continues to be a major public health concern worldwide despite the availability of an effective vaccine and potent antiviral treatments [1]. One-third of the world's population is predicted to have been infected by HBV[2]. The WHO estimates that 257 million people are currently living with a HBV infection and approximately 885 thousand death were cause by HBV-associated complications in 2015[3]. In China, nearly 50% of the Chinese population has a history of HBV infection[4].

To curb hepatitis B epidemic, the universal HBV vaccination has been implemented by Chinese government from 1992 and resulted in HBsAg carrier reduction significant from 10% to <1% in children group over two decades [5]. These vaccinated populations have enrolled in blood donors since 2010. HBV vaccinees were gradually becoming the majority of blood donors in China and impacting blood safety positively. In our previous studies for exploring the true HBV infection in blood donors, 80 were classified as occult HBV infection (OBI) from 307,740 sero-negative blood samples[6], and 121/259(46.7%) non-discriminated reactive samples of 123,280 donations were identified as OBIs, and a number of important mutations were detected additionally[7], Furthermore, 71/159(44.7%) HBsAg ELISA-positive and NAT-negative samples of 101,025 blood donations were confirmed as HBsAg positive[8]. These measurements could interdicted more OBIs with extremely lower viral loads and chronic hepatitis B infections with low level HBsAg in Chinese blood donors, thus reducing the risk of HBV infection after transfusion[9].

However, the previous report suggested that HBV vaccine made of genotype A2 recombinant protein might not be fully efficient when used in other genotype

prevalent areas. Low anti-HBs level induced by vaccination protects against hepatitis B diseases and chronic infection, but favor occult infection [10]. To test this hypothesis, a large group of Chinese blood donors, including presumed vaccinated or non vaccinated recruited by SZBC, were tested and identified for HBV serologic and molecular markers. This was conducted to investigate the recent true epidemiology of HBV infection and the effectiveness of the vaccination program for the prevention of the data of HBsAg+ and/or HBV DNA+ blood donors after individual-donation. This study was intended to examine the effectiveness of the vaccination program and explore the potential threat of vaccination failures to the blood supply.

Material and methods

Ethics statement

This study was approved by the Ethics Committee of the Institute of Blood Transfusion, Chinese Academy of Medical Sciences and Peking Union Medical College. Written informed consent was obtained from each study participant.All methods were carried out in accordance with relevant guidelines and regulations.

Subjects and samples

A total of 26318 blood donors were enrolled in this study from Jan 2016 to Jun 2016.. The presumed vaccinated and non-vaccinated blood donor populations were separated into two groups, according to being born before or after Jan 1, 1992. Blood donors born after Jan 1, 1992, were designated as vaccinated donors. Blood donors born before Jan 1, 1992, were selected as non-vaccinated donors. There 6421 (24.4%) donors for vaccinated groups and 19897 (75.6%) donors for the non-vaccinated groups were available in this study. The pre-donation questionnaire, rapid pre-donation testing and dual ELISA assays for routine screening are described as previous study[7]. Blood samples with HBsAg reactivity were re-tested in duplicate using at least one ELISA assay. Samples were determined to be HBsAg ELISA+ if results were reactive in any assay in re-testing . The donors were defined as first-time blood donors who gave blood for the first time, while donors were defined as repeat-donors who donated blood more than once at the SZBC. Multiple Procleix ultrio plus analysis (Grifols diagnostic solutions, Inc. and hogic) was used to detect

HBV, HCV and HIV-1 genomes in all donors. Individual reactive samples were further tested with a discriminatory Procleix Ultrio plus test to identify the virus responsible for NAT reactivity (HBV, HCV, or HIV-1) as the manufacturer recommends. If necessary, MPX2.0 ID NAT was used as an alternative NAT assay for further identification. Serum and aliquots of the index-retrieved frozen plasma unit for HBsAg ELISA+ and/ or NAT initial reactive were collected for additional determination.

Supplemental serological testing, HBsAg quantitation, and HBsAg+ confirmation

For all HBsAg ELISA+ and/ or NAT initial reactive samples, HBsAg (LOD: 0.05 IU/mL) and anti-HBs (LOD: 2 IU/L) were quantified, hepatitis B e antigen (HBeAg), anti-HBe, and hepatitis B core antibodies (anti-HBc) of were tested by commercially available ECLI (Roche, USA). Any sample testing repeats reactive/ positive by supplemental HBsAg assays with HBV DNA+ was considered HBsAg confirmed positive (HBsAg+).

HBV DNA confirmation

200ul-2500ul HBsAg ELISA + and / or NAT initial reactive samples were extracted for HBV DNA by HighPure Viral Nucleic Acid Large Volume Kits(Roche Diagnostics Gmbh, Mannheim, Germany). The basic core promoter / pre-core (BCP / PC) and S region were amplified by qPCR and nested PCR for further detection[7,8]. As mentioned above, the samples reacted by any two of the five NAT methods were confirmed to be DNA positive. Donations that tested HBsAg+ and HBV DNA+ with anti-HBc were designed as chronic hepatitis infections (CHB), donations that tested HBsAg- and HBV DNA+ with anti-HBc and/ or anti-HBs were OBIs, Donations that tested HBsAg- and HBV DNA+ without any seromarkers were WPs[6].

HBV DNA sequencing, genotyping and comparison

To confirm HBV genotypes, the amplified PCR products obtained from the BCP/PC (295 bp) and the S regions (495 bp) were sent to Shanghai Invitrogen Co., Ltd. (Guangzhou, China) for sequencing. HBV genotype determination was performed by phylogenetic analysis using the MEGA7.0 program. The neighbor-joining method based on Kimura 2-parameter mode and complete deletion for gaps with 1,000 bootstrap replications was used[11]. Bootstrap values of 70% or greater were considered significant. Amino acid sequences isolated from HBsAg+ blood donors from China, Thailand and Malaysia were used as reference sequences[12].

Statistical analyses

The 95% confidence interval (95% confidence interval) of the observed rate was calculated by binomial exact proportion method. Fisher's exact test was used to compare categorical variables. For continuous variables, non parametric Mann Whitney test was used, with P value < 0.05 as the cut-off value of significance.

Results

Demographic Characteristics and Screening results of blood donors samples

There 6421 vaccinated and 19897 non-vaccinated blood donors were enrolled in this study, the demographic characteristics of vaccinated and non-vaccinated donors were shown in Table 1. After screening by dual ELISAs and NAT, there 154 (0.59%) samples were HBsAg ELISA positive including 113 (0.43%) for HBsAg ELISA+/NAT initial reactive (IR), 88 samples were NAT IR/HBsAg ELISA-. Of these 88 samples, there 34 were positive for discriminatory HBV test (dHBV), and 54 were multiplex NAT reactive, but negative for discriminatory HBV/HCV/HIV test (non-repeat reactive, NRR)(Fig.1).

Confirmatory results of HBsAg ELISA+ samples and HBV DNA NAT IR samples

Of 154 HBsAg ELISA positive samples, there 129 (83.8%) cases were confirmed HBsAg+ by ECLI and DNA+ by 4 alternative NATs, including 113/113 (100%) HBsAg ELISA+/NAT IR samples and 18/41 (43.9%) for HBsAg ELISA+/NAT- samples. In 34 HBsAg ELISA-/dHBV+ samples tested by nested PCRs, qPCR and MPX ID NAT, 31 were confirmed HBV DNA+, and 54 HBsAg ELISA-/NAT NRR samples, 31(57.4%) were confirmed DNA+ too. In total, 192 (0.74%) confirmed HBV+ (Table 2).

Statistical result of demographic characteristics and testing results

Successful HBV S gene sequencing followed by genotyping was performed for 124 of the 192 samples, which composed the vaccinated group and non-vaccinated group. The results showed statistically significant differences ($P < 0.05$) in sex, number of first time and repeated time donors, occupation distribution, education distribution, the rate of HBV+, mean HBsAg titers, Anti-HBc+/Anti-HBs+ pattern, and OBI yield rate, the distribution of anti-HBs titers between the two groups (Table 1, Table2); however, no significant differences in the distribution of HBV genotype, and the median of anti-HBs titers between the two groups were found ($P > 0.05$).

Prevalence of HBV, HBsAg, and OBI between blood donors born before and after the universal infant vaccination program

192/26318 (0.73%, [95%CI, 0.61-0.82]) HBV+, 131/26318 (0.50%, [95%CI, 0.41-0.58]) HBsAg+, 58/26318 (0.22%, [95%CI, 0.16-0.28]) OBI were detected and verified in the eligible blood donors population respectively. Of 192 HBV+ blood donors, 36 were vaccinated donors, and 154 were non-vaccinated donors, The rate of HBV+ in the vaccinated donors is lower than in the non-vaccinated donors ($P<0.05$). Of 131 HBsAg confirmed positive donors, 28 (0.44%) were vaccinated donors, and 103 (0.52%) were non-vaccinated donors. Of 58 cases OBIs, 7 (0.11%) were vaccinated donors, and 50 (0.26%) were non-vaccinated donors. There is a significant difference between the two groups ($P<0.05$).

S gene sequencing and phylogenetic analysis

Two hundred forty-two samples were amplified by nested PCR, 124 cases were got s sequences. In the non-vaccinated group, 95 were got s sequences, 79 (83.1%) were genotype B, 16 (16.9%) were genotype C. And in the vaccinated group, 29 samples were got s sequences, 23 (79.3%) were genotype B, 6 (19.7%) were genotype C respectively. There is no difference between the two groups for genotype distribution (P=0.790>0.05).

Mutation analysis within or out of MHR in identified HBV+ donations

S sequences including the major hydrophilic region (MHR) from 124 samples (non-vaccinated group: 79 genotype B and 16 genotype C strains; vaccinated group: 23 genotype B and 6 genotype C strains) were analyzed. Compared with reference amino acids, the occurrence of the donations' amino acid substitutions at each position observed with significant difference (P<0.05) were determined as notable mutations [7] (Table 3). For the amino acid sequence encoded by the S gene from 65 CHBs genotype B samples in non-vaccinated group, N40S/F (40%), I68T/M (6.2%), Q101R/H/K (6.2%), M133L/T/S (27.7%) and F134I/l/S (7.8%) were calculated as notable mutations, and 224 amino-acid substitutions were presented in this group. However, in vaccinated group (CHB), G44D/E (38.1%), M133L (52.4%) and E164G (14.3%) were notable mutations, the frequency of M133L (47.6%) mutation were higher than in non-vaccinated group (CHB, 16.9%, P<0.05). In non-vaccinated OBI group for genotype B, only G44D/E (42.9%) and M133L (64.3%) were observed as notable mutations. However, in vaccinated OBI group, all were found K24R, G44D and M133L mutations. Meanwhile, in genotype C donations, Q30K, S34L, N40S, A45T, T47V, P49H, F55S, I68T, P79H, L175S, V177A were notable mutations in non-vaccinated CHB group. T118K, L175S notable mutations were found in vaccinated CHB group. While 11 OBIs (non-vaccinated group), Q30K, N40S, A45T, T47V/K, P49H/L, S55F, R59H, T113P/S/N, T118K/R, S143T/L, R160K/N, L175S and V177A were determined as notable mutations. G145R, G145A, G145E were observed in 1 genotype C vaccinated CHB donation, and 1 non-vaccinated CHB donation respectively.

Discussion

China is endemic for hepatitis B, and the residual risk of transfusion transmission is significantly higher for HBV than for HIV-1 and HCV. HBV NAT has been preliminarily introduced in some major blood centers in China since 2003, and the HBV NAT yield ranged between 1:1000 to 1:10,000 [22]. In China, neonatal vaccination resulted in a decrease of HBV incidence [5]. However, vaccination may also favor the development of escape mutants and neutralizing anti-HBs antibodies level decrease over time in vaccinated people who may become susceptible to HBV infection [23]. In our study, algorithms of confirmatory testing and supplemental testings are adopted to avoid false-positive results and identified the low-virus-load donations utlmostly as many investigations [22]. Confirmed positivity was based on multiple assay reactivity or sequences generated as accurate as possible. These measurements would give a better comparison between vaccinated blood donors and non-vaccinated blood donors for the prevalence of HBV, HBsAg, and OBI. In this study, 242 reactive by NAT and/or HBsAg ELISA samples from 26318 candidate blood donors were investigated, 23/154 (14.9%), and 27/88 (30.7%) potential false positive in both serology and NAT were identified respectively. In total, of 192 confirmed HBV infected blood donors, 61 (31.3%) cases were HBsAg-/DNA+, and 131 (60.1%) were HBsAg+/DNA+ due to adoption of large volume DNA extraction assay. Therefore, the confirmed HBsAg positive rate is 0.50%, which coincides with the report [24]. Of 54 NRR samples, 30 donations were clarified HBsAg-/DNA+, a higher NAT yield rate was confirmed compared with the multi-regional study [25] and Hongkong study [26].

The true interdiction HBV DNA positive rate by Ultrio Plus ID-NAT screening in combination with HBsAg was 0.73%, higher than true HBsAg positive rate(0.50%, P=0.001), and the true OBI yield was 1:453, nearly had two-fold increase compared with a previous study in Shenzhen [6] due to application of ID NAT. For The Ultrio Plus assay has used a target enhancer reagent, which helps to disrupt viral particles and exposes more single-stranded DNA for the capture probe. This assay modification increased the proportion of OBI yield at least more twofold than the Ultrio assay [26].

Moreover, in the NAT NRR donations, 31.5% were got sequences, and 38% were qPCR positive due to shorter length of the primer, overall, 55% were identified HBV DNA positive in which 100% were low-virus-load OBI, gained another half of OBI cases. In our study with those reported in other regions, the differences of the rates varied considerably depending on HBV epidemiology, the proportion of repeat or the first time donors, NAT sensitivity, and pooling strategy used; for example, 1:624 in Xiamen China [27], 1:3471 in Hongkong in China [26], 1:894 in Taiwan in China [28], 1:4232 in Thailand [29] and 1:770,000 in Germany [30].

During the five months of study, HBV prevalence in vaccinated donors aged 18–24y was lower than in non-vaccinated donors aged 25-60 ($P<0.05$). OBI yield rates were also confirmed lower than in non-vaccinated donors ($P<0.05$). This difference might be related to the increasing cumulative HBV exposure with the ages, and vaccine-related protection would be the definite cause. The OBIs with anti-HBs in present study suggested that OBIs occur primarily in individuals who have recovered from the infection but are unable to develop an effective immune control [31]. Furthermore, among OBI samples, the percentage of those carrying anti-HBs in vaccinated OBI blood donors is lower than the non vaccinated. OBI blood donors ($P<0.05$), suggested that lower level or less of anti-HBs were insufficiently protected and are susceptible to infection associated with breakthrough or occult HBV infections, even when vaccinated at birth. Interestingly, in HBsAg positive vaccinated blood donors, the titers are much higher than non vaccinated blood donors ($P<0.005$), because the infected vaccinated blood donors are no response or warning off vaccinees, HBsAg is secreted more than in usual after infection.

HBV with HBsAg escape mutants are rare but potentially highly infectious and pathogenic, particularly in immune-compromised recipients [32]. The prevalence of the well-known neutralization escape mutation G145R in the HBV envelope protein was as high as 22% in American blood donors [33]. However, in our study, the G145R mutation was not found in genotype B; only three cases harbored G145A/E/R individually were found in genotype C donations. Furthermore, compared to G145R

mutation, several mutations within and out of MHR were detected at too high a prevalence such as M134L and L175S. Surprisingly, the isolates from the CHBs non-vaccinated genotype B group also showed high variability in their S gene sequences in comparison with CHBs vaccinated genotype B group. However, the frequency of M133L (52.4% vs. 16.9%) and E164G (14.3 vs. 3.1%) associated with escape from vaccine-induced immunity was observed higher significantly in CHBs vaccinated group than in the CHBs non-vaccinated group ($P<0.05$). This is because antibodies induced by the current vaccine may not recognize changes in the surface antigen as a result of mutation. In the genotype C non-vaccinated group, lots of mutations out of MHR such as Q34K, N40S, T47V, P49H, S55F, L175S, V177A were detected. These mutations are associated with major histocompatibility complex (MHC) class I-restricted cytotoxic CD8+ lymphocytes (CTLs) epitopes, and it has been experimentally proved that adaptive immune response mediated by CTLs is necessary for controlling HBV infection. This may be because mutations in CTL epitopes can evade cellular immunity and contribute to persistence, and are potentially responsible for vaccine breakthrough infection and HBsAg undetectability [34]. Interestingly, two membrane-embedded C-terminus mutations L175S, V177A were observed at high frequency in genotype C donors in the present study and proved tightly to correlate with OBI, and powerfully to affect HBsAg detection [19].

Conclusion

The prevalence of HBV+ and OBI in vaccinated donors is lower than in non-vaccinated donors ($P<0.05$), suggested that The universal HBV vaccination program markedly reduces the risk of HBV infection in blood donors. Furthermore, there is a high frequency of mutations in the MHR and out of MHR of the HBV S gene, which may cause vaccine escape, diagnosis failure, and failure in HBIg therapy problem and highlights the need for more studies into the prevalence of mutants.

Abbreviations

HBV = Hepatitis B virus; OBI=occult hepatitis B infection; CHB=chronic hepatitis B; NAT=nucleic acid testing; ELISA(s)=enzyme-linked immuno-absorbent assay(s); ECLI= electrochemiluminescence immunoassay; ID = individual donation; LOD = limit of detection; S/CO =sample to cutoff; TMA=transcription-mediated amplification; IR =initial reactive; NRR=non repeat reactive; BCP/PC=basic core promoter/pre-core; MHR=major hydrophilic region; MHC=major histocompatibility complex; HBig=hepatitis B immunoglobulin; CTL=cytotoxic CD8+ lymphocytes.

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Authors' contributions

XY designed the experiments and wrote and reviewed the manuscript. LL reviewed, revised, and edited the manuscript. TL, YL, JZ, RL, XX, XG participated in the study design, performed the experiments, and collected and analyzed the data. All authors read and approved the final manuscript.

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Availability of data and materials

The data sets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Ethics approval and consent to participate

This study was approved by the Ethics Committee of the Institute of Blood

Transfusion, of the Chinese Academy of Medical Sciences & Peking Union Medical College. Written informed consent was obtained from each study participant before the interview, sample collection and testing. All methods were carried out in accordance with relevant guidelines and regulations.

Consent for publication

Not applicable.

Competing interest

The authors declare that they have no competing interests.

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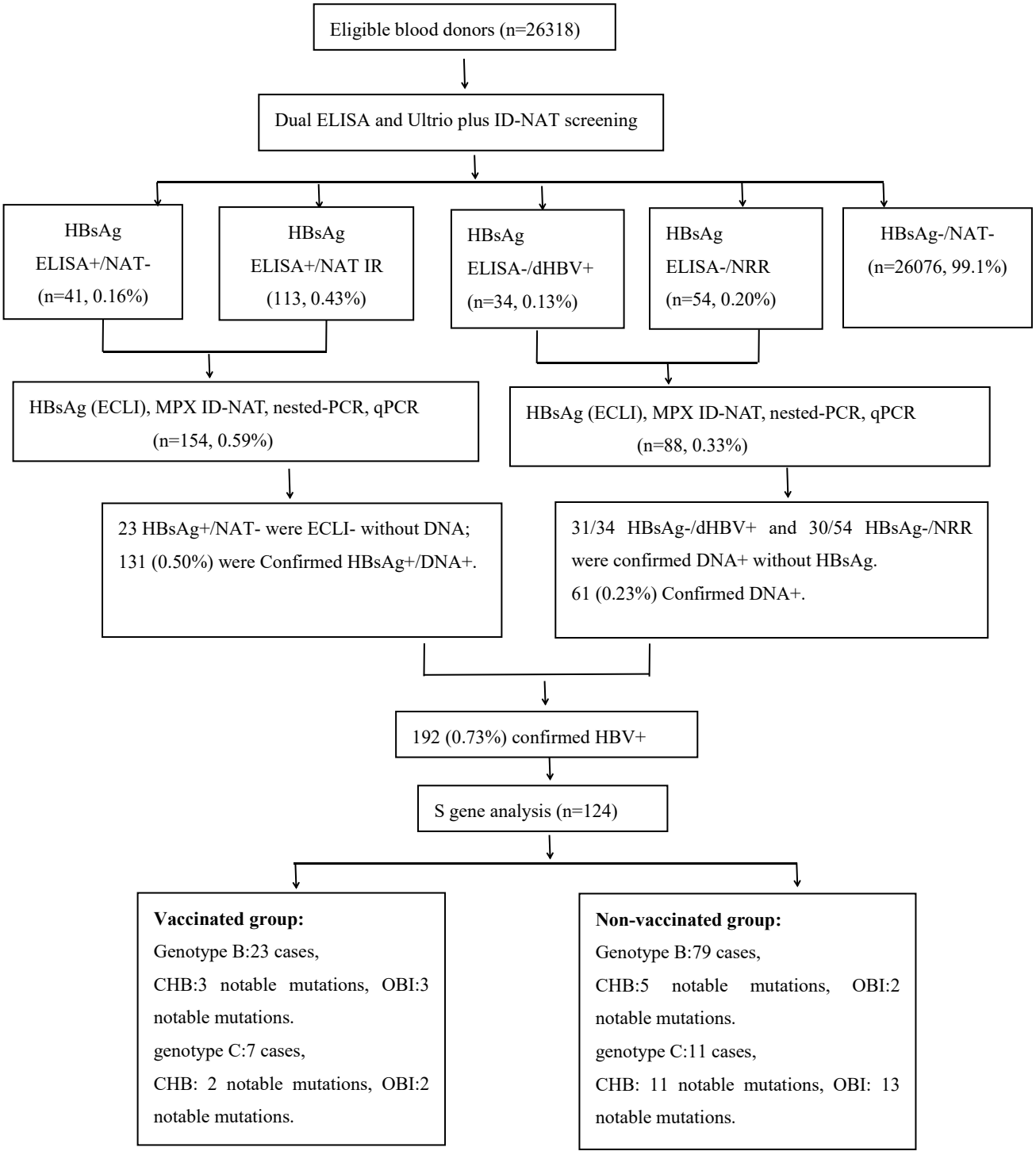

Figure 1. Flowchart for confirmatory testing algorithm of HBsAg ELISA+ and /or NAT IR samples

Table 1. Demographic Characteristics of Vaccinated and Non-vaccinated Donors from Jan. 2016 to Jul. 2016

	Over all Population (%)	vaccinated group (%)	non-vaccinated group (%)	P
Donors Status				
First-time	14738 (56.0)	4810 (74.9)	9929 (49.9)	0.00
Repeat	11580 (44.0)	1611 (25.1)	9968 (50.1)	
Gender				
Male	16975 (64.5)	3501 (54.5)	13475 (67.7)	0.00
Female	9343 (35.5)	2920 (45.5)	6422 (32.3)	
Occuption				
Farmers	482 (18.2)	28 (0.4)	454 (2.3)	
Workers	4173 (15.8)	472 (7.3)	3701 (18.6)	
Students	1778 (6.7)	1653 (25.7)	125 (0.6)	
Soldiers	150 (0.5)	76 (1.2)	74 (0.4)	0.00
Teachers	287 (1.1)	79 (1.2)	208 (1.0)	
Civil servants	255 (1.0)	26 (0.4)	229 (1.1)	
Doctors	369 (1.3)	101 (1.6)	268 (1.3)	
Staff	9920 (37.7)	1797 (28.0)	8123 (40.8)	
Others	8904 (33.8)	2189 (34.1)	6715 (33.7)	
Education				
Below High School	5405 (20.5)	1290 (20.1)	4115 (20.7)	
High School and Assodate Degree	15565 (59.1)	3970 (61.8)	11595 (58.2)	
Bachelor’s Degree	4852 (18.4)	1131 (17.6)	3721 (18.7)	0.00
Master’s Degree	338 (1.3)	22 (0.3)	316 (1.5)	
Others	158 (0.6)	8 (0.1)	150 (0.8)	
Total	26318 (100)	6421 (24.4)	19897 (75.6)	

Table 2. Classification of 192 infected blood donors between vaccinated and non-vaccinated groups

	Over all population	vaccinated group	non-vaccinated group	P
HBV + (%)	192 (0.73)	36 (0.56)	156 (0.78)	0.040
HBsAg+/DNA+ (%)	131 (0.50)	28 (0.44)	103 ((0.52)	0.229
HBsAg titers Median (IU/ml)	82.5	128.8	58.4	
Min.	0.06	0.06	0.06	0.002
Max.	919	919	650.2	
HBsAg-/DNA+	61*(0.22)	8 (0.12)	53 (0.26)	0.031
Anti-HBc-/Anti-HBs+ (%)	3 (0.011)	0 (0.00)	3 (0.015)	0.432
Anti-HBc+/Anti-HBs- (%)	25 (0.095)	4 (0.052)	21 (0.11)	0.234
Anti-HBc+/Anti-HBs+ (%)	30 (0.11)	3 (0.048)	27 (0.14)	0.044
Anti-HBc-/Anti-HBs- (%)	3 (0.011)	1 (0.016)	2 (0.010)	0.568
OBIs	58 (0.22)	7 (0.11)	51 (0.26)	0.04
WPs	3 (0.11)	1 (0.16)	2 (0.10)	0.568
Anti-HBs titer				
<10IU/L (negative)	152	31	121	
10-100IU/L	27	5	22	
>100IU/L	13	0	13	
Median (IU/L)	<2	<2	<2	0.205
Min.	<2	<2	<2	
Max.	>1000	91.7	>1000	
Genotype B (%)	102 (0.38)	23 (0.36)	79 (0.40)	0.254
Genotype C (%)	22 (0.099)	6 (0.093)	16 (0.075)	0.501

* 2 WP and 57 OBI.HBsAg <0.05IU/ml:Negative. Anti-HBs <10IU/L:Negative

Table 3. Notable mutations in envelope proteins of HBV DNA+ donations

Genotype	Infections	Group	Mutation	Frequency (%)	Vaccine escape mutant	Affect Serological diagnosis	Failure in HBIg Therapy	References
B	CHB	Non-vaccinated	N40S/F	(25+1)/65(40)	This study	This study	This study	
			I68T/M	(2+2)/65(6.2)	This study	This study	This study	
			Q101R/H/K	(2+1+1)/65(6.2)	This study	Yes (Q101K)	This study	[13]
			M133L/T/S	(11+5+2)/65(27.7)	Yes (M133L,M133T)	Yes (M133L),M133T)	Yes (M133L)	[14,15]
			F134I/L/S	(3+1+1)/65(7.8)	This study	Yes (F134L)	This study	[15]
		vaccinated	G44D/E	(8+1)/21(38.1)	This study	This study	This study	
			M133L	11/21(52.4)	Yes (M133L,M133T)	Yes (M133L),M133T)	Yes (M133L)	[14,15]
			E164G	3/21(14.3)	Yes(E164G)	Yes(E164G)	This study	[16]
	OBI	Non-vaccinated	G44D/E	(5+1)/14(42.9)	This study	This study	This study	
			M133L	9/14(64.3)	Yes (M133L,M133T)	Yes (M133L),M133T)	Yes (M133L)	[14,15]
		vaccinated	K24R	2/2(100)	This study	This study	This study	
			G44D	2/2(100)	This study	This study	This study	
			M133L	2/2(100)	Yes (M133L,M133T)	Yes (M133L),M133T)	Yes (M133L)	[14,15]
C	CHB	Non-vaccinated	Q30K	3/5(60)	This study	This study	This study	
			S34L	3/5(60)	This study	Yes (S34L)	This study	[17]
			N40S	4/5(80)	This study	This study	This study	
			A45T	3/5(60)	Yes (S45T)	This study	This study	[18]
			T47V	3/5(60)	*	This study	This study	
			P49H	3/5(60)	This study	This study	This study	
			S55F	3/5(60)	This study	This study	This study	
			I68T	3/5(60)	This study	This study	This study	
			P79H	3/5(60)	This study	This study	This study	
			L175S	3/5(60)	*	Yes (L175S)	This study	[19]
			V177A	3/5(60)	This study	Yes (V177A)	This study	[19]
		vaccinated	T118K	1/3(33.3)	Yes (T118R)	Yes (T118R)	Yes (T118R)	[13]
			L175S	1/3(33.3)	*	Yes (L175S)	This study	[19]
	OBI	Non-vaccina	Q30K	4/11(36.4)	This study	This study	This study	
			N40S	5/11(45.5)	This study	This study	This study	

	ted	A45T	5/11(45.5)	This study	This study	This study	
		T47V/K	(5+1)/11(54.5)	*	This study	This study	
		P49H/L	(4+1)/11(45.5)	This study	This study	This study	
		S55F	4/11(36.4)	This study	This study	This study	
		R59H	4/11(36.4)	This study	This study	This study	
		T113P/S/N	(1+1+1)/11(27.3)	This study	This study	This study	
		T118K/R	(2+1)/11(27.3)	Yes (T118R)	Yes (T118R)	Yes (T118R)	[13]
		S143T/L	(2+1)/11(27.3)	This study	Yes (S143L)		[18]
		R160K/N	(7+1)/11(72.8)	This study	This study	This study	
		L175S	4/11(36.4)	*	Yes (L175S)	This study	[19]
		V177A	4/11(36.4)	This study	Yes (V177A)	This study	[19]
	vaccin	L53S	2/3(66.7)	This study	This study	This study	
	ated	I126S/T	(1+1)/3(66.7)	Yes (I126S)	Yes (I126S)	This study	[15]

This study: not determined in this study.* be under positive selection in genotype B and C HBV vaccine escape strains [20]；N40S substitutions found interfere with virion production [21].HBig:hepatitis B immunoglobulin.

Figures

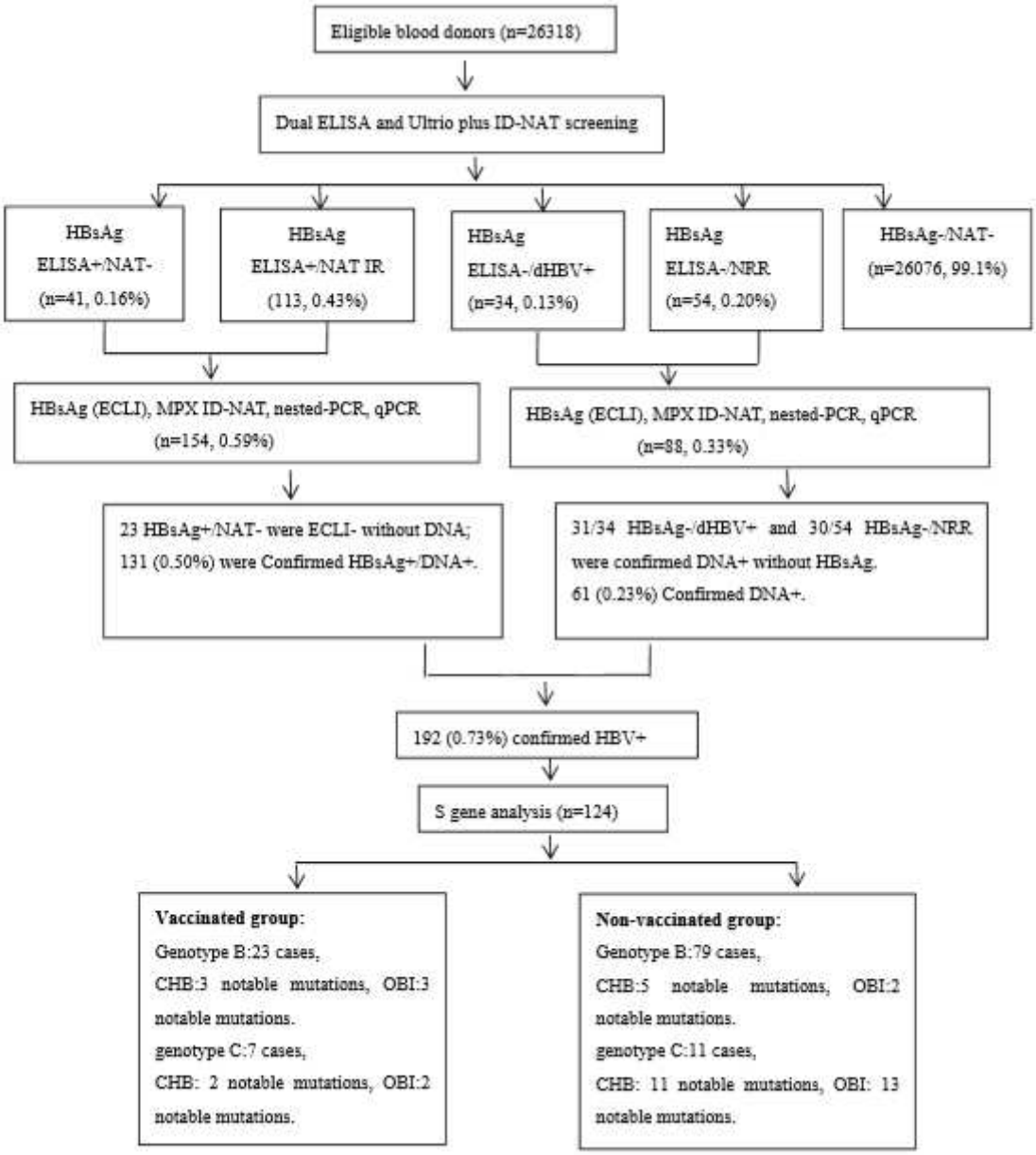

Figure 1

Flowchart for confirmatory testing algorithm of HBsAg ELISA + and/or NAT IR samples