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Molecular Comparison of Screening Technique for Hepatitis B Virus Infection among Blood Donors in Ibadan, South West, Nigeria

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Author's contribution

This work was carried out in collaboration among all authors. Author BOA and AIA prepared and designed the manuscript; performed the laboratory analysis of the sample. Authors OSL and BA performed and interpreted statistical analysis. Author IAS and IMF processed and analyzed the sample. All authors read and approved the final manuscript.

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ABSTRACT

Hepatitis B infection has been a great threat to transfusion medicine and public medicine, especially Nigeria where approximately 18 million Nigerians are chronic carriers. The blood donors in Ibadan are routinely screened with rapid technique or enzyme-linked immunosorbent assay (ELISA) for the detection of Hepatitis B surface antigen (HBsAg), there is paucity of information on the use of Nested Polymerase Chain Reaction (Nested PCR) for the detection of Hepatitis B virus deoxyribonucleic acid (DNA) for the screening of blood donors. This study was aimed at carrying out molecular comparison of the screening techniques for the detection of HBV infection among blood donors in Ibadan, South West, Nigeria.

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Blood samples were collected from 150 potential blood donors at the blood bank, University College Hospital, Ibadan. Rapid immune-chromatographic technique and nested PCR using primer SF, 979 and MF specific for DNA polymerase genome was used to screen the serum of the blood donors. Processing and analysis of data were performed using IBM statistical package for social sciences (IBM SPSS version 21.0 computer software). Descriptive statistics were presented using chart and tables while statistical significance was taken as $P<0.05$

The Rapid Screening test showed that 16 (10.7%) of the blood donors were positive while 134 (89.3%) were negative. The molecular detection of the Hepatitis B virus-DNA using nested PCR showed that 7 (4.7%) of the blood donors were positive while 143 (95.3%) were negative. It was also observed that 5 (71.4%) out of the 16 donors (10.7%) captured by the rapid screening were also detected by the Nested PCR, while the remaining 2 (28.6%) detected by the PCR were negative with the Rapid Screening test. The age range of 30 to 39 years and 40 to 49 years had the higher rate of infection 42.9% respectively. Result of the effects of different risk factors generated with the aid of questionnaire reflected that multiple sex partner have the highest prevalence of 16.7% compared to other risk factors

In conclusion, the detection of HBV-DNA using nested PCR among blood donors that was positive in Ibadan South West, Nigeria has public health implication for prevention of Hepatitis B virus and this confirms the practice of improper screening of blood before transfusion. Nested PCR techniques helps in early detection of hepatitis B virus DNA among blood donors, due to its high specificity and sensitivity than Rapid technique hence it serves as a confirmatory technique.

Keywords: Hepatitis B virus DNA; blood donors; nested PCR; rapid screening.

1. INTRODUCTION

Hepatitis B infection, which causes inflammation of the liver, is one of the major challenges in transfusion medicine. Hepatitis infection can be caused by Hepatitis B virus (HBV). Hepatitis surface antigen (HBsAg) was first discovered in the 1963 [1,2]. It was described in the serum of an Australian aborigine and was referred to as AUSTRALIAN ANTIGEN. The viral genome was sequenced around the early 1980s [3]. The virus is a double stranded DNA virus. It belongs to the *hepadnaviridae* family. It is an enveloped virus with icosahedral symmetry. It represents the only other animal virus with the DNA genome known to replicate by the reverse transcription of the viral RNA intermediate [4,5]. There are four known genes encoded by the genome called C, P, S, and X [5]. The core protein is coded for by gene C (HBcAg). HBeAg is produced by proteolytic processing of the pre-core protein. The DNA polymerase is encoded by gene P. Gene S is the gene that codes for the surface antigen (HBsAg) [6]. Hepatitis B virus is transmitted by percutaneous or mucosal exposure to the blood or body fluids of an infected person, most often through injection-drug use, from sexual contact with an infected person or from an infected mother to her new born during childbirth [7].

The incubation period of the hepatitis B virus is 75 days on average, but can vary from 30 to 180 days. The virus may be detected 30 to 60 days after infection and persists for variable periods of time [8].

Transfusion-transmissible HBV is one of the major threats to blood safety especially in developing countries. HBV can be detected in the serum by testing for series of serological markers of HBV.

Identification of any of these markers is an indication of the presence of the HBV which thus make blood unsafe for transfusion. The techniques employed in the detection of the HBV can be serological or molecular technique.

2. MATERIALS AND METHODS

The study was carried out at the Department of Virology, University College Hospital, Ibadan, One hundred and fifty samples were collected from consenting blood donor at the blood bank section of the tertiary hospital.

2.1 Sample Collection

150 Blood samples were collected from consenting blood donors presenting at the blood bank of University College Hospital, Ibadan. Demographic information was obtained through questionnaire from the participant with an informed consent.

5ml of blood was collected from each donor. Serum was obtained by spinning the blood

sample at 3,000 rpm for 10 minutes using a bench centrifuge. Each sample was labelled with identity number indicating the area of sample collection, the serial number on the questionnaire and date of collection. The collected serum samples were stored under a freezing condition using micro valve container sample bottles.

2.2 HBs Ag Rapid Kit Screening

All the samples were tested using the DiaSpot HBsAg One Step Hepatitis B Surface Antigen Test Strip (manufactured by DiaSpot Diagnostics, USA) The test was performed according to the manufacturer's instructions.

2.2.1 Dna extraction and nested polymerase chain reaction

DNA was extracted according to the manufacturer's instructions, QIAGEN DNA extraction kit (Qiagen, Hilden, Germany). Nested PCR was conducted. The first sets of primers used were HBV_SF-GTGTCTTGGCCAAAATT CGCAGT and HBV_CAAAAGACC CACAATTCT TTGACATACTTTCCAAT while the second sets of primers were HBV_CAAAAGACCCACAA TTCTTTGACATACTTTCCAAT and HBV_MF-TCGGATCCGGTATGTTGCCCGTTTGTCC.

Thermal cycling was performed as follow using the Veriti thermal cycler (Applied Biosystems, California, USA). The procedure works on the principle of temperature-based amplification of template DNA in the presence of Thermus aquaticus (Taq) polymerase, MgCl2, deoxynucleotide triphosphate (dNTPs) and PCR buffer. There are three (3) main stages of the PCR which are denaturation, annealing and extension and each of these stages requires different temperature and duration. However, at the start of the reaction, there's an extended reaction which is usually done at 95°C for 5 minutes. This is then followed by cycles of DNA template denaturation which is at 95°C for20-30 seconds, annealing of forward and reverse oligonucleotide primers targeting a region of Pol gene sequence done at 62-63°C for 20-60 seconds and extension of the primer and synthesis of a new DNA strand at 72°C for 90 seconds. After the last cycle, there is a period of an extended DNA synthesis at 72°C for 10 minutes. This is to ensure that all of the products are at their full length. The reaction is then cooled to 4°C until it is recovered from the thermocycler for detection of the amplified product or for further use. For semi-nested PCR, the amplified products are used for further PCR with different forward and backward primers. These primers are complementary to sequence between the amplified products. This enhances specificity and sensitivity of the PCR, only that the product will be smaller than the first amplicon. The PCR product was placed in 2% agarose gel, stained with ethidium bromide and viewed under ultraviolet trans illuminator to detect the amplified DNAs and the band size to identify the virus.

The confidentiality of participants including personal identity and all personal medical information were maintained. Every effort was made to keep research records private and answers provided to the questionnaire were kept confidential. Pertinent data/information (obtained from the study were kept safe especially those that were HBsAg positive) and data were only made accessible to the Principal Investigator and Institute Research Committee on request.

2.3 Statistical Analysis

Processing and analysis of data was achieved using IBM statistical package for social sciences (IBM SPSS version 21.0 computer software). Descriptive statistics were presented using chart and tables. Statistical significance was taken as $P<0.05$.

3. RESULT

The study was conducted on one hundred and fifty (150) blood donors. The result shows that 10.7% (16) of the blood doors were positive and 89.3% (134) were negative using the rapid kit as the screening method, however, Nested PCR method showed that 4.7% (7) of the blood donors were positive while 95.3% (143) were negative.

It was observed from the result that five donors that were positive using the nested PCR method were positive using the rapid kit method. Two (2) of the donors confirmed positive using Nested PCR were negative using the Rapid kit method. The result shows that sensitivity of Nested PCR over Rapid kit is 71% while the specificity is 99% as seen in table and figures below.

4. DISCUSSION

Most of the blood donors in this study were males and the ratio of male to female in this study is about 1: 10 as seen in Table 2. This

could be due to donor selection criteria which tend to allow more male to donate than females, and some physiological factors in females which include mensuration, breast feeding and pregnancy. Some social and cultural factors do not encourage females to donate blood compare to men in Nigeria society [9].

The age range of blood donors were between 19 and 68 years. The age distribution of 30 to 39 years and 40-49 years had the highest prevalence. This simply showed that men of young or middle age are those who can willingly donate blood. Also the infection is prevalent among them being that majority of them are energetic and active socially and sexually. This result did not correlate with other studies which showed the maximum prevalence in the age groups of 26 to 36 years [10,11,12].

Table 1. Detection of HBV-DNA using nested PCR in relation to rapid screening test

		Rapid screening		Total
		Negative	Positive	
Nested PCR	Negative	132 (92.3)	11 (7.7)	143 (100)
	Positive	2 (28.6)	5 (71.4)	7 (100)
Total		134 (89.3)	16 (10.7)	150 (100)

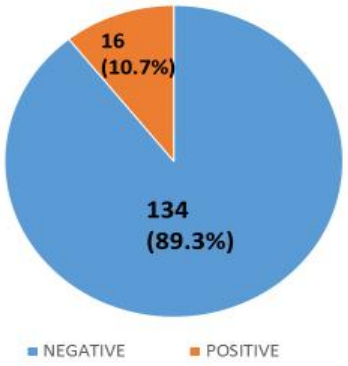

Fig. 1. Prevalence of hepatitis B virus infection using rapid screening method

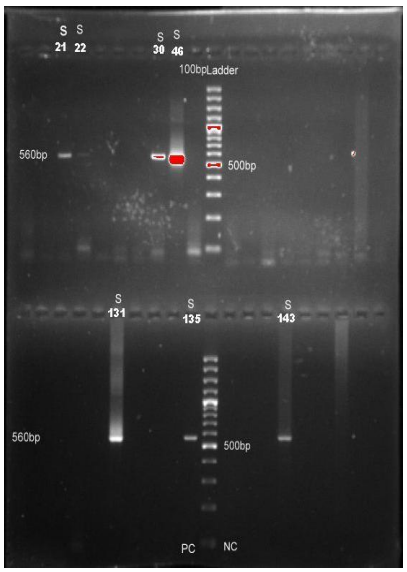

Fig. 2. Nested PCR result of HBV DNA of blood donor samples (S) using primer SF, 979 and 450, length 560bp; NC Negative control; PC Positive control; 100bp ladder marker

Table 2. Detection of HBV-DNA using Nested PCR in relation to gender

		Nested PCR		Total
		Negative	Positive	
Gender	MALE	129 (94.9)	7 (5.1)	136 (100)
	FEMALE	14 (100.0)	0 (0.0)	14 (100)
Total		**143 (95.3)**	**7 (4.7)**	**150 (100)**

Table 3. Effect of different risk factors in the detection of hepatitis B Virus-DNA

Risk factors	Number tested	Number positive	% Positive
Surgery	3	0	0%
Sharing of sharp object	72	4	5.6%
Blood transfusion	11	1	9.1%
Tattoos/marks	2	0	0%
Multiple sex partners	6	1	16.7%
Drug abuse/injection	1	0	0%
Alcohol consumption	5	0	0%
Other risk factors	50	1	2.0%

Table 4. Specificity and sensitivity of the rapid technique

		NPCR		Total	
		POS	NEG		PPV=0.313
Rapid Method	**POS**	5 (A)	11 (B)	16	(Rapid)
	NEG	2 (C)	132 (D)	134	
Total		7	143	150	NPV=0.985 (Rapid)
Sensitivity=0.313 (Rapid)					
Specificity = 0.985 (Rapid)					

Table 5. Cohen's K agreement between NPCR and rapid method

		NPCR		Total
		POS	NEG	
Rapid Method	**POS**	5 (A)	11 (B)	16
	NEG	2 (C)	132 (D)	134
Total		7	143	150
K= 0.913-0.851/1-0.851=0.062/0.149= 0.416				

The prevalence in this study is less compare to prevalence observed among blood donors in other parts of Nigeria e.g Abakaliki, South Eastern Nigeria in which 8.0% were positive and 15% in Zaria. However the 4.7% prevalence is observed to be higher than the findings in Yola, Nigeria with 2.4% [13,14,15].

Detection by the Nested PCR of 2 blood donors out of 134 that were negative to the rapid kit method could be due to the fact that the blood donors were in the early phase of the infection or as a result of occult hepatitis B infection. Antigen amount (HBsAg) is less in window period phase, recovery phase, occult hepatitis B [16,17].

The PCR was also able to confirm 5 out of blood donors that were positive to rapid kit screening technique. This could be due to false positive result on the part of rapid kit screening technique .It should also be noted that it had been documented that sub-standard test kits are mostly used in resource limited settings for transfusion related diagnosis [18].

5. CONCLUSION

This study showed that the nucleic acid method is more effective than the rapid kit method but the cost implication of the nucleic acid method has made it less popular in the developing countries.

This study also showed an overall prevalence of HBV DNA to be 4.7% among blood donors in Ibadan South West, Nigeria. This result has public health implication for prevention of Hepatitis B virus and this confirms the practice of improper screening of blood before transfusion. There is possibility an ongoing horizontal spread of the infection through sex and transfusion of unscreened or poorly screened infected blood is high.

The blood donors that were captured in the positive cases of HBV DNA in this study were contacted, informed and counselled. They were also linked to clinicians for possibly further tests, treatment and follow up.

Nested PCR techniques can help early detection of Hepatitis B virus DNA among blood donors, due to its high specificity and sensitivity than Rapid kit screening technique or using serology hence it serves as a confirmatory technique.

6. RECOMMENDATION

1. Awareness about Hepatitis B viral infection should be created among the general public.
2. Screening and counselling should be made available for blood donors and other members of the general public
3. HBV vaccination should be made compulsory and free for general public.
4. Advocacy for voluntary donors in place of family replacement.
5. Further work is recommended for this category of participants with increase sample size supported by governments and Non-governmental organizations in order to control the spread of HBV.

CONSENT

150 Blood samples were collected from consenting blood donors presenting at the blood bank of University College Hospital, Ibadan. Demographic information was obtained through questionnaire from the participant with an informed consent.

ETHICAL APPROVAL

Ethical approval for this study was issued from Ministry of Health, department of planning, research and statistics division, Oyo State, Nigeria.

COMPETING INTERESTS

Author has declared that no competing interests exist.

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