

Detection of Hepatitis B Virus (HBV) DNA among Blood Donors with HBsAg Positive in Tuban District Based on Nested Polymerase Chain Reaction Technique (Nested PCR)

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Abstract

Hepatitis B virus (HBV) infection is a health problem in the world including Indonesia. It is proven by increasing incident from year to year in detected cases of patients infected with HBV either acute, chronic or on liver cirrhosis, and mild HBV infection is often accidentally detected during blood tests. The blood donors in Tuban district only got serological examination for the detection of Hepatitis surface antigen (HBsAg) but have not been specifically got examination by Nested Polymerase Chain Reaction (Nested PCR) for the detection of HBV DNA. Examination of high number of samples by nested PCR could provide better result (positive). The purpose of this study is for the detection of HBV DNA by nested PCR technique in HBsAg positive blood donors in the Indonesian Red Cross (PMI) of Tuban District. This study is an experimental laboratory research. Blood samples from 13 HBsAg positive samples were obtained from 150 blood donors at Indonesian Red Cross (PMI) of Tuban District. The method used in this study was a nested PCR using primer pair 7 and 8 for first PCR also HBS1 and HBS2 for second PCR. Research was established in the biology laboratory of Universitas Ronggolawe (Unirow) Tuban and Institute of Tropical Disease, Universitas Airlangga (ITD-Unair) Surabaya from March to May 2012. Results showed out of 13 HBsAg positive samples there were 3 (3/13 = 23.08%) HBV DNA detected using primers P7 and P8 then 10 (10/13 = 76.92%) were detected using primers HBS1 and HBS2. Usage two primer pairs in nested PCR can detect all (100%) HBV DNA because the negative PCR results with a single primer pair can be detected with another primer pair.

Keywords: hepatitis B virus DNA, blood donors, Nested PCR

1. Introduction

HBV infection is one of health problem in the world. HBV can be transmitted through sexual contact, blood transfusion or blood products, tissues/organs transplantation, from mother to child during birth (perinatal exposure), as well as other unknown pathway. VHB incubation period is 45-120 days. HBV replication occurred in hepatocytes and then carried by the blood (viremia) (Handajani R, 2000).

Blood donors are a population group potentially transmits HBV. The prevalence of HBV infection among blood donors differ depending on country or area under study. It has been reported that people with HBV among blood donors in eleven major cities of Indonesia ranges from 2.1% to 9.5%, and in Papua province as high as 10.5% (Lucida, M.I., et. al, 2008).

Up to nowadays, hepatitis B virus (HBV) infection is still a health problem in the world including Indonesia proven by incident from year to year in detected cases of patients infected with HBV either acute, chronic or on liver cirrhosis (Handajani R, et.al., 1997). HBV infection is often mild and accidentally detected during blood tests.

Nested PCR is a DNA replication technique by DNA polymerase enzymes using two pairs of primers to amplify fragments. The first primer pair will amplify a fragment that working as other conventional PCR. The second primer pair is usually called nested primers (the primer pair was located in the first fragment) that bind within the fragment of first PCR product to allow for amplification of second PCR product where the result is shorter than the first. Nested PCR is a highly specific amplification of PCR because if there are any false fragments amplified the possibility that the part was amplified a second time by a second primer pair is very low (Chauhan et al. 2009).

Nested PCR is a variation of the conventional Polymerase Chain Reaction (PCR). Nested PCR and conventional PCR are both useful to amplify specific DNA fragments in large quantities. The Nested PCR used 2 primer pairs while conventional PCR only use 1 primer pair. Therefore, the result of DNA fragment on nested PCR is more specific (shorter) than the conventional PCR. The time required in the nested PCR is longer because of the nested PCR requires two times of PCR reaction while on conventional PCR only one time of PCR reaction. Moreover, the advantage of nested PCR is minimizing mistakes amplification of the gene by usage two primer pairs (Chauhan et al. 2009).

The blood donors in Tuban district only got serological examination for the detection of Hepatitis surface

antigen (HBsAg) but have not been specifically got examination by Nested Polymerase Chain Reaction (Nested PCR) for the detection of HBV presence. Nested PCR examination used two different primer pairs, which are the internal and external primers. External primer pair flanking long nucleotide sequence and internal primer pair flanking the same nucleotide sequence but shorter. Examination by nested PCR is expected to provide more positive results, considering where serology examination showed HBsAg positive should also show positive HBV PCR. There is possibility could not detect all HBV DNA while use only one PCR primer pair, so negative PCR results will still found although HBsAg positive.

On the basis of these, researcher aims to detect HBV DNA by nested PCR technique in HBsAg positive blood donors in the Indonesian Red Cross (PMI) of Tuban District. The nested PCR examination was performed in order to get a HBV DNA strands for genotyping determination.

2. Research Methods

This study is an experimental laboratory research. The method used in this study was a nested PCR using primer pair 7 and 8; also primer pair HBS1 and HBS2, research carried out at the Biology Laboratory of Universitas Ronggolawe (Unirow) Tuban and Institute of Tropical Disease, Universitas Airlangga (ITD Unair) Surabaya, from March to May 2012. Blood samples from 13 HBsAg positive samples were obtained from 150 blood donors at Indonesian Red Cross (PMI) of Tuban District.

Methods of laboratory tests performed were as follows:

2.1 DNA Extraction

DNAzol reagent (Invitrogen) was used for HBV DNA extraction from all serum that had been tested HBsAg by ELISA method, according to the instructions of the reagent. Extraction process was also used ethanol, sterile yellow and blue tips, and sterile 1.5 ml eppendorf tubes. Amplification reaction of DNA then was performed. First PCR amplified 541 base pairs (bp) of S gene using 100 picomol (pmol)/mikroliter(μ l) of primer P7 (5'-GTG GTG GAC TTC TCT CAA TTT TC-3') at 256-278 position and primer P8 (5'-CGG TAW^[A/T] AAA GGG ACT CAM^[A/C] GAT-3') at 796-776 position (Lindh M, *et al*, 1997).

2.2 HBV DNA Amplification

Nested PCR had been performed if first PCR result was negative. Second PCR amplified 259 bp of S gene (Lindh M, *et al*, 1997) using 100 pmol/ μ l of primer HBS1 (5'-CAA GGT ATG TTG CCC GTT TG-3 ') at 455-474 position and primer HBS2 (5'-AAA CTG CGA GCC ACC ACT GA-3 ') at 713-694 position (Lindh M, *et al*, 1997). Primer pairs used in this second PCR was different from the first PCR. Nested PCR is basically the same as conventional PCR; the difference is usage two primer pairs.

PCR was performed using "GeneAmp PCR System 2400 (Perkin Elmer)" machine. Each PCR consisted of 40 cycles that were previously carried out a hot start at 94°C for 5 min and PCR condition as follows; denaturation at 94 °C for 60 seconds, annealing at 55 °C for 60 seconds, and elongation at 72 °C for 75 sec.

2.3 PCR results visualization

PCR results were visualized with the process of electrophoresis that uses agarose gel 2% containing ethidium bromide with buffer Tris boric acid EDTA (TBE) 0.5 X, then performed visualization with ultra violet (UV) transilluminator. Marker used 100bp DNA ladder (Fermentas).

2.4 Data analysis

Data was entered and analyzed using descriptive qualitative and percentage.

3. Result

First PCR showed 3 positive results (3/13 = 23.08%) and second PCR showed 10 positive results (10/13 = 76.92%) which can be seen in table 1. Negative PCR results in first PCR as high as 10/13 (76.92%) and the second become zero (0 %) (table 1). PCR results were showed on figure 1 and figure 2.

4. Discussion

PCR using one primer pair did not give accurate results; it was showed in the first PCR using primer P7 and P8 that still got 10 negative results. Samples need to be examined in the second PCR using different primers; HBS1 and HBS2. All 10 negative results in first PCR showed positive in the second. This is important to do because serological examination showed all 13 samples were HbsAg positive, but after first PCR still showed some negative results. It is supposed that HBsAg positive would give also positive PCR results due to more sensitive and accurate detection of the technique, so requiring two different primer pairs for the detection of HBV DNA. One primer pair attached on external part of nucleotide to be duplicated, while the other primer pair only attached on the internal part of external primer.

Hepatitis B is one of several major human diseases and as a global public health serious problem. Approximately two billion people covering more than a third of the world population has been infected with hepatitis B. More than 350 million people of whom a person with hepatitis B virus are mostly located in Asia or

Africa (Lavancy D, 2011).

HBV infection was reported by many researchers after blood transfusions based on examination of HBsAg, including in India as much as 14.6% and UK 0.57%; as much as 12% of negative HBsAg patients suffering post-transfusion hepatitis after cardiac surgery (S Sastri, 2008) .

HBV screening using HBsAg in Taiwan has caused HBV infection after transfusion as many as 200 every 1.000 blood donors (20%) (Chen et al, 2002). Indonesia generally establishes a standard examination of negative hepatitis B surface antigen (HBsAg-) for screening of HBV infection to prevent HBV transmission through blood transfusion (Sastri S, 2008). Antigen amount (HBsAg) is less in *window period* phase, *recovery* phase, *occult hepatitis B* (OHB) (Alkhane, et al, 2002, Allain, JP, 2006) and post-infection, causing HBsAg examination become negative whereas HBV infection still occurred in the phases (Ronald, et al, 2000, Kleinman, SH, et al, 2003, Chen YS, et al, 2002, Paul K Nelson, et al, 2007, P Simmonds, et al, 2002, Chan, HLY, et al, 2003).

In this study, nested PCR examination using two different primer pairs can detect more HBV DNA. Usage of one primer pair can cause HBsAg positive samples become negative in PCR examination, so it is necessary to continue examination using different primer pair to detect the presence of HBV DNA. HBsAg positive samples that become negative in PCR examination can be caused by changes or differences of nucleotide sequence of attached place of primer, so it is not complementary and will result in a negative PCR. The advantageous of nested PCR is negative PCR result using one primer pair can be detected using another primer pair and gives a positive result.

It is known that the PCR examination for the detection of HBV DNA can give earlier positive result than using serological HBsAg (S Sastri, 2008). HBV DNA detection among blood donors is actually very important to ensure hepatitis B infection, completing HBsAg examination, it is due to using only HBsAg serology may cause false negative, but up to now HBV DNA PCR is still too expensive to be applied for screening examination among blood donors.

5. Conclusion

Based on the results of research and discussion, it can be concluded that nested PCR examination using two different primer pairs can detect all HBV DNA among blood donors with HBsAg positive. Nested PCR techniques can help early detection of hepatitis B virus DNA among blood donors.

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Figure 1. First PCR result of HBV DNA using primer P7 and P8, length 541 bp; lane 5,7,8 positive PCR; KN negative control; KP positive control; M marker.

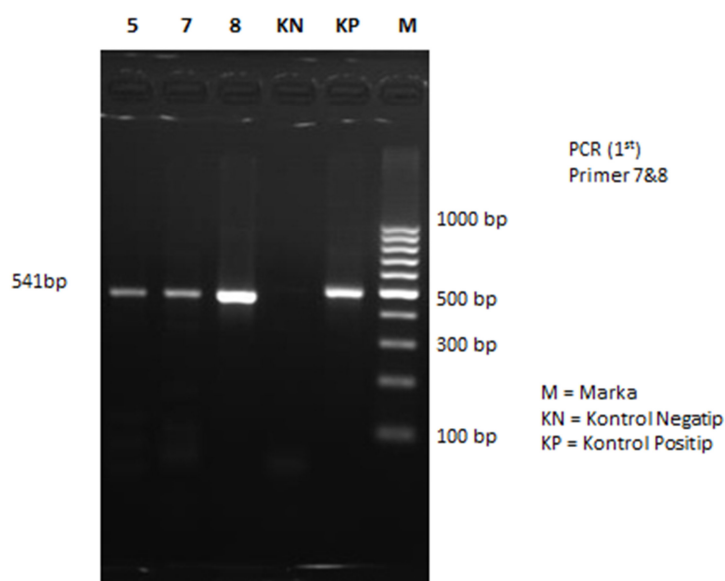


Figure 2. Second PCR result of HBV DNA using primer HBS1 and HBS2, length 259 bp; lane 1,2,3,4,6,10,11,12,13 positive PCR; KN negative control; KP positive control; M marker.

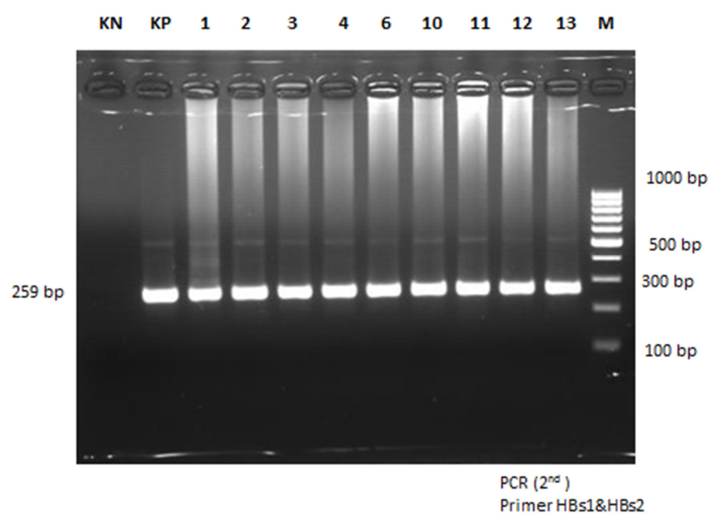


Table 1. Nested PCR results of blood donor samples with HBsAg positive in Indonesian Red Cross of Tuban Distric.

Primer Pair	Nested PCR result	
	Positive	Negative
P7 and P8	3/13 (23,08%)	10/13 (76,92%)
HBS1 and HS2	10/13 (76,92%)	0/13 (0 %)
Total	13/13 (100%)	