

Detection of Hepatitis B Virus DNA among Abdominal Typhus Patients with Hepatitis B Virus Co-Infection in Tuban District Based on Nested PCR Technique

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Abstract

Typhoid fever is often known by the name of abdominal typhus that may cause liver disruption so that arise disease complication which was known as typhoid hepatitis. Detection of abdominal typhus generally using routine blood test techniques that based on antigen and antibody reactions which is take a long time to obtain positive results so that these test cannot diagnose quickly and accurately. The detection of this disease in Tuban district is only using simple routine blood tests (eg Widal), urine and feces; it is difficult to diagnose the disease if there is complication. Abdominal typhus patients in Tuban district never got a specific examination technique by Nested Polymerase Chain Reaction (Nested PCR) to detect the occurrence of hepatitis B virus co-infection.

The purpose of this study to detect hepatitis B virus DNA among abdominal typhus patients with hepatitis B virus co-infection by using Nested PCR techniques.

This study is an experimental laboratory research. Blood samples from 9 positive HBsAg samples, which was obtained from 30 abdominal typhus patients in R. Kusma Hospital of Tuban District. The method used in this study was a nested PCR using primers P1 and P2 for the first PCR also primers HBV1 and HBV2 for the second PCR. Research carried out at the Biology Laboratory of Universitas Ronggolawe (Unirow) Tuban and Institute of Tropical Disease, Universitas Airlangga (ITD Unair) in Surabaya, from March to May 2009.

The results showed out of 9 positive HBsAg samples there were 4 (44.44%) HBV DNA detected using primer pair P1 and P2 then 1 (11.11%) were detected using primer pair HBS1 and HBS2. The use of two pairs of primers in nested PCR can detect more HBV DNA, because of the negative PCR results using a single primer pair can be detected using another primer pair.

Conclusion of the study is usage of nested PCR technique using two different primer pairs can detect more HBV DNA in abdominal typhus patients with hepatitis B virus co-infection as much as 55.55%.

Keywords: hepatitis B virus DNA, abdominal typhus patients, Nested PCR

1. Introduction

Salmonella typhi as causal agent of abdominal typhus (typhoid fever) in humans can cause more than 600,000 mortality per year. According to the data typhoid fever is a widespread disease throughout the world, especially in tropical climates, which is still a worldwide health problem (Thong et al, 2002, Mirza et al, 2000, Wain, et al, 2003, Ackers, 2000). Typhoid fever is often known by the name of abdominal typhus that may cause liver disruption so that arise disease complication which was known as typhoid hepatitis (Medical Tribune, 2001).

Prevalence of abdominal typhus and hepatitis B in Tuban District is high. Data from medical records of R. Kusma Hospital in 2004 as many as 205 abdominal typhus patients and 43 hepatitis patients, in 2005 increased as many as 354 *abdominal* typhus patients and 437 hepatitis patients. Detection of abdominal typhus generally using routine blood test techniques that based on antigen and antibody reactions which is take a long time to obtain positive results so that these test cannot diagnose quickly and accurately. The detection of this disease in Tuban district is only using simple routine blood tests (eg Widal), urine and feces; it is difficult to diagnose the disease if there is complication. Abdominal typhus patients in Tuban district never got a special examination technique by Nested Polymerase Chain Reaction (Nested PCR) to detect the occurrence of hepatitis B virus co-infection.

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 wrong 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).

Abdominal typhus patients in Tuban district never got a specific examination technique by Nested Polymerase Chain Reaction (Nested PCR) to detect the occurrence of hepatitis B virus co-infection for detection of Hepatitis B Virus (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 positive HBsAg should also show positive HBV PCR. While usage only one PCR primer pair, there is the possibility could not detect all HBV DNA, so negative PCR results will still found, although positive HBsAg.

On the basis of these, researcher aims to detect hepatitis B virus DNA in abdominal typhus patients with hepatitis B virus co-infection by Nested PCR techniques.

2. Research Methods

This study is an experimental laboratory research. The method used in this study was a nested PCR using primer pair 1 and 2; also primer pair HBV1 and HBV2, research carried out at the Biology Laboratory of Universitas Ronggolawe (Unirow) Tuban and Institute of Tropical Disease, Universitas Airlangga (ITD Unair) in Surabaya, from March to May 2009. Blood samples from 9 positive HBsAg samples, which was obtained from 30 abdominal typhus patients with hepatitis B virus co-infection in R. Kusma Hospital of Tuban District.

Methods of laboratory tests performed were as follows:

2.1 DNA Extraction

HBV DNA extraction of all serum of abdominal typhus patients who have been diagnosed with hepatitis B virus co-infection by a specialist in internal medicine and has been tested HBsAg by ELISA, using DNAzol reagent (Invitrogen) according to the instructions of reagents. In the extraction process was also used ethanol, sterile yellow and blue tips, and sterile 1.5 ml Eppendorf tubes.

2.2 HBV DNA Amplification

HBV DNA amplification reactions used: 10X Tth buffer solution; Tth enzyme DNA polymerase; dNTPs solution; Primer pair I, ie P1 and P2 correspond to the S region of HBV genomic, used in PCR I. P1 nucleotide sequences (sense) were: 5'-GTG GTG GAC TTC TCT CAA TTT TC - 3' and P2 (antisense): 5'-CGG TA (A / T) AAA GGG ACT CA (A / C) GAT-3' (Lindh, 1997) with 542 nucleotides product including the primer. PCR condition used: Denaturation temperature 94°C for 1 min, annealing 50 ° C for 1 min, extension 72 ° C for 2 min, 35 cycles of PCR. Negative result of HBV PCR using primer pair I were continued to Nested PCR using primer set II, ie HBV-1 (sense): 5'-CAA GGT ATG TTG CCC GTT - 3' and HBV-2 (antisense): 5' - CTA GCC AAA ACC ACT CGA -3 (Talent, 1997), with 261 nucleotides product including the primer. S gene region have been selected based on previous publications that showed can be used to determine the HBV genotype.

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

There were 9 positive HBsAg samples out of 30 samples of abdominal typhus patients in R.Kusma Hospital of Tuban District. Those 9 samples then performed PCR using external primers P1 and P2. Results of PCR using external primers P1 and P2 can be seen in Fig. 1.

Negative result of PCR using external primer then continued to nested PCR using different internal primer pair. Internal primer used in this study (HBV1 and HBV2) resulted in positive HBV DNA PCR (Figure 2). Out of 9

positive HbsAg samples, 4 samples were positive PCR using primer pair P1 and P2 (44.44%) (ie sample number 10, 24, 45 and 49) that produces 542 bp nucleotides, whereas one sample (11, 11%) (Sample number 2) was positive after nested PCR using primer pair HBV1 and HBV2 that produces 261 bp nucleotides that can be seen in Table 1.

4. Discussion

In this study, there were positive HbsAg in abdominal typhus patients after serological test. Research showed the examination with only one primer pair not give accurate results because with the primer pair P1 and P2 in the first round of PCR there were negative PCR results as many as 5 samples that need to be examined by Nested PCR, a second round of PCR with different primer ie the primary HBV1 and HBV2. Of 5 samples, by PCR using primers HBV1 and HBV2, HBV DNA detected only in one sample means not all positive HBsAg samples were detected HBV DNA, this could happen because of the diversity of the HBV genome so that the primers be incompatible. In this study proved Nested PCR was found to give more positive PCR results, which initially detected 4 samples, can be increased to 5 samples. This is consistent with research by 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.

Hepatitis B is one of several major human diseases and is 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 become hepatitis B patients and mostly located in Asia or Africa (Lavancy D, 2011).

This study proved that in addition to suffering from abdominal typhus, patients may also suffer from hepatitis B simultaneously. This proved by co-infection cases (HBV) in patients, in which patients' entry to the hospital only by complaint or diagnosis of *Salmonella typhi* infections. Transmission of HBV infection can occur by injection or transfusion, so there is possibility the patient can be infected from other patients by injection or transfusion which the occurrence cannot be explained in this study.

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 from post-transfusion hepatitis after cardiac surgery (S Sastri, 2008) . It has been reported that people with HBV among blood donors in eleven major cities in Indonesia ranges from 2.1% to 9.5%, and in Papua province was 10.5%. (Lucida M.I., et.al.2008).

The study suggests that in patients with a diagnosis of *Salmonella typhi* should also be checked of HBsAg and if necessary and capable also to be checked PCR of HBV and *Salmonella typhi*.

5. Conclusion

Based on the results and discussion, it can be concluded that the nested PCR examination using two different primer pairs can detect more HBV DNA among abdominal typhus patients with hepatitis B virus co-infection as much as 55.55%.

Nested PCR techniques can help early detection of hepatitis B virus DNA co infection of patients with abdominal typhus.

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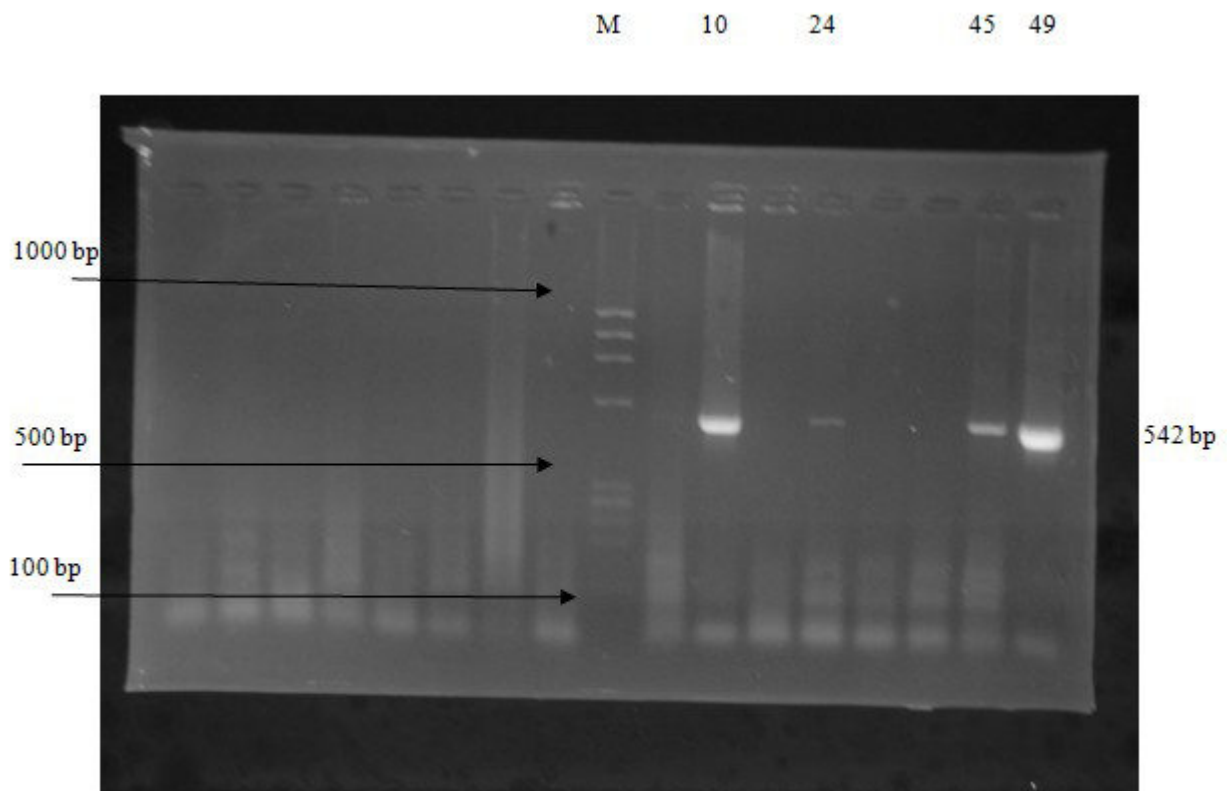
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**Figure 1. PCR result of HBV DNA using primer pair P1 and P2 with length 542 bp.
Note: Lane no. 10, 24, 45 and 49 are positive HBsAg samples and positive PCR of HBV DNA.**

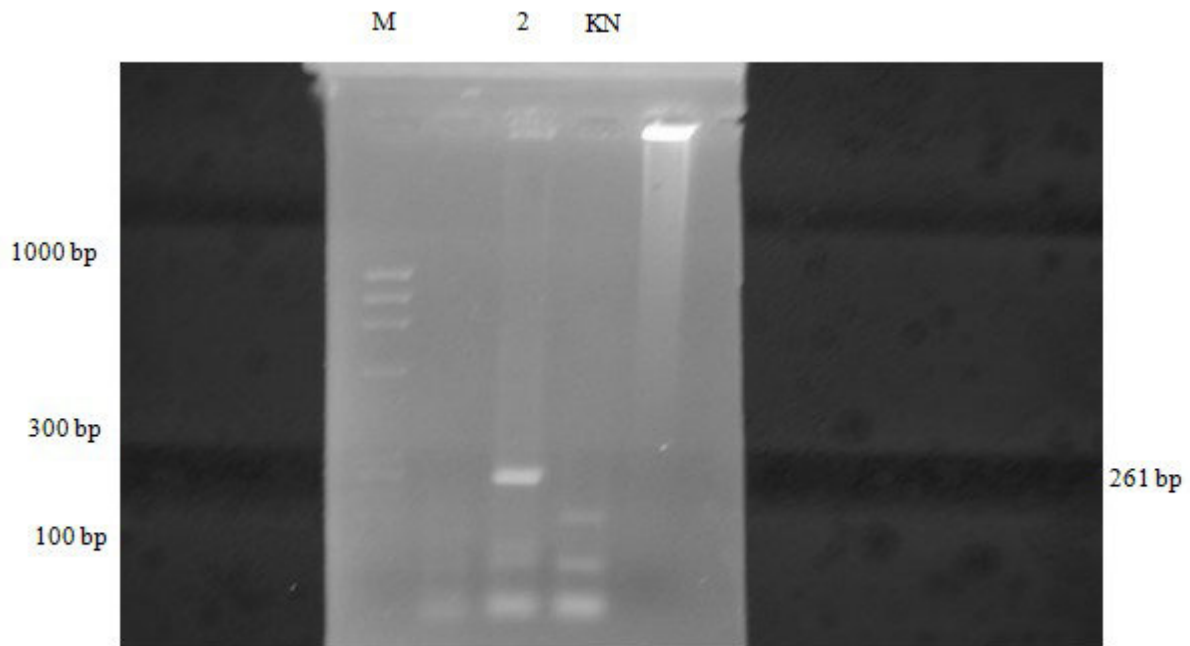


Figure 2. Nested PCR result of HBV DNA using primer pair HBV1 dan HBV2 with length 261 bp
Note: Lane no. 2 is positive HBsAg sample and positive PCR of HBV DNA.

Table 1. Nested PCR result of abdominal typhus patients with hepatitis B virus co-infection and positive HbsAg in Indonesian Red Cross of Tuban District

Pasangan <i>Primer</i>	Hasil <i>nested PCR</i>	
	Positif	Negatif
P1 dan P2	4/9 (44,44%)	5/9 (55,55%)
HBV1 dan HBV2	1/9 (11,11%)	4/9 (44,44%)
Total	5/9 (55,55%)	