Hepatitis B infection and genotype hepatitis B virus among drug abused in an Indonesian Teaching Hospital

Article · January 2013

DOI: 10.7813/2075-4124.2013/5-1/A.21

6 authors, including:

Lina Lukitasari
Airlangga University
6 PUBLICATIONS **1** CITATION
SEE PROFILE

Lilik Herawati
Airlangga University
17 PUBLICATIONS **8** CITATIONS
SEE PROFILE

Erwin Astha Triyono
Airlangga University
13 PUBLICATIONS **4** CITATIONS
SEE PROFILE

Retno Handajani
Faculty of Medicine. Airlangga University
41 PUBLICATIONS **422** CITATIONS
SEE PROFILE

L. Lukitasari, L. Herawati, E.A. Triyono, H.M. Margono, Soetjipto, R. Handajani. Hepatitis B infection and genotype hepatitis B virus among drug abused in an Indonesian Teaching Hospital. International Journal of Academic Research Part A; 2013; 5(1), 134-138. ***DOI:*** *10.7813/2075-4124.2013/5-1/A.21*

HEPATITIS B INFECTION AND GENOTYPE HEPATITIS B VIRUS AMONG DRUG ABUSED IN AN INDONESIAN TEACHING HOSPITAL

Lina Lukitasari[1]*, Lilik Herawati[2], Erwin Astha Triyono[3,5], Hendy Muagiri Margono[4,5], Soetjipto[1,6], Retno Handajani[1,6]

[1]Medical Biochemistry Department, School of Medicine, Airlangga Universiy, Surabaya,
[2]Physiology Department, School of Medicine, Airlangga Universiy, Surabaya,
[3]Internal Medicine Departement, School of Medicine, Airlangga Universiy, Surabaya,
[4]Psychiatry Departement, School of Medicine, Airlangga Universiy, Surabaya,
[5]Dr. Soetomo Hospital, Surabaya,
[6]Hepatitis Study Group of Tropical Diseases Center, Airlangga University, Surabaya **(INDONESIA)**
***Corresponding author:** linalukitasari@gmail.com

DOI: 10.7813/2075-4124.2013/5-1/A.21

ABSTRACT

South East Asia has high level endemicity of hepatitis B infection in the world. The hepatitis B virus (HBV) is transmitted by parenteral exposure from infected blood or body fluid. Drug abused group is one of the highest risk expose group of HVB infection. According to Indonesian National Narcotics Board, the drug abused case always rise every year. The aim of this study was to determine the hepatitis B infection among drug abused used *Polymerase Chain Reaction* (PCR) method, then sequence to determine the HBV genotype. First, hepatitis B infection determined by ELISA method in 101 sera from *drug abused.* Sera which had positive HBsAg will investigate later to determine the HBV DNA based on surface gen. Sera which had positive DNA HBV will be sequenced and analyzed using computer program version 9. In the result, Seven (6,93%) sera were detected had positive HBsAg from 101 sera and four (57,14%) sera were detected had HBV DNAs from 7 sera. Genotype B were detected in all positive HBV DNAs (100%). Conclusion, We had identified HBV DNA in HBsAg positive sera and others are not known whether it was hepatitis B infection or not. Thus, it is likely that genotype B of HBV is predominant in Indonesia. It needs further investigation to explore hepatitis B prevalence by molecular detection and genotype distribution with greater participant in HBsAg positive and negative.

Key words: hepatitis B, drug abused, genotype

1. INTRODUCTION

Infection by hepatitis B virus (HBV) is a common infection in developing countries. In 2000, Core Working Party for Asia Pacific Consensus on Hepatitis B and C states Indonesia has an endemicity level of hepatitis B from moderate to high around 3-20%. Numbers of chronic carriers of hepatitis B virus in the province of East Java in 2007 - 2009 was 4.5% (21/463) (1). Patient with drug abused is one of the high-risk groups for contracting HBV. HBV infection can develop progressively cause liver diseases, such as liver fibrosis, liver cirrhosis and liver cancer with approximately 1 million deaths due to liver cancer caused by HBV infection. In addition, hepatitis B also causes epidemic outbreaks of hepatitis B in Asia and Africa. Areas with the highest endemicity in the world are China and Southeast Asia (2).

Hepatitis B virus infection occurs because of the exposure with blood or body fluids containing HBV. High risk group of contracting HBV are the husband or wife with acute infection, health workers who interact with blood specimens, patients who received blood transfusions especially for those who received blood transfusions repeatedly (for example hemophilia patients), drug abuse syringes, and vertical transmission (from mother to child at birth) (3).

Hepatitis B virus has eight genotypes (A to H) and 4 subtypes (adw, adr, ayr, ayw). The kind of genotype and subtype is influenced by geography distribution, ethnicity, and clinical manifestations. HBV with B and C genotype from the results of previous research often causes hepatocellular carcinoma (3-5).

According to BNNRI (*Badan Narkotika Nasional Indonesia Republik Indonesia*/ Indonesian National Narcotics Board), Indonesia is a transit route of drug trafficking, to enter and exit from Indonesia, Myanmar, Thailand and Laos. Currently, Indonesia is not only a transit country but also has become a drugs producer. The incidence of drug abuse in Indonesia is increasing, proved in 2004 there were 930 cases with 1262 suspects; in 2005 there were 1.242 cases with 2.009 suspects; in 2006 there were 1772 cases with 2042 suspects. Thus, the risk to be infected by HBV in patients with drug abuse also increased (7).

By seeing the fact mentioning above and based on researchers knowledge, there is no data which describe the incidence of HBV infection in patients with drug abused in Surabaya. Thus, it is necessary to conduct research

with the goal of molecular detection of hepatitis B virus infection and HBV genotype distribution in patients with drug abused in Surabaya. This can be done by examining HBV DNA to detect the presence of hepatitis B infection with the method of Polymerase Chain Reaction (PCR) and sequencing to determine the HBV genotype distribution in patients with drug abused in Surabaya infecting by HBV.

This research is expected to contribute to other researchers and government (especially) in the efforts of developing prevention strategies and appropriate rescue for drug abused patients infecting by hepatitis B virus.

2. MATERIALS AND METHODS

This study is a cross sectional study. Calculation of the sample uses the population estimation formula according to (8), based on the number of people with hepatitis B virus in the province of East Java in 2007 to 2009 was 4.5% (21/463) (1). In collecting of 101 samples of serum, informed consent was done for drug abuse patients who came to the outpatient clinic of Dr Soetomo Hospital, Surabaya, Indonesia and has received ethical viability from the local hospital ethics committee.

Identification of HBV infection is done by examining the hepatitis B surface antigen (HBsAg) using the Enzyme Linked Immunosorbent Assay (ELISA).

HBV DNA genomic isolation

Genomic isolation of HBV DNA uses DNAzol ® procedure based on the novel guanidine-detergent lysing solution that hydrolyzes RNA and performs selective precipitation of DNA from cell lysate (10). The procedure is developed by P. Chomczynski (1), (U.S. patent no. 5945515). In doing genomic isolation of DNA, negative and positive control (containing a known sample of HBV) is done simultaneously.

Amplification by Polymerase Chain Reaction (PCR)

HBV DNA amplification is done by PCR technique (if necessary it can be nested PCR) with 40 cycles with preliminary temperature at 94°C for 5 minutes, then for each cycle consists of denaturation at 94°C for 1 minute, annealing at 52°C for 1 minute, elongation at 72°C for 2 minutes using primers P7 and P8 VHB for the first PCR and primers HBS1 and HBS2 for the second PCR (9,11). Primers P7, P8, HBS1, and HBS2 are taken from conserved HBV surface areas and have been published so the expectation level for PCR success is high. After doing HBV DNA amplification, electrophoresis (ELP) is done on 2% agarose in 0.5 X TBE containing ethidium bromide. This phase is intended to detect whether HBV DNA is present or not.

Nucleotide sequences

Purification of HBV DNA from PCR results performed by QIAquick-spin PCR Purification Kit from Qiagene or by phenol-chloroform purification method, after gaining a pure HBV DNA continued by ELP applications on low melting agarose by using 2% agarose in 0.5 X TBE buffer solution containing ethidium bromide and separated DNA electrophoresis results are examined under ultraviolet light to detect whether DNA is still present or not after purification. After purification, labelling HBV DNA performed by ddNTPs and Bigdye termination Kit with PCR pro sequencing technique used previous primer. Purification of HBV DNA from pro sequencing results by presipitation using etanol and sodium asetat then analysed the nucleotide used direct sequencing with sequencer mecine.

3. RESULTS AND DISCUSSION

In this research, 101 serum samples have been collected from drug abused patients gaining from the outpatient in Soetomo Hospital, Surabaya, Indonesia. The detail of gender and age group of drug abused patients who blood samples are taken from is presented in table 1 and the detail of gender and age group of drug abused people infecting by HVB examined by PCR is presented in table 2.

Table 1. Gender and age group of drug abused patients

Gender	Number of Patients	Age (years)
Male	97 (96,04%)	21 – 44
Female	4 (3,96%)	27 – 30
T O T A L	101 (100%)	

The youngest drug abused patients in this research is 21 years old and the oldest is 44 years, with an average age of 23.60 years and male patients is obtained (97 people) more than female patients (4 people).

In this research, drug abused patients with positive HBV DNA are found at the age of 29 years (the youngest) and the oldest is 41 years old, and they are found in male patients (4 people).

In the 101 serum of patients, all serum has been examined for SGPT concentrations. On the results of positive HbsAg, PCR examination is done to detect the presence of HBV DNA continued by sequencing to determine HBV genotypes.

PCR examination used in this research is nested PCR with 2 sets of primer pairs namely primary P7, P8 and HBS1, HBS2. Serum samples that give negative results with the primer pair P7 and P8 will be done a detection by using the second primers, HBS1 and HBS2 (see table 2) and electrophoresis results can be seen in figures 1 and 2.

Table 2. Gender and age group drug abused patients with positive HBV DNA & Amplification result by nested HBV PCR with 2 primer pairs in positive HbsAg drug abused patients

Gender	Age (years)	Number of Patients	PCR positive	
			Primer P7&P8	Primer HBS1&HBS2
Male	29 – 41	4 (3,96%)	2/4 (50%)	2/4 (50%)
Female	-	0 (0%)	-	-
T O T A L		4/101 (3,96%)	2/4 (50%)	2/4 (50%)

This suggests that serum samples with negative results for primers P7 and P8 (50%), but give negative results on both primer pairs, namely HBS1 and HBS2 (50%), it shows that all samples contain HBV DNA and that results also shows that there is a possibility of nucleotide sequences mutation for primers P7 and P8 so that primer can not do the annealing then the annealing will be done with primer HB2 HBS1 obtained positive PCR results.

For P7 and P8 primers that can give a positive PCR amplification results, next sequencing can be done. HBS1 dan HBS2 primer pairs can give a positive PCR amplification results, next sequencing can be done. The obtaining nucleotide sequences can be used to determine the genotype of the serum samples by comparing nucleotide sequences which have been published, for HBS1 and HBS2 primer pairs that have a shorter nucleotide fragments will be done the determination of genotypes based on the short nucleotide.

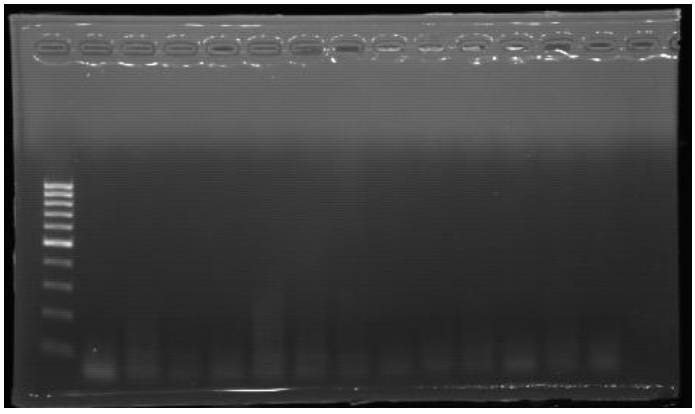

Fig. 1. *Electrophoresis result with negative HBV PCR*

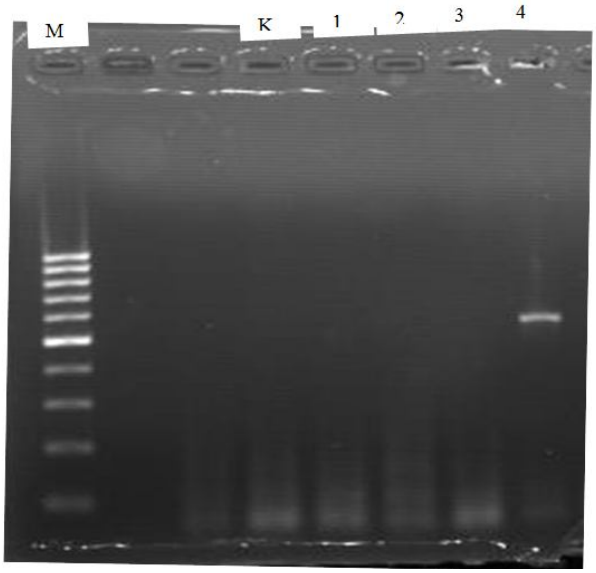

Fig. 2. *Electrophoresis result with negative HBV PCR*

Notes:
M : Marker
1 : Sample 1 with negative result
2 : Sample 2 with negative result
K : Negative control
3: Sample 2 with negative result
4: Sample 2 with positive result

Table 3. Amplification result by nested HBV PCR in drug abused patient with HbsAg

	HbsAg	PCR positive	PCR negative
HBsAg Positive HBsAg Negative	7/101 (6,93%) 94/101 (93,07%)	4/7 (57,14%) 0	3/7 (42,86%) 0
Total	101/101 (100%)	4/7 (57,14%)	3/7 (42,86%)

Currently, there are ten detected genotypes (A-J). Genotype I has been detected in Laos. This genotype has phylogenetic which is almost the same with aberrant Vietnamese strains that have a complex recombinant genome. In Japan, isolated HBV research was done to hepatocellular carcinoma patients who have living history of involving in military action in Borneo during World War II. The result is new genotypes, genotype J, are acquired. This genotype is based on the sequence divergence approximately 10.7 to 15.7% of another genotype, unique phylogenetic between man and ape animals, suggesting the existence of a strong recombination (6). As for A - H genotypes are based on more than 8% of the entire nucleotide sequence divergence (5). Hepatitis B virus with B and C genotypes was found in regions of Asia and the dominant genotype in Indonesia are B and C genotypes.

From the analysis of nucleotide and phylogenetic tree with genetyx program ver 9, the HBV genotypes in this research (4 samples) are included in the HBV with B genotype. From previous research, the detection of HBV from healthy blood donors who came from Indonesia, it is obtained B, C, and D genotypes. So HBV genotype in drug abused patients with positive HBV DNA was B genotype.

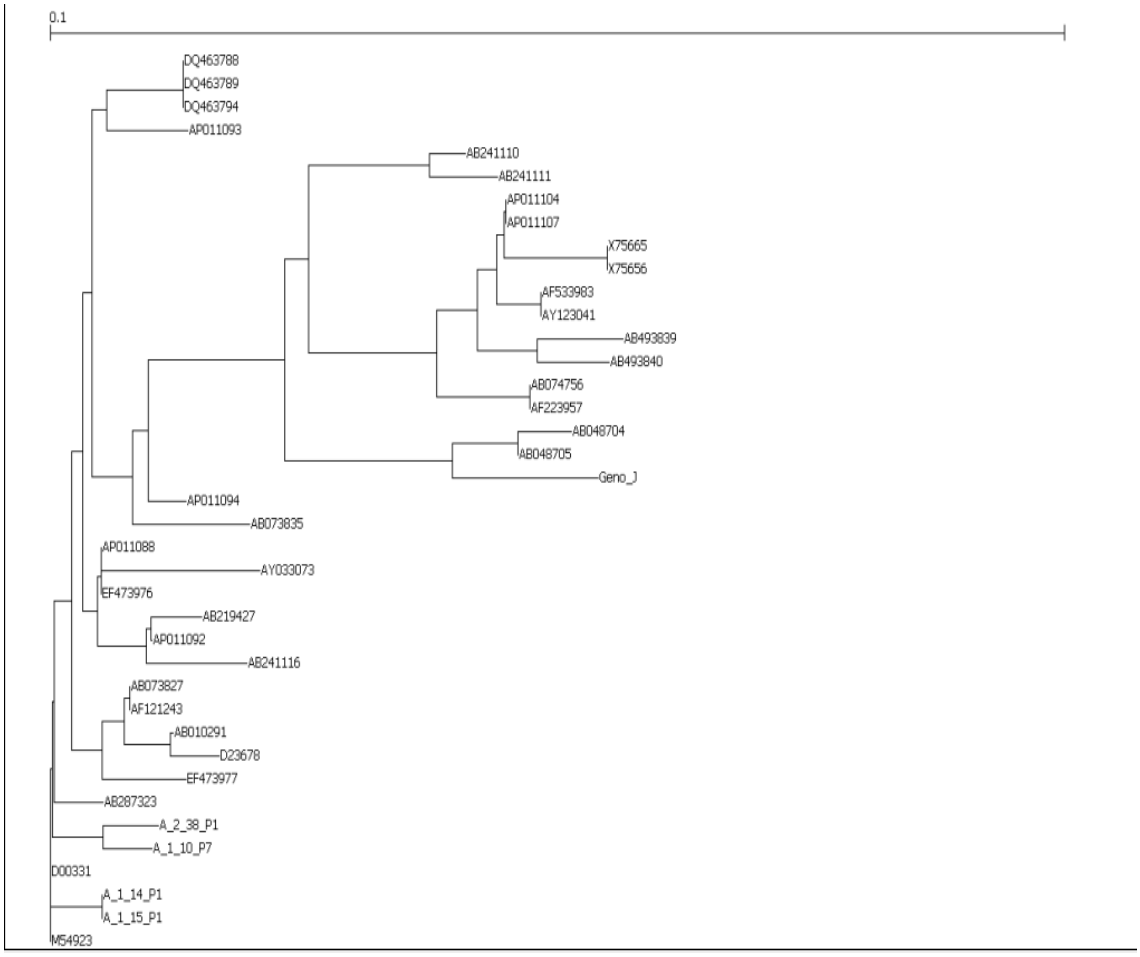

Fig. 3. *HBV moleculer analysis in phylogenetic tree*

4. CONCLUSION

From the research entitled "Hepatitis B Infection and Genotype Hepatitis B Virus among Drug Abused in an Indonesian Teaching Hospital" it can be concluded that drug abused patients with HBsAg-positive as much as 6.93% but drug abused patients with positive HBV DNA is 3.96%. HBV genotype in drug abused patients with positive PCR was B genotype. To obtain the prevalence data of hepatitis B with molecular detection and HBV genotype distribution in patients with drug abused in Surabaya, it is necessary to be done a research with more sample.

REFERENCES

1. U. Takako, M.I. Lusida, Juniastuti, M. Amin, Soetjipto, Y. Yano, H. Hotta, Y. Hayashi. Investigation of Hepatitis B Virus Infection in Indonesia. International Symposium on Infectious Diseases. Indonesia-Japan Collaboration. September 30th (2010).
2. A.R. Zekri, M.M. Hafez, N.I. Mohamed, Z.K. Hassan, M.H. El-Sayed, M.M Khaled, T. Mansour. Hepatitis B Virus Genotypes in Egyptian Pediatric Cancer Patients with acute and Chronic Active HBV Infection. Journal of Virology. Jul 15;4:74 (2007).
3. S. Specter. Viral Hepatitis Diagnosis, Treatment, Preventation. Humana Press Inc., Totowa NJ. (1999).
4. H. Lik-Yuen Chan, M.L. Wong, A.Y. Hui, L. Cheung-Tsui Hung, F. Ka-Leung Chan, and J. Jao-Yiu Sung. Hepatitis B Virus Genotype C Takes a More Aggressive Disease Course Than Hepatitis B Virus genotype B in Hepatitis B e Antigen-Positive Patients. Journal of Clinical Microbiology. 41 : 3 : 1277-1279 (2003). http://dx.doi.org/10.1128/JCM.41.3.1277-1279.2003.
5. K.Tatematsu, Y. Tanaka, F. Kurbanov, F. Sugauchi, S. Mano, T. Maeshiro, T. Nakayoshi, M. Wakuta, Y. Miyakawa, and M. Mizokami. A Genetic Variant of Hepatitis B Virus Divergent from Known Human and Ape Genotypes Isolated from a Japanese Patient and Provisionally Assigned to New Genotype. Journal Of Virology. Oktober : 83 : 20 (2009).
6. Z. Wang, Y. Tanaka, Y. Huang, F. Kurbanov, J. Chen, G. Zeng, B. Zhou, M. Mizokami, and J. Hou. Clinical and Virology Caharacteristic of Hepatitis B Virus Subgenotypes Ba, C1, and C2 in China. Journal of Clinical Microbiology. Mei : 45 : 5 :1491-6 (2007).
7. D. Mawuntyas.. Aparat Ungkap 776 Kasus Narkoba di Jawa Timur. Tempo Interaktif, Rabu, 04 Juli 2007 http://www.tempointeraktif.com/hg/nusa/jawamadura/2007/07/04/brk,20070704-103064,id.html (2007).
8. Lameshow dan Lwanga. Sample Size Determination in Health Studies. World Health Organization. (1999).
9. L.M. Anderson and A. Gusdal. Genotypes, nt 1858 Variants, and Geographic Origin of Hepatitis B Virus large-scale Analysis Using a New Genotyping Method. The Journal of Infectious Diseases. 175: 1285-93 (1997). http://dx.doi.org/10.1086/516458.
10. Anonymous. DNAzol® - Genomic DNA Isolation Reagent. Manufacturer protocol, 1999. Molecular Research Center,Inc.,Cincinnati,OH. DNAzol® is a trademark of Molecular Research Center, Inc. Copyright© 1998-2003 Molecular Research Center. (1999).
11. P.F.S. Talenta, G.P. Poggio, J.L. Lopez, J. Gonzales, A. Lemberg and R.H. Campos. Increased Prevalence of Genotype F Hepatitis B Virus Isolates in Buenos Aires, Argentina. Journal of Clinical Microbiology. Juli : 1873-75 (1997).