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Original Article

Genotype Distribution of Hepatitis C Virus in West Sumatera, Indonesia

Almurdi[1], Ellyza Nasrul[1], Yanwirasti[2], Jamsari[3]
Department of Clinical Pathology Faculty of Medicine[1], Department of Anatomy Faculty of Medicine[2],
Molecular Biology Department of Biotechnology[3], Andalas University/
Dr. M. Djamil Hospital Padang, Indonesia.

Abstract

Background: The genomes of HCV variants display considerable sequence divergence and have been classified into six genotypes and over 100 subtypes. Relatively well conserved regions of the genome such as NS5B have been used as the basis for HCV classification. HCV genotypes display different geographic distribution worldwide, a marker of response to antiviral therapy and serves as a guideline for the duration of therapy.
Study design, settings and duration: Serum samples were retrieved from clinical labs of West Sumatera between June to December 2013.
Materials and Methods: Sample from 50 anti-HCV positive blood donors and clinical laboratory patients were analyzed. HCV RNA and genotyping were carried out using PCR for NS5B region.
Results: HCV RNA was detected in 36 samples (72.0%). Genotype analysis demonstrated three different genotypes of HCV found in West Sumatera. There were genotype 1, 2 and 3. The most frequent subtypes were 1a (54.3%) followed by subtype 1c (17.4%), 1b (11.4%), 3k (11.4%) respectively.
Conclusion: HCV subtype 1a was the most prevalent in the samples from West Sumatera.
Key words: HCV, PCR, genotype.

Introduction

Infection with hepatitis C virus (HCV) is a major health problem in the world as the majority of patients infected with this virus failed to recover and will progressively suffer from chronic hepatitis which can progress to cirrhosis and hepatocellular carcinoma. This virus infects approximately 3% of the population and approximately 200 million people have been infected worldwide. It is estimated that more than 350,000 people die each year as a result of infection with hepatitis C virus.[1-3]

The prevalence of HCV infection in Indonesia is between 0.5 - 3.4%. According to Inoue *et al*., in blood transfusion unit about 2.1% were infected with HCV.[4] In Indonesia, a study in several hospitals of Jakarta, reported that hepatitis C virus genotype 1 is the most prevalent about 72.7% , composed of subtype 1b 47.3%, subtype 1c 18.7% and subtype 1a 6.7%.[5]

HCV genotype determination is important for patient management, determine the duration of therapy, determine the response to antiviral therapy, and geographic distribution. Various examination techniques to determine the genotype of the virus can be done through: restriction fragment length polymorphism (RFLP), using specific primers and hybridization with specific oligonucleotide probes. Reference method for determining the genotype of the virus can be determined by direct sequencing.[6]

Some areas targeted HCV genome has been used to determine the genotype of hepatitis C virus, such as the core area, envelope, and NS5B. In this way, HCV genotypes and subtypes[7,8] can be distinguished. Examination of hepatitis C virus RNA by reverse transcriptase PCR is a highly sensitive and specific method for detecting HCV infection and is the gold standard.[9] The aim of the current study was to evaluate the HCV genotype distribution among blood donors and patients with anti-HCV positive in clinical laboratory examination of West Sumatera, Indonesia.

Materials and Methods

Serum samples were obtained from 50 blood donors and clinical laboratory patients for anti-HCV

Corresponding Author:
Almurdi
Department of Clinical Pathology Faculty of Medicine
Andalas University/ Dr. M. Djamil Hospital Padang
Indonesia.
Email: aalmurdi@yahoo.com

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Authors Contribution

A, EN and J have done the conceptualization of project. A and EN did data collection. A also did the literature search and statistical analysis. Drafting, revision, manuscript writing was done by A and Y.

positive using third-generation HCV enzyme immunoassay in West Sumatera, between June to December 2013. Blood samples were collected from each patient. Serum was separated and stored at -80ºC.

Serum samples stored at -80ºC were retrieved for analysis. HCV RNA was extracted from 140 ul serum or plasma using QIAamp viral RNA mini kit (Qiagen, Inc) according to the manufacturer's protocol and stored at -80ºC until further analysis.

RT-PCR was performed using one-step RT-PCR kit (Invitrogen, Inc). The NS5B region was amplified by PCR with forward primers NS5B-1: 5'-TATGAYACCCGYTGCTTTGAC-3' and reverse primers NS5B-2: 5'-GAGGAGCAAGATGTTATCAGCTC-**3'**.[10] DNA amplification was performed for 40 cycles each consisting of 94ºC for 15 s, 55ºC for 30 s, and 72ºC for 1 minute in a thermal cycler (Biorad, Inc). The last cycle was followed by a 5-min extension step at 72 C. One microliter of amplicons was used for second-round PCR using Go Taq Green (Promega). The following cycling parameters were used for 35 cycles.

Amplicons were purified by using the QIA quick PCR purification kit (Qiagen, Inc) and analyzed by ethidium bromide agarose gel electrophoresis. Samples showing a band of the appropriate size (449 bp) (Figure), were further analyze by DNA sequencing in Macrogen, Korea Republic.

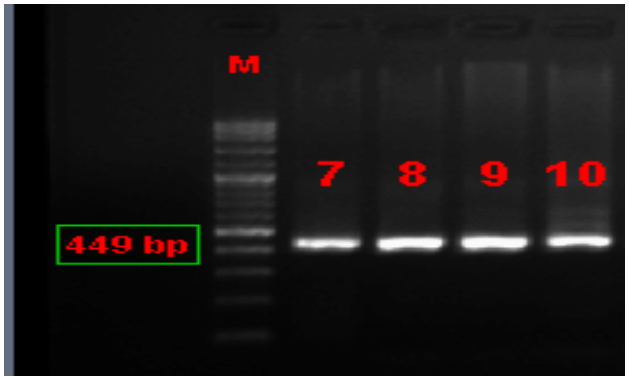

Figure: Agarose gel electrophoresis of PCR product (449 bp), M = marker (100 bp), 7-10 = positive samples

HCV genotyping was based on NS5B, the reference sequences were retrieved from the DNA Data Bank. The sequences were aligned using Geneous software.

Statistical analysis were performed using SPSS 15.0, *p* values < 0.05 were considered statistically significant.

Results

Sera were obtained from 50 anti-HCV positive blood donors and also from clinical laboratory. From these samples, it has been analyzed that 36 samples (72.0%) showed HCV specific positive signal by PCR. Genotypes of all HCV RNA positive samples were carried out using sequencing methods for NS5B region. Findings of the genotype analysis are summarized in Table-1.

Table 1: The prevalence of HCV genotype in West Sumatera.

Genotype	Subtype	No. of Samples	%
1		29	82.86
	1a	19	54.29
	1b	4	
	1c	6	
2	2e	1	2.86
3		5	14.29
	3a	1	2.86
	3k	4	11.43
Undeterminate		1	
Total		36	100.00

Thirty-five of 36 samples analyzed appeared to be readily classified as those belonging to individual genotypes 1a, 1b, 1c, 2e, 3a, and 3k. The predominant HCV genotypes in this population were genotype 1a (54.3%), followed by genotype 1c (17.1%), genotype 1b and 3k (11.4%) respectively. One sample (2.9%) could not be typed using genotyping method.

Analysis of the subtype distribution according to age showed that patients infected by subtype 1a (mean age 37.2 years) and 3a (35.0 years) were younger than patients infected by subtypes 1b (42.3 years), subtypes 1c (43.0 years) and others, but the differences between subtypes were not significant, *p* value = 0.43 (Table-2).

Tabel 2. The distribution of HCV subtype based on the mean of patient age.

Subtype	*No. of Patients*	*Mean age (yr)*	*Min. (yr)*	*Max. (yr)*	*p Value*
1a	19	37.2	28	50	0.43
1b	4	42.3	26	60	
1c	6	43.0	28	55	
2e	1	52.0	52	52	
3a	1	35.0	35	35	
3k	4	45.3	26	69	
Total	35	40.0			

Discussion

Determination of hepatitis C virus genotype is not only associated with the geographic distribution, but also related to the efficacy of interferon and ribavirin therapy. Hepatitis C virus genotype 1 and genotype 4 tend to exhibit lower virologic response (SVR) compared to genotype 2 and genotype 3. The response to treatment of HCV genotype 2 and genotype 3 is approximately

88.0%, whereas genotype 1, genotype 4, genotype 5 and genotype 6 had a 48.0% response to therapy.[11]

In this study, hepatitis C virus subtype 1a is the largest subtype of 19 specimens (54.3%). This is in contrast with previous studies in Indonesia carried out by Inoue *et al.*,[4] with largest subtype of 1b as much as 57.8% of blood donors. Research conducted by Utama *et al.*,[5] also obtained subtype 1b as the dominant subtype in Indonesia (47.3%). Another study conducted by Anggorowati *et al.*,[12] found subtype 1a as much as 52.0%, while the research conducted by Prasetyo *et al.*,[13] found subtype 1a was 46.7%, subtype 1c was 16.7%, subtype 1b was 3.3%. This study reveals a decrease in subtype 1b percentage as the previously dominant subtype in Indonesia. It also occurs in some cities such as Manila, Hanoi and Ho Chi Minh City formerly with predominant subtype 1b, shows the prevalence of subtype 1a becomes dominant.[13]

An increase in HCV subtype 1a and subtype 1c can be due to a phenomenon quasispecies and high mutation rate.[14] The high mutation rate is because of billions of virus replications occur per day.[15] HCV is known to have marked genetic heterogeneity with nucleotide substitution rate 1.44-1.92 x 10^{-3} per site per year. Accumulation of nucleotide substitution in the HCV genome results in diversification and evolution into different types.[11]

Subtype 1a and subtype 3a often associated with intravenous drug users, whereas subtype 1b associated with blood transfusion or nosocomial infection. Infection with genotype 2 is often associated with geriatric patients.[16] The use of needles together has become the primary transmission route in industrial countries while blood transfusion remains as a source of contamination in developing countries as the blood screening is not widely implemented.[16]

Changes in the distribution of subtypes also occurred in Italy, beginning with the most prevalent subtype is subtype 1b and genotype 2 replaced by subtype 1a and subtype 3a. These changes are also observed in Russia, subtype 1b is progressively replaced by subtype 3a. Studies in Germany showed subtype 1b is dominant among elderly patients, whereas subtype 1a is more dominant in the younger population.[16]

The genotype of 1 sample was undeterminate, likely due to the low viral load,[17] specimens with low viremia is required examination to confirm the diagnosis.[18]

Conflict of interest: None declared.

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