THE CORRELATION BETWEEN THE POSITIVITY OF ANTI-HCV AND HEPATITIS C VIRUS RNA IN SERA AMONG BLOOD DONORS, PATIENTS ON MAINTENANCE HEMODIALYSIS, AND PATIENTS WITH HEPATOCELLULAR CARCINOMA IN SURABAYA

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ABSTRACT:

Hepatitis C virus (HCV) has been known to be a major causative agent of chronic liver disease such as chronic hepatitis and liver cirrhosis, which often leads to hepatocellular carcinoma (HCC). Determination of the prevalence of liver disease caused by HCV helps provide an understanding of the virulence of these virus. Hence, we performed seroepidemiological and molecular epidemological study of HCV in Surabaya. The anti-HCV antibodies shows that the patients has been exposed to HCV, but we don't know whether this virus still in the blood or not, and detection of HCV RNA in the blood shows that the patients still infectious. The aim of this study is to know the correlation between the positivity of anti-HCV antibodies and HCV RNA among blood doors, patients on maintenance hemodialysis, and patients with hepatocellular carcinoma (HCC). The positivity of HCV RNA in anti-HCV-positive sera from blood donors, patients on maintenance hemodialysis, and patients with HCC were 84%, 97%, and 95%. These data shown that HCV RNA could be detected in more than 80% anti-HCV-positive sera, and it's means that the sera were still infectious.

Keywords : hepatitis C virus, anti-HCV, HCV RNA

INTRODUCTION:

Hepatitis C virus (HCV) has been known to be a major causative agent of chronic liver disease such as chronic hepatitis and liver cirrhosis, which often leads to hepatocellular carcinoma (HCC). The genomic structure of HCV resembles, to some extent, that of flaviviruses and pestiviruses, and therefore, the virus is considered to represent a new genus of the family Flaviviridae (Houghton *et al,* 1991). On the other hand, it was recently proposed that HCV should be classified into a new virus family, named Hepciviridae, because of its comparatively large sequence diversity from other members of the family Flaviviridae (Shukla *et al, 1995)*). The HCV genome of about 9.5 kb

has a long open reading frame, flanked with 59 and 39 untranslated regions (UTRs), which encodes a polyprotein precursor consisting of about 3,010 to 3,030 amino acid residues. The polyprotein is cleaved by the host signal peptidase and two other virally encoded proteases to generate at least 10 viral proteins: 4 structural proteins such as the core protein, the E1 envelope glycoprotein, and two types of E2 envelope glycoproteins (types A and B), and 6 nonstructural proteins (NS2, NS3, NS4A, NS4B, NS5A, and NS5B) (Grakoui *et al,* 1993; Hijikata *et al,* 1993; Mizushima *et al,* 1994).

Considerable sequence variation has been observed with different HCV clones. On the basis of the sequence diversity, HCV is now classified into at least six major genotypes, each of which can be further divided into a number of subtypes (2, 3, 9, 14). The prevalence of each HCV subtype has been reported to vary in different geographical areas (Bukh *et al,* 1995; Doi *et al,* 1994; Mello *et al,* 1995; Simmonds *et al,* 1993). It is still possible to identify sequence variants that could represent novel types or subtypes of HCV by performing extensive surveillance in previously overlooked areas. Viral pathogenicity and sensitivity to interferon treatment have been reported to vary with different subtypes (Pistello *et al,* 1994; Pozzat *et al,* 1991). Determination of the prevalence of liver disease caused by HCV helps provide an understanding of the virulence of these virus. Hence we performed seoepidemiological study of HCV in Surabaya. The anti-HCV antibodies give information to us that the patients has been exposed to HCV, but we don't know whether this virus still in the blood or not, and detection of HCV-RNA in the blood shows that the patients might be still infectious

The aim of this study is to know the further correlation between the positivity of anti-HCV antibodies and HCV-RNA among blood donors, patients on maintenance hemodialysis, and patients with hepatocellular carcinoma (HCC) in Surabaya.

MATERIALS AND METHODS

Serum samples. Sera were obtained from 2,234 healthy blood donors at the Red Cross Blood Transfusion Center, Surabaya, 76 patients on maintenance hemodialysis, and 34 patients with HCC at the Dr. Soetomo Hospital, Faculty of Medicine, Airlangga University, Surabaya. The sera were tested for antibodies against HCV by second-generation enzyme-linked immunosorbent assays (ELISAs; UBI HCV EIA, United Biologicals, Inc.; Ortho HCV Ab ELISA Test II, Ortho Diagnostics, Inc.). Serum alanine aminotransferase (ALT) levels were determined by using the Granutest ALAT (Merck, Darmstadt, Germany) according to the manufacturer's instructions. Normal ALT levels were $\leq$ 23 U/liter for men and $\leq$ 19 U/liter for women when tested at 25° C.

RT-PCR to detect HCV RNA in serum samples. Anti-HCV-positive sera were subjected to reverse transcription-PCR (RT-PCR) to detect HCV RNA, as described previously (Apichartpiyakul *et al,* 2994, Doi *et al,* 1994, Hotta *et al,* 1994, Katayama *et al,* 1996). Briefly, we performed RT-PCR for portions of NS5B region sequences using different sets of primers so that we could amplify the corresponding sequences from as many serum samples as possible. When NS5B region sequences could not be

amplified by the RT-PCR described above, we then tried to amplify 5'UTR sequences in order to check for the presence or absence of HCV RNA in the samples. These methods have been shown to be highly sensitive for the detection of HCV RNA in 90 to 100% of anti-HCVpositive sera from patients with chronic liver disease in Japan (Doi *et al,* 1994) and Indonesia (Shukla *et al,* 1995) and in 80 to 90% of anti-HCV-positive sera from blood donors in Thailand (Apichartpiyakul *et al,* 1994) and the Philippines (Katayama *et al,* 1996). The PCR products were electrophoresed in a 2% agarose gel containing ethidium bromide and were visualized by UV illumination.

RESULTS AND DISCUSSION:

Prevalence of anti-HCV antibodies among healthy blood donors, patients on maintenance hemodialysis, and patients with HCC in Indonesia. Sera obtained from 2,234 healthy blood donors were divided into two groups on the basis of their ALT titers. Two hundred sixty serum samples showed elevated ALT titers (>23 U/liter), and the remaining 1,974 serum samples showed normal ALT titers (d"23 U/liter). Twenty-three (8.8%) of the 260 serum samples with elevated ALT titers were positive for anti-HCV antibodies, whereas 9 (1.4%) of the 646 serum samples randomly picked from the normal ALT group were positive by the same test (Table 1).

TABLE 1. Prevalence of anti-HCV antibodies among blood donors, patients on maintenance hemodialysis, and patients with HCC in Surabaya

Group	No. positive/no.tested (%)
Blood donors	
Normal ALT level	9/646 (1.4) (28/1,974)b
Elevated ALT level	23/260 (8.8)c
Total	51/2,234 (2.3)
Hemodialysis patients	
Normal ALT level	31/40(77.5)
Elevated ALT level	27/36 (75.0)
Total	58/76 (76.3)
HCC patients	
Without advanced liver cirrhosis	14/22 (63.6)
With advanced liver cirrhosis	8/12 (66.7)
Total.	22/34 (64.7)

a A total of 646 serum samples randomly picked from the 1,974 serum sample with normal ALT levels and all of the 260 serum samples with elevated ALT levels were tested.

b Estimated values obtained by calculation.

cP , 0.05 (x2 test with Yates' correction) compared with blood donors with normal ALT levels. P , 0.001 (x2 test with Yates' correction) compared with patients on maintenance hemodialysis or patients with HCC.

d Diagnosed by ultrasonography.

It was estimated by calculation that a total of 28 serum samples would have been positive for anti-HCV antibodies among the 1,974 serum samples with normal ALT titers. Therefore, the total number of anti-HCV-positive individuals among the 2,234 blood donors was estimated to be 51, with the overall anti-HCV prevalence being 2.3%. The prevalence of anti-HCV positivity in blood donors with elevated ALT titers was significantly higher than that in those with normal ALT titers (P , 0.05), but it was significantly lower than that in patients on maintenance hemodialysis and patients with HCC (P ,0.001). The prevalence of anti-HCV antibodies among patients on maintenance hemodialysis was 76.3%, and in contrast to blood donors, there was no significant difference in anti-HCV prevalence between those with normal ALT titers and those with elevated ALT titers. A high prevalence of anti-HCV antibodies was also observed with HCC patients, whether or not the patients had advanced liver cirrhosis as diagnosed by ultrasonography.

Detection of HCV RNA by RT-PCR for the NS5B and 5' UTR in sera obtained from healthy blood donors, patients on maintenance hemodialysis, and patients with HCC. Anti-HCV-positive sera from 32 blood donors (31 males and 1 female; mean age, 42.3 years), 33 patients on maintenance hemodialysis (28 males and 5 females; mean age, 48.1 years), and 22 patients with HCC (16 males and 6 females; mean age, 59.3 years) were further analyzed for HCV RNA. Twenty-four (75%) of the 32 serum samples obtained from blood donors were positive for NS5B amplification with one or more of the different primer sets (Table 2). We did not retest the negative samples for NS5B amplification. The 8 serum samples that had been negative for NS5B amplification were subjected to RTPCR for the 5'UTR. Three of them became positive for the 59 UTR, but the other five serum samples remained negative after two independent amplifications. In total, 27 (84%) of the 32 blood donor samples were positive for HCV RNA. Of the 33 serum samples from patients on maintenance hemodialysis, 25 (76%) were positive for NS5B amplification and 7 others were positive for the 59 UTR. In total, 32 (97%) of the 33 serum samples were determined to be positive for HCV RNA. Similarly, 18 (82%) of the 22 serum samples from HCC patients were positive for NS5B and 3 others were positive for the 59 UTR, resulting in positive detection of HCV RNA in 21 (95%) of the 22 serum samples.

TABLE 2. Positivity of HCV RNA in anti-HCV-positive sera from blood donors, patients on maintenance hemodialysis, and patients with HCC in Surabaya

Target region	No. Positive/no. tested)%)		
	Blood donors	Hemodialysis patients	HCC patients
NS5B	24/32 (75)	25/33 (76)	18/22 (82)
5'UTR	3/8 a	7/8 a	3/4 a
TOTAL	27/32 (84)	32/33 (97)	21/22 (95)

a Samples negative for the NS5B amplification were examined.

From this results were found that the prevalence of anti-HCV antibodies among blood donors, patients on maintenance hemodialysis, and patients with hepatocellular carcinoma was 2,3 %, 76,3%, and 64,7%. The positivity of HCV-RNA among blood donors, patients on maintenance hemodialysis, and patients with hepatocellular carcinoma with positive anti-HCV antibodies was 84%, 97%, and 95%. Anti-HCV antibodies positive means that the patients has been exposure with HCV whether the HCV-RNA still positive or not. If the HCV-NA positive, it means that viremia might be still exist in those patients. From this research works we know that more than 80% patients with positive anti-HCV antibodies, were also positive for the HCV-RNA, and it's means that the patients might be still have viremia or still infectious.

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