

Screening of Blood Donors for HCV RNA by RT-PCR

Emine Sönmez, MD¹, Catherine Troisi, PhD¹, F. Blaine Hollinger, MD¹, Hsiang Ju Lin, PhD¹, Mohammed Naem, PhD¹

A total of 376 blood donors have been tested for anti-HCV by ELISA (second generation) test. 164 of 376 (40.95%) specimens were anti-HCV negative. 212 (59.05%) sera were anti-HCV positive. Seventy-six of total sera (20.21%) were found positive for HCV RNA. In 70 of 212 (33.01%) anti-HCV positive sera, HCV RNA was found positive. Six of 164 anti-HCV negative sera (3.65%) were considered positive for HCV RNA. These results indicate that reactivity in second generation assays for hepatitis C virus antibodies correlates with viremia, but they are not sensitive enough to screen hepatitis C virus carriers with low viremia. [Journal of Turgut Özal Medical Center 1996;3(4):303-305]

Key Words: HCV RNA, blood screening, HCV antibody

Donör kanlarının RT-PCR ile HCV RNA yönünden taranması

Toplam 376 donör kanı anti-HCV yönünden ELISA ile (ikinci jenerasyon) test edildi. 164 örnekte (%40.95) anti-HCV negatif iken 212 serumda (%59.05) anti-HCV pozitif bulundu. 76 serum (%20.21) da HCV RNA pozitif bulundu. Anti-HCV negatif serumların 6'sında (%3.65) HCV RNA pozitif bulundu. Bu sonuçlar HCV antikorlarını gösteren ikinci kuşak ELISA testleri ile viremi arasında korelasyon olduğunu gösterir, fakat düşük viremili HCV taşıyıcılarını tespit etmek için bu testler yeterince sensitif değildir. [Turgut Özal Tıp Merkezi Dergisi 1996;3(4):303-305]

Anahtar Kelimeler: HCV RNA, kan tarama, HCV antikor

The presence of antibodies to hepatitis C virus (anti-HCV) in the serum of blood donors; detectable by enzyme-linked immunosorbant assay (ELISA) and confirmed by recombinant immunoblot assay (RIBA) or polymerase chain reaction (PCR), is correlated with the risk for post-transfusion hepatitis (1,2). However, anti-HCV screening of blood donors with second-generation ELISA did not prevent completely the risk for post-transfusion C hepatitis (3). The detection of HCV RNA by PCR has been shown to be a better marker of viremia and infectivity than the detection of anti-HCV antibodies by ELISA or RIBA (4). Indeed, some anti-HCV negative blood donors with or without increased serum alanine aminotransferase (ALT) activity have detectable serum HCV RNA (5-7).

The objective of this study was to show the role of the second generation ELISA and PCR for screening of HCV infection and to detect HCV RNA prevalence in blood donors.

MATERIAL AND METHOD

In this study; 376 blood donors were detected for HCV RNA by reverse transcriptase (RT)-PCR, in department of Molecular Virology of Baylor College of Medicine, Houston, Texas-USA. These samples have been tested for anti-HCV, HBsAg, anti-HBc, ALT level in Gulf Coast Regional Blood Center-Houston, previously.

¹ Baylor College of Medicine, Department of Molecular Virology, Houston-Texas, USA

RT-PCR assay for HCV RNA procedure

A total of 376 specimens were pooled into 94 pools, each pool having 200 µL of serum from 4 serum samples (800µL per pool). Pools were numbered 1 through 94 and tested for HCV RNA by RT PCR. Some modifications were made in the extraction of RNA in the methods used by HJ Lin (8).

GLX reagent was made with 37.5 mL LS (phenol and guanidinium), 2 mL water, 5 mL yeast tRNA (10 µg/mL) and 0.5 mL micro-carrier gel. 800 µL GLX and 200 µL of the pooled serum were mixed. All the other steps were performed according to our routine protocol for patient samples except the RNA pellet was dissolved in 50 µL of TKNPRID (a mixture of 1.5 mL 20% NP-40, 1.5 mL 1 M KCl, 0.2 M Tris, 0.12 mL BPB and 27 mL water with RNase inhibitor at 0.7 units/µl) (8).

Another modification was made in the positive control, instead of using pooled positive serum, it was diluted 1:4. Primers used were the same as published by HJ Lin (8).

The sera tested in the positive pools were tested individually according to the standard protocol used in the laboratory for testing patient samples. This method utilizes 100 µL of serum samples and four sequences in the 5' noncoding region of the HCV genome.

PCR round a: Primer 57 and primer 321

PCR round b: Primer 126 and primer 299

RT and two rounds of amplification are carried out in a single tube.

After phenol-chloroform extraction, PCR a reagent ((PCR a mix: Mg, 10x buffer, dNTPs, primer 57, primer 321, BPB), DTT, RNase inhibitor, Taq polymerase- Boehringer Mannheim,

Maloney Murine reverse transcriptase- Boehringer Mannheim) were used and amplified as follows:

37°C	45 min
94°C	3 min
94°C	1 min
60°C	1 min
72°C	1 min, 30 cycles
4°C	soak

After round a, PCR b reagent was added (10 X buffer, dNTPmix, primer 126, primer 299, taq polymerase) and reamplified as following:

94°C	5 min
94°C	1 min
64°C	1 min
72°C	1 min, 25 cycles
72°C	5 min
4°C	soak

Each pooled sera and individual sera were tested duplicate extraction and quadriplex amplification for RT-PCR. Amplification products were shown in agarose gel (2%) with ultraviolet illumination.

RESULTS

Mean age of 376 donors was 36.40±7.50 and 266 of 376 were male, 110 of 376 were female. In all samples, HBsAg and anti-HBc were negative. 212 of 376 samples (59.05%) were anti-HCV positive and ALT levels were < 54 IU/L (upper normal limit), at the same time. Remains were anti-HCV negative and ALT levels were ≥ 54 IU/L (Table 1). HCV RNA positivity were found in 76 of all samples (20.21%), 70 of these sera were anti-HCV positive, 6 of these sera were anti-HCV negative (Table 2).

DISCUSSION

HCV antibodies are closely correlated with presence of viremia, whereas this is not always the case in a blood donor population (9-11).

There have been some reports suggesting that second-generation anti-HCV assays, based on a combination of structural and nonstructural recombinant HCV proteins, correlate well with the detection of serum HCV RNA by PCR in blood

Table 1. Summary of blood donors and samples

N	376
Male/female	266/110
Age (mean)	36.40 ± 7.50
ALT < 54 IU/L	212
ALT ≥ 54 IU/L	164
HBsAg (+)	----
Anti-HBc (+)	----
Anti-HCV (+)	212 (59.05%)
Anti-HCV (-)	164 (40.95%)
HCV RNA (+)	76 (20.21%)
HCV RNA (-)	300 (79.78 %)

Table 2. Relation of HCV RNA and anti-HCV

Anti-HCV	Total		HCV RNA (+)		HCV RNA (-)	
	n	%	n	%	n	%
Negative	164	43.6	6	3.65	158	93.35
Positive	212	56.4	70	33.01*	142	66.99

* The correlation was evident between serum HCV RNA levels and anti-HCV positivity ($p < 0.05$).

donors (12-15). We found a correlation between serum HCV RNA levels and anti-HCV positivity ($p < 0.05$), but in 6 of anti-HCV negative sera, HCV RNA was positive. Our findings, however, indicate that second generation anti-HCV assay are not sensitive enough to screen all asymptomatic HCV carriers. Previous studies reported that second generation anti-HCV assays may serve to detect highly viremic HCV carriers; low viremic HCV carriers can escape from this screening (3,16).

We found HCV RNA positivity as 20.21% in blood donors. Shirahi, *et al.* (17) found HCV RNA positivity as 60.97% and Yuki, *et al.* (16) found HCV RNA as 24.24% in blood screening. Different results may be depend on different geographic, sociocultural population of blood donors (*i.e.* iv drugs user, homosexuality) and different laboratory conditions. The results indicate that reactivity in second-generation assays for hepatitis C virus antibodies correlates with virus replication; but they are not sensitive enough to screen hepatitis C virus carriers with low viremia.

REFERENCES

1. Van der Poel CL, Reesink HW, Schaasberg W, *et al.* Infectivity of blood seropositive for hepatitis C virus antibodies. *Lancet* 1990;335:558-60.
2. Marcelin P, Martinot-Peignoux M, Gabriel F, *et al.* Chronic non A, non B, non C hepatitis in French blood donors assessed with HCV third-generation tests and polymerase chain reaction. In: Nishioka K, Suzuki H, Mishiro S, Oda T (eds). *Viral Hepatitis and Liver Disease*. Tokyo. Springer - Verlag 1994:85-8.
3. Aarh RD, Cladd ES, Blaine-Hollinger F, *et al.* Hepatitis C virus infection in post-transfusion hepatitis. An analysis with first and second generation assays. *N Engl J Med* 1991;325:1325-9.
4. Garson JA, Tedder RS, Briggs M, *et al.* Detection of viral hepatitis C viral sequences in blood donations by 'nested' polymerase chain reaction and prediction of infectivity. *Lancet* 1990;335: 1419-22.
5. Weiner AJ, Kuo G, Bradley DW, *et al.* Detection of hepatitis C viral sequences in non-A, non-B hepatitis. *Lancet* 1990;335:1-3.
6. Villa E, Ferreti I, De Palm M, *et al.* HCV RNA in serum of asymptomatic blood donors involved in post-transfusion hepatitis. *J Hepatol* 1991;13:256-9.
7. Sugitani M, Inchauspe G, Shindo M, *et al.* Sensitivity of serological assay to identify blood donors with hepatitis C viremia. *Lancet* 1992;339:1018-9.
8. Lin HJ, Shi N, Mizokami M, *et al.* Polymerase chain reaction assay for hepatitis C virus RNA using a single tube for reverse transcription and serial rounds of amplification with nested primer pairs. *J Med Virol* 1992;38:220-5.
9. Yuki N, Hayashi N, Kasahara A, *et al.* Antibodies to a putative hepatitis C virus poly protein in Japanese patients with chronic liver disease. *J Med Virol* 1992;38:86-91.
10. Yuki N, Hayashi N, Hagiwara H, *et al.* HCV RNA and antibody to HCV core protein in Japanese patients with chronic liver disease. *Dig Dis Sci* 1992;37:1483-8.
11. Yuki N, Hayashi N, Hagiwara H, *et al.* Improved serodiagnosis of chronic hepatitis C in Japan by a second generation enzyme-linked immunosorbent assay. *J Med Virol* 1992;37:237-40.
12. Van der Poel CL, Cuypers HTM, Ree Sink HW, *et al.* Confirmation of hepatitis C virus infection by new four antigen recombinant immunoblot assay. *Lancet* 1991; 337:317-9.
13. Hsu HH, Gonzalez M, Fong SKH, *et al.* Antibodies to hepatitis C virus in low risk blood donors: Implication for counseling positive donors. *Gastroenterology* 1992; 101:1724-7.
14. Esteban JI, Lopez-Talavera JC, Genesca J, *et al.* High rate of infectivity and liver disease in blood donors with antibodies to hepatitis C virus. *Ann Intern Med*, 1991;115:443-9.
15. Repicetta M, Attili Mele A, De Santris A, *et al.* Prevalance of hepatitis C virus antibodies and hepatitis C virus RNA in urban population. *J Med Virol* 1992;37:87-92.
16. Yuki N, Hayashi N, Hagiwara H, *et al.* Blood screening for asymptomatic Hepatitis C virus carriers with second generation hepatitis C virus antibody assays. In: Nishioka K, Suzuki H, Mishiro S, Oda T (eds). *Viral Hepatitis and Liver Disease*. Tokyo, Springer-Verlag 1994:352-4.
17. Shiraki H, Ishida C, Matsumoto K, *et al.* Antibody profile of blood donors infected with hepatitis C virus. In: Nishioka K, Suzuki H, Mishiro S, Oda T (eds). *Viral Hepatitis and Liver Disease*. Tokyo, Springer - Verlag 1994:341-5.

Yazışma adresi : Yrd.Doç.Dr. Emine SÖNMEZ
İnönü Üniversitesi Tıp Fakültesi
Klinik Mikrobiyoloji ve Bakterioloji ABD
44100 MALATYA