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Diagnostic Potential of Combined HCV Antigen For Hepatitis C Virus Infection

Jayendrakumar M. Patel[a,*], **Preeti Sharma**[a]

[a] Department of Biotechnology, Veer Narmad South Gujarat University, Surat, Gujarat, India

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Abstract

Background: Hepatitis C virus (HCV) is main cause of Hepatitis (Hepa= Liver and itis= inflammation) which leads to blood transmitted chronic hepatitis, liver cirrhosis, and hepatocellular carcinoma in both developed and developing countries. For screening of HCV infection, anti-HCV antibodies detection assays are used. These assays utilizing HCV antigens as capture molecules for capturing anti-HCV antibodies from patient blood. The combined antigen is artificial composite proteins constructed from diagnostically relevant antigenic regions derived from different HCV proteins. **Methods:** In present study diagnostic potential of combined HCV antigen was determined by its comparative performance study with individual recombinant proteins of HCV (i.e. Core, NS3, NS4, NS5) in the format of Immunofiltration assay. Performance study included 102 samples positive for anti-HCV antibodies and 891 samples negative for anti-HCV antibodies determined by HCV Version 3.0 (#930740) ELISA (Ortho Diagnostic Ltd., India). In addition to these samples, sero-conversion panels and performance panel from Seracare Lifesciences [PHV911 (M), PHV913, WWHV303] were also tested. **Results:** Combined HCV antigen was found 100% sensitive and 100% specific. A cocktail of individual recombinant antigens was found 100% sensitive and 99.55% specific.
Conclusion: Combined HCV antigen is more specific and it has good diagnostic potential for HCV infection.

Key words: HCV; Immunofiltration assay; ELISA; Liver cirrhosis; Hepatocellular carcinoma.

Introduction

Hepatitis C virus (HCV) is positive RNA strand containing enveloped virus, classified within the genus Hepacivirus in the Flaviviridae family [1, 2]. HCV is considered as the main cause of liver diseases in both developed and developing countries (total global HCV prevalence is 2.5%) and contributes to the increasing risk of liver failure and hepatocellular carcinoma (HCC) [3, 4, 5, 6].

HCV causes subclinical acute hepatitis. In 80% cases of HCV infected persons it gradually developed into chronic hepatitis which has a long time course, often extending for decades [2,7]. Chronic HCV infection is often associated with the development of liver cirrhosis, hepatocellular cancer (HCC) and death [3,8]. Ongoing changes in viral sequences enable the virus to persist and evade immune surveillance or antiviral therapies [9].

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Genome of HCV is made up of 9600 nucleotides encoding a poly protein of about 3000 aminoacids which is proteolytically cleaved to produce structural proteins (Core, E1, E2 and p7) and non structural proteins (NS2, NS3, NS4A, NS4B, NS5A and NS5B) [10,11].

Envelope proteins are very low immunogenic. Highest antibody response is observed against NS4 antigen of HCV, whereas responses to NS5 were generally the lowest and the most likely to be missing. HCV Core antigen is most conserved antigen. Whereas, NS3 and NS5 show high sequence diversity among HCV subtypes and genotypes [1, 12].

Anti-HCV assay using HCV proteins, which detect antibodies against HCV and used as a screening test to detect HCV in blood and blood products, are performed in order to prevent transmission of HCV [13]. The US Center for Disease and Prevention (CDC) recommends screening all individuals with risk factors for HCV infection for antibodies[7].

The first generation HCV enzyme immuno assay (EIA) detected only antibodies against the nonstructural region 4 (NS4) with recombinant antigen c100-3. In second-generation tests, additional antigens from the core region (c22-3), the NS3 region (c33c) and a part of c100-3 (named 5-1-1) from the NS4 region were used. Third-generation EIA anti-HCV test includes an additional antigen from the NS5 region and a reconfiguration of the core and NS3 antigens [14, 15, 16].

The combined HCV antigen is artificial composite proteins constructed from diagnostically relevant antigenic epitopes from different immunodominant structural and nonstructural proteins[17]. It was proposed that unique combination of combined HCV antigen having three dimensional structure provides high specificity and sensitivity in diagnostic assays. In case of different HCV recombinant poly peptide chains, its aberrant folding may hide immunodominant epitopes or create some epitopes which are not present in native antigen and thus resulting into loss of sensitivity or false reaction of the protein respectively [18].

In present study, potential of combined HCV antigen was determined by its functional testing with numbers of HCV positive and HCV negative samples in comparison with a cocktail of individual recombinant HCV antigens.

Materials and Methods

Serum samples were collected from clinical laboratories and blood bank of Surat, Gujarat, India. For determination of diagnostic potential of combined HCV antigen (Fapon Inc.), its comparative performance study was performed with a cocktail of individual recombinant HCV antigens (Core, NS3, NS4, NS5) [Prospec protein specialist] in Immunofiltration assay. For performance study, optimum concentration of each combined HCV antigen and individual recombinant HCV antigens were determined in Immunofiltration assay. Different concentrations of each antigen were prepared in 10mM Carbonate buffer, pH 9.6. Cocktail of individual HCV recombinant antigens was prepared with optimum concentration of each individual HCV recombinant antigen. For performance study, combined HCV antigen and a cocktail of individual HCV antigen were coated on the nitrocellulose membrane having 0.45µ pore size (Nupore) of immunofiltration devices. Protein A coupled with colloidal gold particles by using protocol mentioned by Lishan et al., 2014.

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The assay procedure for testing of immunofiltration assay was initially add 2 drops of wash buffer [0.2% non-ionic detergent (Tween 80) in Phosphate buffer and preservative) to wet the membrane which is followed by addition of 1 drop of patient sample (serum/plasma), add 2 drops of wash buffer to remove unbound materials, add 2 drops of detector reagent (protein A coupled colloidal gold conjugate) to detect captured anti-HCV antibodies, at last 3 drops of wash buffer to remove unbound detector reagent and clear background.

All serum samples were simultaneously tested with Ortho HCV Version 3.0 (#930740) ELISA, Immunofiltration devices containing Combined HCV antigen and Immunofiltration devices containing a cocktail of recombinant HCV antigens. Serum samples were tested with Ortho ELISA as per instructions provided by its manufacturer.

In performance study 102 samples positive for anti-HCV antibodies by Ortho HCV Version 3.0 including 68 mix infected samples (11 HCV and HIV positive, 18 HCV and HBsAg positive and 39 HCV and syphilis positive) and 891 samples negative for anti-HCV antibody by Ortho HCV Version 3.0 including 489 normal healthy donors samples, 54 other diseases positive samples, 105 interfering substances containing samples, 95 samples collected from different regions of the India, 148 clinical samples were tested. In addition to above mentioned samples, two HCV seroconversion panels [PHV911 (M), PHV913] and HCV worldwide performance panel WWHV303 procured from Seracare Lifesciences, USA were also tested.

Results and Discussion

All 102 samples positive for anti-HCV antibody by Ortho HCV Version 3.0 ELISA were detected positive by both immunofiltration test devices containing combined HCV antigen and a cocktail individual HCV antigen (Table 01). No interference was observed with mix infected samples. So sensitivity of combined HCV antigen and a cocktail individual HCV antigen were not compromised by presence of other infection.

In seroconversion panels testing with HCV antigens, performance of both combined HCV antigen and a cocktail individual HCV antigen were found comparable with the results of Ortho HCV Version 3.0 ELISA (Table 02 and Table 03). In testing of HCV worldwide performance panel WWHV303 containing HCV positive serum samples of different genotype, combined HCV antigen and a cocktail individual HCV antigen detected 18 samples out of 20 samples which was found comparable to results of Ortho HCV Version 3.0 ELISA (Table 4). So sensitivity of combined HCV antigen and a cocktail individual HCV antigen were found comparable to each other and with Ortho HCV Version 3.0 ELISA.

All 891 anti-HCV antibody samples negative for HCV by Ortho HCV Version 3.0 ELISA were detected negative by combined HCV antigen. These samples including 489 normal healthy donors samples, 54 other diseases positive samples, 105 interfering substances containing samples, 95 samples collected from different regions of the India and 148 clinical samples and thus it was found from these results that specificity of combined HCV antigen was not affected by presence of other disease, presence of

interfering substances, samples from different regions and with clinical samples. In case of a cocktail of recombinant HCV antigen, 4 clinical samples out of 891 samples were detected positive and thus its specificity was found 99.55% (Table 05).

Conclusion

Combined HCV antigen was found 100% sensitive and specific. Combined HCV antigen was found more specific than a cocktail of individual recombinant HCV antigens in immunofiltration assay as false positive reactions with a cocktail of individual recombinant HCV antigens were observed (specificity 99.55%) whereas in case of combined HCV antigen, no false positive reactions observed (specificity 100%) hence it has good diagnostic potential.

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Tables

Table 01: Testing of anti-HCV antibodies positive serum samples.

Samples		Immunofiltration assay			
		Individual recombinant HCV antigens		Combined HCV antigen	
		Positive	Negative	Positive	Negative
ORTHO® HCV Version 3.0 (#930740) ELISA positive serum samples n= 102	HCV positive samples n = 34	34	00	34	00
	HCV and HIV positive n = 11	11	00	11	00
	HCV and HBsAg positive n = 18	18	00	18	00
	HCV and Syphilis positive n = 39	39	00	39	00
Total numbers of samples n = 102		102	00	102	00
Sensitivity		100%		100%	

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Table 02: Testing of HCV Seroconversion panel [Modified PHV911 (M)].

Panel Member	Bleed date	Days since	ELISA	Immunofiltration assay			
			ORTHO® HCV	Individual recombinant HCV		Combined HCV antigen	
				Control	Test	Control	Test
PHV911-02	02-Nov-96	3	-	+	-	+	-
PHV911-03	13-Nov-96	14	+	+	+	+	+
PHV911-04	20-Nov-96	21	+	+	+	+	+
PHV911-05	23-Nov-96	24	+	+	+	+	+

Where, "+" = Positive, "- " = Negative

Table 03: Testing of HCV Seroconversion panel (PHV913).

Panel Member	Bleed date	Days since 1st bleed	ELISA	Immunofiltration assay			
			ORTHO® HCV Version 3.0 #930740	Individual recombinant HCV antigens		Combined HCV antigen	
				Control	Test	Control	Test
PHV913-01	27-Feb-97	0	-	+	-	+	-
PHV913-02	01-Mar-97	2	-	+	-	+	-
PHV913-03	06-Mar-97	7	+	+	+	+	+
PHV913-04	08-Mar-97	9	+	+	+	+	+

Where, "+" = Positive, "- " = Negative

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Table 04: Testing of Worldwide Performance Panel WWHV303.

Panel Member	Genotype	ELISA	Immunofiltration assay			
		ORTHO® HCV Version 3.0 #930740	Individual recombinant HCV antigens		Combined HCV antigen	
			Control	Test	Control	Test
WWHV303-01	1a	+	+	+	+	+
WWHV303-02	1b	+	+	+	+	+
WWHV303-03	1b	+	+	+	+	+
WWHV303-04	2a	+	+	+	+	+
WWHV303-05	2a	+	+	+	+	+
WWHV303-06	2b	-	+	-	+	-
WWHV303-07	2b	-	+	-	+	-
WWHV303-08	3, no subtype	+	+	+	+	+
WWHV303-09	3, no subtype	+	+	+	+	+
WWHV303-10	3a	+	+	+	+	+
WWHV303-11	3a	+	+	+	+	+
WWHV303-12	4, no subtype	+	+	+	+	+
WWHV303-13	4, no subtype	+	+	+	+	+
WWHV303-14	4a	+	+	+	+	+
WWHV303-15	4a	+	+	+	+	+
WWHV303-16	5a	+	+	+	+	+
WWHV303-17	5a	+	+	+	+	+
WWHV303-18	6, no subtype	+	+	+	+	+
WWHV303-19	6a	+	+	+	+	+

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WWHV303-20	1	+	+	+	+	+

Where, "+" = Positive, "-" = Negative

Table 05: Testing of anti-HCV antibody negative serum samples.

Samples			Immunofiltration assay			
			Individual recombinant HCV antigens		Combined HCV antigen	
			Positive	Negative	Positive	Negative
ORTHO® HCV Version 3.0 (#930740) ELISA negative serum samples n= 891	Normal Healthy donor samples N = 489		00	489	00	489
	Other diseases positive n = 54	HIV Positive n = 15	00	15	00	15
		Hepatitis B virus positive n = 29	00	29	00	29
		Syphilis positive n = 10	00	10	00	10
	Interfering substances containing samples n = 105	RA Positive samples n = 29	00	29	00	29
		High billirubin containing samples n = 21	00	21	00	21
		High SGPT containing samples n = 3	00	3	00	3
		High cholesterol containing samples n = 38	00	38	00	38
		High hCG containing samples n = 3	00	3	00	3
		ANA positive samples n = 4	00	4	00	4
		CRP positive samples n = 7	00	7	00	7
	Samples collected from different regions of the India n = 95		00	95	00	95
	Clinical samples n = 148		4	144	00	148
Total numbers of samples			4	887	00	891
Specificity			99.55%		100%	

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*Corresponding author. Mailing address: Department of Biotechnology, Veer Narmad South Gujarat University, Surat, Gujarat-395007, India: E-mail address: **jayendrap@ymail.com**