Comparison of Serological and Nucleic Acid Based Assays Used to Diagnose Hepatitis C Virus (HCV) Infection in Acute and Chronic Liver Diseases

M. Irshad, I. Dhar, Khushboo, Shiwani Singh, S. Kapoor
Clinical Biochemistry Division, Department of Laboratory Medicine
All India Institute of Medical Sciences, New Delhi (India).

Abstract:

Background: This study reports a comparative diagnostic potential of three different assay systems used to detect HCV infection in acute and chronic liver diseases.

Methods: A total number of 364 patients with various types of liver diseases were analyzed for hepatitis C virus (HCV) core antigen using Enzyme Immuno Assay (EIA), HCV-RNA by RT-PCR and anti-HCV antibodies by third generation EIA system. Simultaneously these patients were also tested for markers of other hepatitis viruses, notably, hepatitis A, B, C, D and E. In some cases, even transfusion transmitted virus (TTV) was tested using TTV-DNA as the marker of TTV infection.

Results: Analysis of results demonstrated the presence of hepatitis B, C and E in different proportions of patients belonging to these liver diseases. Hepatitis A and D infections could not be detected in these cases TTV infection was prevalent in different liver diseases in different proportions. Though none of control sera demonstrated hepatitis A-E infection, however, TTV infection was noted in control group also. When we analysed all the sera for HCV infection using these different assay systems, we found HCV core, HCV-RNA and anti-HCV antibodies in 18.3%, 18.3% and 5.83% cases of acute viral hepatitis (AVH), 13.3 %, 13.3% and 46.6% cases of chronic viral hepatitis (CVH), 23.8%, 23.8% and 23.8% cases with cirrhosis of liver and 20%, 17.5% and 10% cases respectively, of fulminant hepatic failure (FHF) patients. Whereas HCV core and HCV-RNA assays were comparable and predominantly positive in acute cases (AVH and FHF), anti-HCV antibodies were detected in high proportions in chronic liver diseases. Cirrhosis patients showed all the markers in equal proportions. This pattern of HCV markers remains unaffected by co-infection of HCV with other hepatitis viral infections.

Conclusion: In conclusion, where HCV core and HCV-RNA are best diagnostic markers in acute liver diseases, anti-HCV diagnoses high proportion of HCV cases in chronic liver diseases. This diagnostic pattern is not changed on co-infection of HCV with other viral infections.

Keywords: HCV, Core, Hepatitis, PCR, TTV.

Correspondence:
Dr. M. Irshad
Professor
Clinical Biochemistry Division
Department of Laboratory Medicine
P.O. Box – 493, A.I.I.M.S.
New Delhi-110029 (India).

Introduction

HCV a member of the Flaviviridae family, is an enveloped virus with a positive single-stranded 9.6-kb RNA genome.[1] This virus has been identified as the major causative agent of non-A, non-B hepatitis [2] that persistently infects several people throughout the world. Although acute phase HCV infection is asymptomatic in most cases, the virus frequently establishes a persistent infection and this condition is often associated with serious liver diseases. More than 170 million people throughout the world are infected with HCV. Persistence of the virus in the liver leads to chronic hepatitis in 70% of infected patients, which can progress to cirrhosis and liver cancer. [3]

Virological test for the diagnosis and management of HCV infection included EIAs and confirmatory immunoblot assays for the detection of anti-HCV antibodies and qualitative and quantative nucleic acid techniques (NAT) for the detection of HCV-RNA. At present, the virological diagnosis of HCV begins with the detection of anti-HCV antibodies using EIA. The anti-HCV antibodies can be detected 7-8 weeks after infection and usually persist for life. A negative anti-HCV is sufficient to exclude chronic HCV infection. [4] Given the high sensitivity and specificity of current anti-HCV EIAs, immunoblots are now considered to be of no use. [5,6] Qualitative NAT is the key diagnostic method for distinguishing the individuals who have resolved HCV infection (HCV-RNA negative) from the patients with active/ ongoing HCV infection (HCV-RNA positive).[6] However, there are at least three clinical situations when the screening for HCV infection cannot rely only on serology, but should also include a sensitive qualitative NAT. These are acute Hepatitis C, HCV infection after occupational exposure and immunocompromised situation. [6] Since NAT is labor intensive, prone to contamination and also expensive, efforts have been made to identify a test that could supplement or eventually replace NAT in the management of Hepatitis C.[7,8] Among the potential candidate HCV proteins, the HCV nucleoprotein / core protein has attracted the attention of researchers because it is a structural HCV protein and its sequence is highly conserved. [9] Assays for the detection of HCV core antigen have been developed recently and are being used globally for HCV diagnosis.

Present study describes the comparative role of serological assays detecting anti-HCV antibodies and HCV core antigen in serum, as well as, nucleic acid based assays showing presence of HCV-RNA in serum, in diagnosing HCV infection in different forms of liver diseases. This is aimed to select the most appropriate assay to diagnose HCV infection in the given clinical presentation of liver disease.

Methods

Patients and blood samples

A total number of 364 patients of both sexes and in adult age group were included in the present study. 120 patients (age range : 25-45 years) were diagnosed as having AVH ; 120 patients (age range : 18-70 years) with CVH, 84 patients (age range : 20-65 years) with cirrhosis of liver and 40 patients (age range : 15-60 years) with FHF. All these patients attended either outpatient department or were admitted to the liver unit of All India Institute of Medical Sciences, New Delhi, India from October 2001 to February 2006. They were evaluated clinically and biochemically and their sera were tested for hepatitis viral markers. The diagnosis of different types of liver diseases was based on accepted clinical, biochemical and histological criteria as outlined elsewhere. [10] AVH was diagnosed when patients exhibited overt jaundice and / or increased alanine aminotransferase levels (at least 3 times above the normal value) documented at least twice at a one week interval without any history of pre-existing liver disease. None of the patients had a past history of alcohol intake or using any drug. We also could not find any clinical or serological evidence of autoimmune diseases or biliary infection in these patients. The patients with CVH and cirrhosis of liver were diagnosed by histopathological criteria laid down by international study group on chronic hepatitis. [11] All these CVH patients had persistent elevation of transaminases level (at least twice the upper limit of normal range) for more than six months and histologic evidence of chronic hepatitis on liver biopsy at the beginning of follow-up. Fulminant hepatic failure was diagnosed if the patients developed hepatic encephalopathy within four weeks of the onset of acute hepatitis as outlined elsewhere.[10] One hundred age and sex matched healthy subjects were used as controls.

From each of the above patients, 6-10 mL of venous blood was drawn and aliquoted in plain tubes without anticoagulant. Serum was separated after centrifugation and then stored at -70°C until further analysed. Repeated freezing and thawing of serum was avoided as far as possible. These sera samples were used to

analyse various hepatitis markers, liver function tests and HCV core protein. Control sera were also analyzed simultaneously for all the test-parameters as those of patient sera.

Hepatitis viral markers

Sera were investigated for hepatitis B surface antigen (HBsAg) and IgM antibodies to hepatitis A virus (IgM anti-HAV), hepatitis B core antigen (IgM anti-HBc), HDV (IgM anti-HDV) and HEV (IgM anti-HEV). Similarly, all these sera were also tested for total antibodies against HCV (anti-HCV). The serological analysis was done using EIA kits of high sensitivity and specificity obtained from internationally known firms. Kits for HBsAg, IgM anti-HBc and IgM anti-HAV were purchased from Abbot Laboratories, USA. Anti-HCV was tested using highly sensitive third generation ELISA kit from Ortho diagnostics. This anti-HCV kit used peptides versus core, NS3, NS4 and NS5 regions of HCV genome, as antigen to coat the ELISA plate. IgM antibody to HDV was tested using an EIA kit from Wellcome, U.K. Similarly, IgM anti-HEV was tested using third generation ELISA kit from Genelabs and Diagnostics, Biotechnology, Singapore.

HCV Core Ag Assay

Sera samples were assayed for HCV core protein according to the manufacturer's instructions, using EIA kit from Ortho Diagnostics, UK. One hundred μL of samples and controls were mixed with 100 μL of a pretreatment buffer. For the ELISA reaction, 200 μL of pretreated samples and controls were incubated for 95 minutes at 37°C with continuous shaking in the antibody-coated wells of a microtiter plate. The plates were washed and incubated for 30 minutes at 37°C with 200 μL of conjugate, washed again, and incubated for 30 minutes at 37°C with 200 μL of substrate. The optical densities (ODs) were read in a spectrophotometer at 490 nm using a 620 nm reference. The samples and controls were tested in duplicate and the mean OD of each duplicate testing was used. The samples that exhibited more than 25% variation between the two ODs were considered invalid and retested. As recommended by the manufacturer, the lower detection cutoff was established for each run and corresponded to the mean OD of the 2 negative controls plus 0.040. A sample was considered positive only when the mean OD was higher than the cutoff OD of the corresponding run.

Detection of HCV-RNA by RT-PCR

Total RNA was isolated from 100-μL serum or plasma using High Pure Isolation kit from Roche, Germany, according to the method given in instructions manual. It was immediately used in RT-PCR experiments or stored at -70°C. Five μg of the isolated RNA was applied to reverse transcription and nested PCR with primers located in the highly conserved 5` noncoding region (5` NCR) using BIOHCV kit (B&M Labs., Madrid, Spain). The reverse transcription mixture was incubated for 1 min at 85°C, followed by 30 min at 60°C. First PCR was performed in whole content after adding 40 μl of HCV amplification mixture. Thermal cycler was programmed as follows: 85°C for 30 s, 94°C for 2 min followed by 40 cycles of 94°C for 30 s, 55°C for 30 s and 72°C for 30 s and then incubation of samples for 5 min at 72°C. Five μL of first PCR product was subjected to nested PCR using nested PCR mixture containing second round primer and enzymes etc. The protocol on thermo cycler was the same as mentioned in first PCR. The PCR product was subjected to electrophoresis in 20g/L agarose containing ethidium bromide and was visualized under UV. A positive control provided in the kit was used as control. All positive and negative controls were tested in parallel with test samples throughout the entire procedures, starting with RNA extraction.

Detection of TTV-DNA

Total DNA was extracted from 200 μL of serum using DNA isolation kit from Roche Diagnostics GmbH, Germany: This isolation method utilizes the ability of nucleic acids to absorb to silica (glass) in the presence of a chaotropic salt. Serum sample was treated with buffer containing proteinase K & silica particles where nucleic acids are bound to silica surface of magnetic particles. Since the binding process is specific for nucleic acids, the bound nucleic acids are purified from salts, proteins and other impurities by washing steps. A low salt buffer is used to elute the DNA. Using 5μL of the DNA solution as a template, TTV DNA of the open reading frame (ORF-1) sequence, was detected by PCR employing the semi-nested primers reported by Okamoto et al.[12] The first-round PCR was carried out for 35 cycles (94°C, 45 s; 60°C, 45 s; 72°C, 60 s; with additional 7 min in the last cycle) using NG059 primer (sense:5'- A C A G

A C A G A G G A G A A G G C A A C A T G - 3') and NG063 primer (antisense: 5' C T G G C A T T T T A C C A T T T C C A A A G T T -3'). Thereafter, the second-round PCR was carried using 1μL of the first-round PCR product, NG061 primer (sense: 5'-G G C A A C A T G Y T R T-G G G A T A G A C T G G-3', where Y= T or C; R = A or G), and NG063 primer for 25 cycles under the same conditions described above. The PCR product (10 μL) was electrophoresed on 20g/L agarose gel, stained with ethidium bromide, and observed under ultraviolet light. The product of the first-round PCR was 286 base pairs (bp) and that of the second-round PCR was 271 bp.

Diagnosis of viral hepatitis

Liver function tests including transaminases levels (AST and ALT) in serum were performed on autoanalyser Hitachi-917 using the established techniques. Similarly, hemogram and coagulation profiles were performed using routine assays established in our laboratory. The diagnosis of different types of viral hepatitis was established as follows: The diagnosis of HAV infection was confirmed by the presence of IgM anti-HAV in serum. HBV infection was established by finding IgM anti-HBc in sera of AVH and FHF patients and by the persistent HBsAg antigenemia in sera of CVH and cirrhosis cases. Similarly, anti-HCV and IgM anti-HDV in sera samples were used for the diagnosis of HCV and HDV infections, respectively. All anti HCV sera were also tested for HCV-RNA using nested PCR as described above. However, final diagnosis of HCV was based on anti HCV antibodies in serum. Active or recent HEV infection was diagnosed by the presence of IgM anti- HEV in serum. Sera positive for HBsAg but negative for all other viral markers were labeled as HBV-carriers. Absence of all the markers including HBsAg labeled the patients with hepatitis non-ABCDE infection on exclusion criteria. HCV-RNA in serum was used to confirm active HCV infection.

Results

Sera samples from a total number of 364 patients with both acute and chronic liver diseases were analyzed for markers of various hepatitis viral infections. The different liver disease groups included in this study were AVH, CVH, cirrhosis of liver and FHF. Hepatitis viral infections whose markers were tested in these disease groups were HAV, HBV, HCV, HDV and HEV infections. Since many patients still remain unaccounted by the known A-E viral infections, we tested for marker of TTV (TTV-DNA) in some patients in each group. The results of analysis are shown in Table 1.

Table (1). Etiological profile of viral hepatitis in different liver diseases groups

Type of Infection	AVH			CVH			Cirrhosis			FHF		
	No. tested	Positive		No. tested	Positive		No. tested	Positive		No. tested	Positive	
		No.	%		No.	%		No.	%		No.	%
Hepatitis - A	120	0	Nil	120	0	Nil	84	0	Nil	40	0	Nil
Hepatitis – B	120	86	71.66	120	57	47.5	84	40	47.61	40	16	40
Hepatitis – C	120	30	25	120	58	48.33	84	52	61.9	40	5	12.5
Hepatitis – D	120	0	Nil	120	0	Nil	84	0	Nil	40	0	Nil
Hepatitis – E	120	42	35	120	9	7.5	84	12	14.29	40	19	47.5
Hepatitis - non A-E	120	13	10.83	120	22	18.33	84	27	32.14	40	4	10

It is clear from the results that adult population in India rarely shows HAV infection. Similarly, HDV infection is also very rare in this country. Of course, HBV, HCV and HEV infections are quite common and are found in all categories of liver diseases. Moreover, co-infection with two or more viruses in these disease groups is also common. A low percent population in each patient group remains without any marker and is labeled as non-A-E hepatitis group. To evaluate the prevalence of TTV in Indian population, we analyzed sera for TTV-DNA and found TTV infection also quite common in all liver diseases. TTV-DNA was detected in 17 of 60 (28%) patients with AVH, 11 of 65 (17%) with CVH, 29 of 58 (50%) with cirrhosis, 4 of 32 (8%) with FHF and 28 of 100 (28%) healthy controls, respectively. Whether TTV causes hepatitis, is still uncertain, however it was tested to assess its impact on HCV diagnosis.

All the patients were analyzed for three different markers of HCV infections i.e. anti-HCV antibodies, HCV-RNA and HCV core antigen. This was aimed to find out the diseasewise diagnostic potential of each marker and also assess the impact of co-infection on HCV diagnosis by these methods. Relative presence of HCV core, HCV-RNA and anti-HCV in different liver diseases are shown in Table 2.

In AVH, HCV core was detected in 22 of 120 (18.3%) cases, HCV-RNA in 22 of 120 (18.3%) cases and anti-HCV in 7 of 120 (5.83%) cases, respectively. In CVH, these 3 markers were detected in 16 of 120 (13.3%) cases, 16 of 120 (13.3%) cases and 56 of 120 (46.6%) cases, respectively. Of 84 cases with cirrhosis, HCV core, HCV-RNA and anti-HCV antibody were detected in 20 cases (23.3%) each. Similarly, of 40 FHF patients, HCV core was present in 8 (20%) cases, HCV-RNA in 7 (17.5%) cases and anti-HCV in 4 (10%) cases, respectively. All the sera samples from 100 healthy controls analyzed under similar conditions were found to be negative for all hepatitis markers including HCV markers mentioned above.

In each disease group, detection of each HCV marker could not show any difference arising out of the absence or presence of other viral co-infection. This implies that course of development of HCV-marker and also its detection remains unaffected by the simultaneous co-infection of patients with other A-E hepatitis viruses in the liver disease groups.

Table (2). HCV core protein in relation to anti-HCV antibodies in different liver diseases.

Disease Group	*No. tested*	*HCV Core positivity*		*HCV-RNA positivity*		*Anti-HCV positivity*	
		No.	*(%)*	*No.*	*(%)*	*No.*	*(%)*
Acute viral hepatitis	120	22	18.3	22	18.3	7	5.83
Chronic viral hepatitis	120	16	13.3	16	13.3	56	46.6
Cirrhosis of liver	84	20	23.8	20	23.8	20	23.8
Fulminant hepatic failure	40	8	20.0	7	17.5	4	10.0
Control	100	0	NIL	0	NIL	0	NIL

Discussion

There are three common assay procedures used to diagnose HCV infection. These include anti-HCV antibody assay, HCV-RNA detection and recently introduced HCV core antigen assay. Reports from various studies indicate the presence of HCV core protein in nearly 80-92% patients positive with anti-HCV antibody.[13,14] At the same time, the concordance between HCV core and HCV-RNA was noted upto 93-95%.[14] In few studies, HCV core assay has been reported to be less sensitive than anti-HCV or HCV-RNA assay, though all those reports found it to be more specific as compared to these techniques.[14] Total HCV core antigen quantification is an accurate and precise indirect marker of HCV replication in HCV infected patients. However, HCV core assay cannot detect HCV replication for HCV-RNA value below 20,000 IU/ml.[15] Despite several merits of HCV core assay over HCV-RNA detection by PCR, core assay has been reported to be less sensitive in different studies. Whereas the sensitivity of HCV-RNA assay was found to be 99%, that of HCV core assay was noted as 98%.[16] Tanaka et al[17] also reported a sensitivity of 98% for the core assay. It was comparable to that of HCV-RNA. However, Zanetti et al[18] found only 82% sensitivity of core as compared to that of HCV-RNA assay.

The study by Lorenzo et al., [19] also assessed the clinical usefulness of the hepatitis C core antigen assay for monitoring of patients being treated for chronic HCV infection. Levels of HCV-RNA and HCV core antigen were determined simultaneously and in parallel, to compare both techniques. A good linear correlation was observed between both techniques. Maximum correlation, with significant difference, was found between patients infected with the 1a genotype and other genotypes. In conclusion, the HCV core antigen assay is useful for the diagnosis of early infection; however, its use for determining the exact timing of viral elimination during treatment is clearly unsuitable.[19]

In view of these contradicting reports about the usefulness of a single type of assay in diagnosing HCV infection in patients with given clinical condition, there was a need to assess each assay system for its optimum diagnostic utility for a particular liver disease condition.

We analyzed our cases in context of various finding reports on comparative diagnostic significance of these HCV markers and related our results with grade or type of disease presentation. The results of this study could draw some important conclusions. The diagnostic value of an individual HCV marker depends not only on comparative sensitivity or specificity of the assay, but at the same time, on presentation or severity of liver disease also. In this study, we found HCV core protein and HCV-RNA in equal proportion (18% each) and in the same patients belonging to AVH group. On the other hand, anti-HCV was detected in less number (6%) and different cases than those having presence of HCV core protein or HCV-RNA. In CVH, again an interesting picture emerged where HCV core protein and HCV-RNA were not only detected in same proportions and similar cases, but were also present in significantly low percent population as compared to overall anti-HCV prevalence (47%). Thus, whereas acute presentation predominantly has detectable HCV core and HCV-RNA, it cannot be diagnosed by anti-HCV antibody that possibly could not develop during this period. In chronic cases, the picture is reverse and thus, HCV diagnosis may be better made with the presence of anti-HCV antibodies in serum. Chronic persistence of disease provides enough time to immune system to respond and produce anti-HCV antibodies. In patients with cirrhosis, all the three markers were detected in equal proportions and same cases. Therefore, we can safely use any one marker to diagnose HCV infection in patients with cirrhosis of liver. In FHF, anti-HCV antibody was again detected in low percent populations of patients as compared to HCV core and HCV-RNA. Moreover, HCV core was detected in one additional case where HCV-RNA was absent. Although it is not possible to explain why HCV core was present without HCV-RNA; may be due to less sensitivity of assay; however, low incidence of anti-HCV in FHF patients is not an unlikely phenomenon, particularly because presentation of disease is too prompt to incite immune response against viral related epitopes. Antibody formation takes some time which is much longer than the time taken in fulminant presentation of liver disease. This may be the possible reason of low anti-HCV prevalence as compared to HCV core and

HCV-RNA, the marker of live / replicating HCV virions, in FHF patients. Our findings in AVH and FHF have relevance with the report[4] which shows that HCV core and HCV-RNA detect HCV infection between 40 and 50 days earlier than the current third generation HCV antibody assay. Besides, this study also shows that HCV core closely tracks HCV-RNA dynamics.

Our study also focuses on the possible impact of viral co-infection on HCV diagnosis by these assays. A high proportion of liver diseases in India, have viral co-infection than a single infection. Since India is endemic for nearly all types of hepatitis viral infections, co-infection by two or more viruses in all types of liver diseases is a common phenomenon. To evaluate the effect and relation of co-infection with HCV detection by these techniques in different forms of liver diseases, we applied all the assays in all liver diseases and found a straight relation between diagnostic value of assays system and the type of liver disease, rather than a visible effect of co-infection of HCV with other hepatitis causing viruses. This implies that detection of HCV infection depends on the assay used in a given clinical condition and not on the simultaneous presence of other hepatitis viruses.

From this study, it is concluded that choice of assay to detect HCV infection in different liver diseases should be made with the type of liver disease. Whereas it is more appropriate to use HCV core or HCV-RNA based assays for acute liver diseases, anti-HCV antibody based screening for HCV is the best choice for patients with chronic liver diseases and cirrhosis of liver. Using any one assay to diagnose HCV infection in all types of liver diseases may miss a significant proportion of HCV infected cases and so choice of technique be made cautiously with presentation of liver diseases.

To evaluate the impact of TTV co-infection with HCV on the outcome of disease and diagnosis of HCV in different categories of patients needs more detailed studies which are under process at our centre.

Acknowledgement

The authors thank and appreciate the financial aid provided by ICMR, New Delhi, India to conduct this study. Authors are also thankful to Mrs. Suman Rawat for preparing this manuscript and Mr. Bijendra to help in test performance.

References

1. Murphy FA, Fauquet CM, Bishop DHL, Ghabrial SA, Jarvis AW, Martelli GP, Mayo MA and Summers MD1995. Virus Taxonomy - The Classification and Nomenclature of Viruses: Sixth Report of the International Committee on Taxonomy of Viruses. Springer-Verlag, Vienna pp 424-426.
2. Choo QL, Kuo G, Weiner AJ, Overby LR, Bradley DW, Houghton M.Isolation of a cDNA clone derived from a blood-borne non-A, non-B viral hepatitis genome. Science. 1989; 244: 359 - 362.
3. Poynard T, Yuen MF, Ratziu V, Lai CL. Viral hepatitis C. Lancet. 2003; 362: 2095 -2100.
4. Seme K, Poljak M, Babic DZ, Mocilnik T, Vince A. The role of core antigen detection in management of hepatitis C: a critical review. J Clin Virol. 2005; 32: 92 - 101.
5. Pawlotsky JM. Use and interpretation of virological tests for hepatitis C. Hepatology. 2002; 36 (5 Suppl 1) : S65 - 73.
6. Pawlotsky JM. Clinical virology on hepatitis C. In: Marcellin P, editor. Management of patients with viral hepatitis. Paris: APMAHV; 2004, p 21 – 34.
7. Alter HJ. Hepatology highlights Hepatology. 2002; 35: 741 - 742.
8. Mondelli MU. Monitoring response to antiviral treatment by serum hepatitis C virus core antigen: too early to take shortcuts? J Hepatol. 2004; 40: 536 - 538.
9. Simmonds P. Viral heterogeneity of the hepatitis C virus. J Hepatol. 1999; 31 Suppl 1 : 54 - 60.
10. Tandon BN, Joshi YK, Tandon M. Acute liver failure: experience with 145 patients. J.Clin Gastroenterol 1986; 8: 664 - 668.
11. Acute and chronic hepatitis revisited. Review by International Study Group on chronic hepatitis Lancet 1977; ii: 914 - 919.
12. Okamoto H, Nishizawa T, Kato N, Ukita M, Ikeda H, Lizuka H, Miyakawa Y, Mayumi M. Molecular cloning and characterization of a novel DNA virus (TTV) associated with posttransfusion hepatitis of unknown etiology. Hepatology Research 1998; 10: 1 – 16.

13. Ruff A, Guarnieri F, Stageley-O'Carroll K, Siliciano R, August J. The enhanced immune response to the HIV gp 160-LAMP chimeric gene product targeted to the lysosome membrane protein trafficking pathway. J. Biol. Chem. 1997; 272: 8671 - 8678.
14. Kashiwakuma T, Hasegawa A, Kajita T, et al. Detection of hepatitis C virus specific core protein in serum of patients by a sensitive fluorescence enzyme immunoassay (FEIA). J. Immunol. Methods 1996; 190: 79 - 89.
15. Arduino JM, Stuver SO, Spiegelman D, et al. Assessment of markers of Hepatitis C virus infection in a Japanese adult. J. Infect. Dis. 2001; 184: 1229 - 1235.
16. Megali Bouvier A, Patel K, Dahari H, Beaucourt S, et al. Clinical utility of total HCV Core antigen quantification: A new indirect marker of HCV replication. Hepatology 2002 ; 36: 211 - 218.
17. Tanaka E, Ohue C, Aoyagi K, Yamanguchi K, Yagi S, Kiyosawa K and Alter HJ. Evaluation of a new enzyme immunoassay for hepatitis C virus (HCV) core antigen with clinical sensitivity approximating that of genomic amplification of HCV RNA. Hepatology 2001; 32: 388 - 393.
18. Zanetti AR, Romano I, Brunetto M, Colombo M, Belati G and Tackney C.Total HCV core antigen assay: a new marker of hepatitis C viremia for monitoring the progress of therapy. J. Med. Virol. 2003; 70: 27 - 30.
19. Lorenzo J, Castro A, Aguilera A, Prieto E, Lopez-Calvo S, Regueiro B, Pedreira J. Total HCV core antigen assay. A new marker of HCV viremia and its application during treatment of chronic hepatitis C. J. Virol. Methods. 2004; 120: 173 -177. PMID: 15288960.