OPEN ACCESS Journal of Applied Sciences

ISSN 1812-5654
DOI: 10.3923/jas.2018.

Research Article
Correlations of Viral Load Toward HCV Core Antigen and Aminotransferase in Hepatitis C Virus Genotype 1 Infection

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Abstract

Background and Objective: Hepatitis C becomes a problem in liver disease over the world. The diagnosis of hepatitis C infection is carried out by detecting of antibodies against the virus using an immunoassay but unreliable in the seroconversion phase of infection, due to the long window period. Real time PCR examination and hepatitis C virus core antigen (HCVcAg) detection by ELISA offer an alternative method to detect the virus infection. The aim of this study was to identify the viral load correlation with the number of HCV core antigen and aminotransferase levels in HCV infection. **Materials and Methods:** This study combined the molecular examination for determining the genotype of HCV and viral load with q-PCR and ELISA method for investigating the HCVcAg. The correlation between number of viral load with HCVcAg and Alanine aminotransferase (ALT) and aspartate transaminase (AST) were analyzed with Pearson's correlation test. **Results:** There was a significant correlation between the number of viral load with HCVcAg levels in hepatitis C patients but levels of ALT (49.71 U mL^{-1}) and AST (31.83 U mL^{-1}) serum were not correlated with the number of viral load. **Conclusion:** HCVcAg can be applied as an alternative examination to detected viral load in hepatitis C infection to prevent the infection of HCV lead to cirrhosis and even hepatocellular carcinoma.

Key words: Viral load, hepatitis C virus core antigen, alanine aminotransferase, aspartate transaminase, genotype-1, hepatitis C virus

Citation: Eti Yerizel, Almurdi and Gusti Revilla, 2018. Correlations of viral load toward HCV core antigen and aminotransferase in hepatitis C virus genotype 1 infection. J. Applied Sci., CC: CC-CC.

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Republic of Indonesia has a substantial burden of HCV infections. Efforts have been made and supported with increasing commitment by the government[1]. The report of IRC (Indonesian Red Cross) revealed in 2010-2014, the individual diagnosis of HCV annually through blood donation between[2] 8,400 and 12,100. Based on IRC and the Indonesian National Health Survey (Riskesdas) data, it was estimated that there were 1,284,000 (447,000–2,047,000) viremic individuals[3] in 2014. Total viremic infections were estimated to increase slightly to 1,303,000 by 2023 before returning to 1,288,000 by 2030. In 2014, an estimated 9% of the viremic population experienced cirrhosis, HCC or liver transplant eligibility[4]. Infection with HCV virus can either resolve spontaneously or develop into chronic liver disease. Chronic hepatitis can lead cirrhosis and even hepatocellular carcinoma. HCV infects about 3% of the world's population, the WHO estimates that approximately 200 million people worldwide are infected with HCV[5-7].

Hepatitis C virus is an envelope RNA virus, 50 nm in size, belonging to the family Flaviviridae. The virus has 9600 nucleotides, consisting of 3000 amino acids, divided into 6 genotypes based on phylogenetic analysis. The virus consists of 3 structural proteins, namely core protein, envelop E1 and E2 and seven nonstructural proteins (NS), NS1, NS2, NS3, NS4A, NS4B, NS5A and NS5B [8].

The diagnosis of hepatitis C virus infection is currently done by detecting the presence of antibodies against the virus by using enzyme immunoassay but antibody detection is not a reliable marker of the infection preseroconversion phase, since the window period ranges from 58 up to 70 days[9-10]. The examination to detect anti-HCV cannot distinguish between acute infection and chronic infection, because IgM anti-HCV levels vary in acute infection, whereas the antibody is still detected in chronic infection[11].

The examination of HCV RNA by reverse transcriptase PCR is a very sensitive method for detecting HCV infection and established as a gold standard. Amplification of nucleic acids with PCR is a reliable examination with high sensitivity and specificity but this method consumes much time, cost and complete equipment requirements. In contrast, the ELISA examination for the detection of HCV core antigens is simpler and inexpensive[12-13].

Several study were previously performed to investigate the correlation of HCV with ALT[14]. However, the RNA level of HCV does not correlate directly with liver injury and the duration of infection. The ALT examination only describes the degree of liver necroinflammation which can also be caused by non-infectious factors such as induced drugs, alcohol, etc. Beld *et al.*[15] investigated the difference HCV RNA load profiles following seroconversion among injecting drug users without correlation with HCV genotype and serum alanine aminotransferase levels, the study observed that during seroconversion there is a decrease in the amount of virus in the blood because the initial formation of antibodies against the virus. The current study determines the number of viruses in the beginning of infection and for follow-up therapy. Quantitative examination of PCR to determine viral load is very useful for measuring the amount of virus in the blood of people with HCV, HBV and HIV infection, because this examination provides initial information on viral load, reduction in the number of viruses during therapy and to determine the response to persistent virology. Although, the viral load examination using the PCR method is the intended method, the assay requires a lot of time, high costs and complete equipment, while the HCV core antigen examination only uses the Elisa format and affordable and can be performed mainly in the developing country.

Liver is the central of protein synthesis and amino acids are distributed into other biochemical pathways. Liver is one of organ with the most abundant aminotransferase contain. Serum aminotransferase is a sensitive marker to detect the damage in liver. Alanine aminotransferase (ALT) is found in large quantities in liver cells and is excreted to the circulation during the damage occurs, it's due to the inflammation reaction which caused by the infection or non-infectious things. ALT is found in the cytosol, as a liver enzyme widely used as an indicator of hepatocellular damage in acute and chronic hepatitis[16-18]. The aim of this study was to identify the viral load correlation with the number of HCV core antigen and aminotransferase levels in HCV infection.

MATERIALS AND METHODS

A total of 75 samples were collected in all branches of Red Cross Indonesia and in Clinical Pathology Laboratory, Dr. M.D. General Hospital during June, 2014-December, 2015. The analysis of ELISA was conducted in Regional Health Laboratory, Padang. Molecular analysis was conducted in Biomedical Laboratory, Faculty of Medicine, Andalas University, Padang.

Preparation of samples: Blood samples were collected from each patient who visited in clinical pathology laboratory for anti-HCV positive diagnosis using third-generation HCV enzyme immunoassay (Abbot Laboratories, USA). Serum was separated and stored at -80°C. The study was approved by

Ethics Committee of Medical Faculty, Andalas University, Padang, Indonesia.

Viral RNA extraction and RT-PCR: Serum samples stored at -80°C were retrieved for analysis. The HCV RNA was extracted from 140 µL serum or plasma using QIAamp viral RNA mini kit (Qiagen, German) according to the manufacturer's protocol and stored at -80°C until further analysis.

RT-PCR was performed using one-step RT-PCR kit (Invitrogen, Inc). The NS5B region was amplified by PCR to identify the HCV genotype with forward primers NS5B-1: 5'-TATGAYACCCGYTGCTTTGAC-3' and reverse primers NS5B-2: 5'-GAGGAGCAAGATGTTATCAGCTC-3'[19]. DNA amplification was performed for 40 cycles each consisting of 94°C for 15 sec, 55°C for 30 sec and 72°C for 1 min in a thermal cycler (Biorad, USA). 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, USA). The following cycling parameters were used for 35 cycles.

Quantification of HCV RNA was performed using the Bio-Rad HCV RT-PCR kit according to the manufacturer instructions.

DNA purification and sequencing: Amplicons were purified by using the QIAquick PCR purification kit (Qiagen, German) and analyzed by ethidium bromide 2% agarose gel electrophoresis. Samples showing a band of the appropriate size (449 bp) were further analyze by DNA sequencing in Macrogen, Republic of Korea. The HCV genotyping was based on NS5B, the reference sequences were retrieved from the NCBI GeneBank.

Alignment of sequence target: The sequence of DNA was edited using Geneious's version R 7.

HCV core antigen assay: Sera samples were assayed for HCV core antigen according to the manufacturer's instructions using an EIA kit (Cell-Biolabs, USA). Briefly, 100 µL of samples and controls to anti-HCVc Ag antibody were coated in plate, incubated for 2 h and wash five times with 250 µL wash buffer. One hundred microliters of FITC-Conjugated anti HCVcAg were added to each well, incubated and washed, 100 µL HRP-conjugated anti-FITC monoclonal antibody was added to all well. A substrate solution was added and stopped the enzyme reaction by adding stop solution. The absorbance of each well was measured using ELISA reader at 450 nm.

Biochemical tests: Liver function tests, including alanin aminotransferase (ALT) and aspartate aminotransferase (AST) were performed on an autoanalyzer (Selectra E-Mrk, Germany) by manufacture' protocol.

Statistical analysis: Statistical analysis were performed using SPSS 15.0, the correlation between parameters was analyzed by Pearson's correlation test. Pooled data are presented as means. Data were compared using Student t-test. p-values <0.05 were considered statistically significant.

RESULTS

In this study, 75 samples were obtained from anti-HCV positive blood donors and from clinical laboratory, 53 samples (70.67%) with HCV specific positive signal by PCR, consist of 39 genotype-1 (78.0%), 2 genotype-2 (4.0%), 9 genotype-3 (18.0 %) and three samples were undetermined.

The number of viral load of patients infected with hepatitis genotype-1 virus (6.82 log copy/mL) was significantly different (p = 0.000) compared with other genotypes (6.26 log copy mL^{-1}). The number of HCV core antigens (HCVcAg) of patients infected with hepatitis C virus genotype 1 did not differ significantly with other HCVcAg (p>0.169) (Table 1). ALT serum levels of patients infected with hepatitis C virus were slightly above normal, while AST serum levels were within normal limits.

A significant correlation was showed in viral load of patients infected by genotype-1 hepatitis C virus with HCVcAg (p = 0.000) with r = 0.773. This correlation indicated positive linear, the higher the viral load was followed by the elevation of HCV core antigen levels.

A significant correlation (p = 0.001) between viral load of all genotype hepatitis C-infected patients with HCVcAg levels presents moderate correlation (r = 0.474) as shown in Fig. 1a and b.

There was no significant correlation (p = 0.10) between hepatitis C viral load with serum ALT level, the strength of correlation was moderate (r = 0.426) and no significant correlation (p = 0.126) between hepatitis C viral load with AST serum levels and the strength of correlation was less (r = 0.259) (Fig. 2a, b).

Table 1: Average of several parameters on HCV infection

Parameters	Mean (SD)	p-value
Viral load (log copy mL^{-1})		
Genotype 1	6.82±6.85	0.000
Other genotype (2/3)	6.26±6.30	
HCVcAg (log pg mL^{-1})		
Genotype 1	5.78±5.09	0.169
Other genotype (2/3)	5.74±5.27	
ALT (U mL^{-1})	49.71±28.8	
AST (U mL^{-1})	31.83±18.1	

ALT: Alanine aminotransferase and AST: Aspartate transaminase

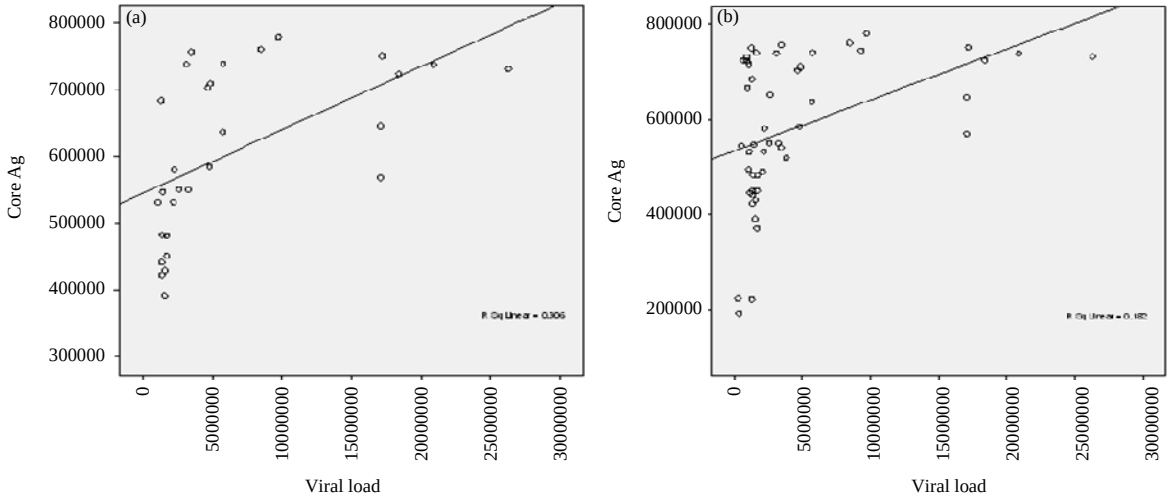

Fig. 1(a-b): (a) Relationship between viral load HCV and viral load of all genotype and (b) Relationship between genotype-1 and HCVcAg (r = 0.773, p <0.05) and HCVcAg (r = 0.474, p <0.05)

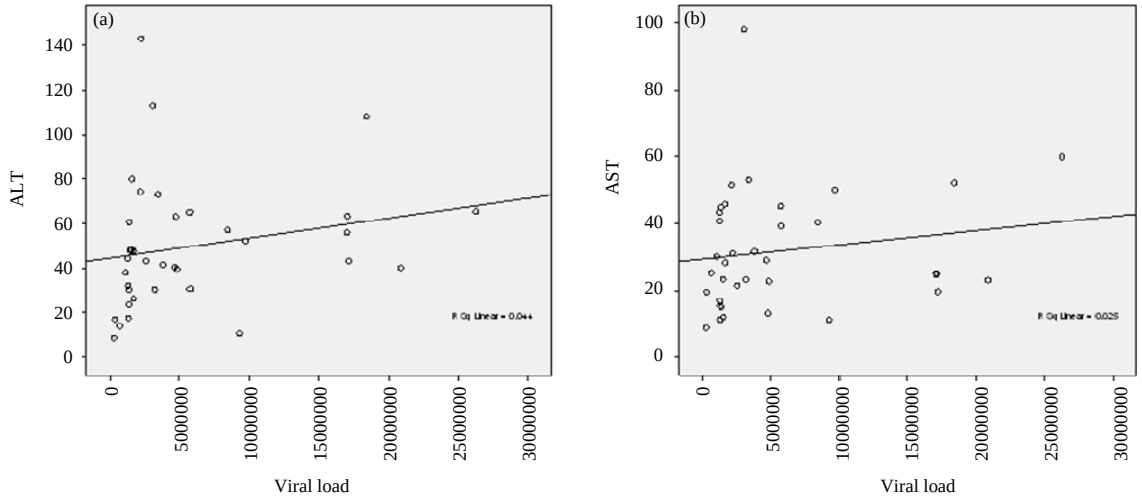

Fig. 2(a-b): (a) Correlation between serum ALT and AST and viral load (r = 0.426, p >0.05) and (b) Correlation between serum viral load (r = 0.259, p >0.05)

DISCUSSION

Quantitative examination of PCR to determine viral load is very useful for measuring the amount of virus in the blood of people with HCV, HBV and HIV infection, because this examination provides initial information on viral load, reduction in the number of viruses during therapy and to determine the response to persistent virology. Although the viral load examination using the PCR method is the intended method, the assay requires a lot of time, high costs and complete equipment, while the HCV core antigen examination only uses the Elisa format and affordable and can be performed mainly in the developing country.

Hepatitis C virus infection has always been diagnosed with anti-HCV test, performed in a window period between infections with seroconversion for 58-70 days. For early diagnosis of infection, developed countries have applied a nucleic acid test (NAT) to avoid the risk of infection associated with blood transfusion. However, the NAT test are relatively expensive, the equipment is sophisticated and requires trained personnel [20].

The HCV RNA detection can identify hepatitis C virus infection several weeks to several months before the response of antibody is detected. This examination is also useful as a marker of treatment efficacy characterized by a quantitative reduction in the number of viral load and is used as a gold

standard. The persistence of HCV after acute infection is often undiagnosed causing chronic hepatitis, liver cirrhosis and even death. Spontaneous clearance of HCV infection is only found in about 17% of untreated patients found to be anti-HCV reactive with negative HCV RNA. The benefit of HCV protein cores test is to use the immunology format, requiring no sample processing such as molecular examination, no carry-over risk and relatively cheap checking rates.

In this study, there was a significant difference between the numbers of viral load of patients infected with genotype-1 hepatitis virus with other genotypes. Gremion and Cerny[21] reported that genotype-1 HCV is often associated with severe liver disease, while the genotype-2 viral infection is found in asymptomatic careers. Low number of viral load can also be found in patients undergoing antiviral therapy or in patients with hemodialysis; because the particles of virus can be broken as they pass through the dialysis membrane[22].

There was a significant correlation between the numbers of viral load of hepatitis C-infected patients with HCVcAg levels, because HCVcAg can be found in serum of HCV-infected patients in virion and also in core free RNA protein form. Several studies have indicated that HCVcAg kinetics is proportional to HCV RNA in various infections and HCVcAg concentrations with HCV RNA has a good correlation. HCVcAg examination performance is similar to HCV RNA examination which can decrease window period, besides it can also be used to monitor antiviral therapy in patients with chronic infection[23].

The detection and quantification of HCV RNA has become the standard method for diagnosis and monitoring of chronic HCV infection and for assessing the response of antiviral treatment to HCV infection[24]. In addition, RNA viral is also used as an acute infection marker before anti-HCV seroconversion. HCVcAg examination is recommended as an alternative examination to determine the viral load in patients with hepatitis C virus infection[25].

The liver is an organ that contains many aminotransferase enzymes, this enzyme will be released in large quantities to the circulation during liver damage due to inflammation. High level of ALT serum in blood indicates the occurrence of necro-inflammation. Liver damage appears in the process of immune clearance, long response of immune processes will increase the risk of liver cirrhosis. The examination of ALT enzymes provides important information about the state of liver function. According to Fabris *et al.*[26] that the levels of ALT enzyme increase after 10-14 weeks of initial infection and the elevation reaches up to 20 times of the normal limit level but a slight increase in ALT levels is often found mainly in chronic infections, as found in this study.

In this study, there was no significant correlation between viral load and aminotransferase levels, both ALT and AST levels. This could be due to inadequate of immune response, especially in chronic infection. According to Maheshwari *et al.*[27] the failure to produce an effective immune response during acute infection is a key factor in the development of HCV infection becomes chronic hepatitis.

The average levels of ALT was higher compared with AST levels in the study, it is caused by ALT as a cytosolic enzyme that is found with high concentrations and more specific in the liver. AST enzymes are found in the liver, also in heart muscle, skeletal muscle, kidney, brain, pancreas and lung. This enzyme is less specific for the liver.

This study is very useful for determining the number of viruses in the beginning of infection and for follow-up therapy. When quantitative viral load testing is not available, HCV core antigen examination can be used as an alternative assay.

CONCLUSION

Based on the results of the study, it can be concluded that a significant correlation between hepatitis C viral load and HCV core antigen levels indicates that HCVcAg examination can be used as an alternative assay to determine viral load in hepatitis C viral infection, in order to follow up therapy.

SIGNIFICANCE STATEMENT

The examination of HCV RNA by reverse transcriptase PCR is a very sensitive method but consumes much time, cost and complete equipment requirements. In contrast, the ELISA examination for the detection of HCV core antigens is simpler and inexpensive. By combining the molecular examination for determining the genotype of hepatitis C virus and viral load with q-PCR and ELISA method for investigating the core HCV (HCVcAg) antigen, this study can be the novel alternative to prevent the infection of HCV lead to cirrhosis and even hepatocellular carcinoma.

ACKNOWLEDGMENT

The authors thank to Ministry of Research and Technology through DIPA fund of Andalas University for financing of fundamental research in 2016 with contract number: 105/BPPT/ PNP-FK-Unand-2016.

REFERENCES

1. Muljono, D.H., 2017. Epidemiology of hepatitis B and C in Republic of Indonesia. Euroasian J. Hepatogastroenterol., 7: 55-59.

2. Liakina, V., S. Hamid, J. Tanaka, S. Olafsson and A.I. Sharara *et al*., 2015. Historical epidemiology of Hepatitis C Virus (HCV) in select countries-volume 3. J. Viral Hepat., 4: 4-20.
3. Ministry of Health Republic of Indonesia, 2010. Report on national basic health research (RISKESDAS) 2007. The National Institute of Health Research and Development, Jakarta.
4. NIHRD., 2014. National report on basic health research (RISKESDAS) 2013. Ministry of Health, National Institute of Health Research and Development, Jakarta, Indonesia.
5. Cheng, C.H., H.L. Chen, I.T. Lin, C.H. Wu, Y.K. Lee, M.W. Wong and M.J. Bair, 2015. The genotype distribution of hepatitis C in southeastern Taiwan: Clinical characteristics, racial difference and therapeutic response. Kaohsiung J. Med. Sci., 31: 597-602.
6. Boltjes, A., D. Movita, A. Boonstra and A.M. Woltman, 2014. The role of Kupffer cells in hepatitis B and hepatitis C virus infections. J. Hepatol., 61: 660-671.
7. Luo, S.C., C.C. Wu, S.B. Jheng and Z.Y. Huang, 2016. Spontaneous remission of hepatocellular carcinoma without any treatment. J. Cancer Res. Pract., 3: 128-131.
8. Irshad, M., I. Khushboo, S. Singh and S. Singh, 2008. Hepatitis C Virus (HCV): A review of immunological aspects. Int. Rev. Immunol., 27: 497-517.
9. Rustgi, V.K., 2007. The epidemiology of hepatitis C infection in the United States. J. Gastroenterol., 42: 513-521.
10. Maudar, K.K., P. Gandhi, P.K. Mishra, S. Varshney, R. Punde and A. Bhargav, 2012. Novel approach for quantification of hepatitis C virus in liver cirrhosis using real-time reverse transcriptase PCR. J. Gastrointestinal Surg., 16: 142-147.
11. Ansaldi, F., B. Bruzzone, G. Testino, M. Bassetti, R. Gasparini, P. Crovari and G. Icardi, 2006. Combination hepatitis C virus antigen and antibody immunoassay as a new tool for early diagnosis of infection. J. Viral Hepat., 13: 5-10.
12. Sasase, N., S.R. Kim, K.I. Kim, M. Taniguchi and S. Imoto *et al*., 2008. Usefulness of a new immunoradiometric assay of HCV core antigen to predict virological response during PEG-IFN/RBV combination therapy for chronic hepatitis with high viral load of serum HCV RNA genotype 1b. Intervirology, 51: 70-75.
13. Kowala-Piaskowska, A., I. Mozer-Lisewska, M. Figlerowicz, L. Machowska and W. Służewski, 2004. Interleukin 6 and 12, alanine aminotransferase activity and HCV viral load in children with chronic hepatitis C treated with interferon and ribavirin. Inflammation, 28: 319-325.
14. Liu, P., Y. Li and C.M. Sun, 2009. Correlations of serum hepatitis C virus RNA and alanine transaminase with liver histopathological changes in patients with chronic hepatitis C. Lab. Med., 40: 167-169.
15. Beld, M., M. Penning, M. McMorrow, J. Gorgels, A. van den Hoek and J. Goudsmit, 1998. Different Hepatitis C Virus (HCV) RNA load profiles following seroconversion among injecting drug users without correlation with HCV genotype and serum alanine aminotransferase levels. J. Clin. Microbiol., 36: 872-877.
16. Limdi, J.K. and G.M. Hyde, 2003. Evaluation of abnormal liver function tests. Postgrad. Med. J., 79: 307-312.
17. Wong, G.L.H., V.W.S. Wong, P.C.L. Choi, A.W.H. Chan and A.M.L. Chim *et al*., 2008. Evaluation of alanine transaminase and hepatitis B virus DNA to predict liver cirrhosis in hepatitis B e antigen-negative chronic hepatitis B using transient elastography. Am. J. Gastroenterol., 103: 3071-3081.
18. Ergunay, K., B. Sener, A. Alp, J. Karakaya and G. Hascelik, 2011. Utility of a commercial quantitative hepatitis C virus core antigen assay in a diagnostic laboratory setting. Diagn. Microbiol. Infect. Dis., 70: 486-491.
19. Ozkan, T.B., F. Budak, G. Erdemir, T. Ozgur and S. Aker, 2010. Do liver IL-12 levels predict sustained response to IFN-α therapy in children with chronic hepatitis B? J. Interferon Cytokine Res., 30: 433-438.
20. Drexler, J.F., B. Kupfer, N. Petersen, R.M.T. Grotto and S.M.C. Rodrigues *et al*., 2009. A novel diagnostic target in the hepatitis C virus genome. PLoS Med., Vol. 6, No. 2. 10.1371/journal.pmed.1000031.
21. Gremion, C. and A. Cerny, 2005. Hepatitis C virus and the immune system: A concise review. Rev. Med. Virol., 15: 235-268.
22. Miedouge, M., K. Saune, N. Kamar, M. Rieu, L. Rostaing and J. Izopet, 2010. Analytical evaluation of HCV core antigen and interest for HCV screening in haemodialysis patients. J. Clin. Virol., 48: 18-21.
23. Daniel, H.D.J., P. Vivekanandan, S. Raghuraman, G. Sridharan, G.M. Chandy and P. Abraham, 2007. Significance of the Hepatitis C Virus (HCV) core antigen as an alternative plasma marker of active HCV infection. Indian J. Med. Microbiol., 25: 37-42.
24. Gupta, E., M. Bajpai and A. Choudhary, 2014. Hepatitis C virus: Screening, diagnosis and interpretation of laboratory assays. Asian J. Transfus. Sci., 8: 19-25.
25. Freiman, J.M., T.M. Tran, S.G. Schumacher, L.F. White and S. Ongarello *et al*., 2016. Hepatitis C core antigen testing for diagnosis of hepatitis C virus infection: A systematic review and meta-analysis. Ann. Internal Med., 165: 345-355.
26. Fabris, P., V.M. Fleming, M.T. Giordani and E. Barnes, 2008. Acute hepatitis C: Clinical aspects, diagnosis and outcome of acute HCV infection. Curr. Pharm. Design, 14: 1661-1665.
27. Maheshwari, A., S. Ray and P.J. Thuluvath, 2008. Acute hepatitis C. Lancet, 372: 321-332.