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Existence of HCV NS5A Proteins in Peripheral Blood Mononuclear Cells of Responder Patients is Associated with Viral Relapse

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Abstract

Background: Hepatitis C Virus (HCV) RNA and proteins have been detected in peripheral blood mononuclear cells (PBMCs). The replication of HCV in PBMCs and its role in relapse is still not confirm. Objective: The objective of the study was to detect the HCV NS5A Protein in PBMCs of responder patients and to find out its association with viral relapse.

Method: A total of 77 chronic HCV patients who cleared HCV RNA in plasma after completion of 6 months of Interferon (IFN) plus Ribavirin (RBV) therapy were enrolled in the study. PBMCs were isolated from whole blood and the expression of NS5A protein in PBMCs was checked. After six months of completion of treatment, viral RNA was tested in the blood samples of all patients to trace the relapse.

Results: At the end of treatment stage, HCV NS5A protein was detected in 22 (28.6%) of the 77 responder patients. After 6 months from end of treatment, 62 (80.5%) of the responder patients had undetectable HCV RNA in plasma (SVR) while 15 (19.5%) patients were detected with viral RNA in their blood (relapse). Out of the 22 patients positive for NS5A protein in PBMCs at end treatment stage, 50% (11) showed viral relapse as compared to only 7.3% (4) of the patients negative for NS5A protein in PBMCs at same stage. There was a significant difference (P=0.000) of relapse rate between both groups.

Conclusion: There is an association of HCV NS5A protein in PBMCs and risk of viral relapse in future.

Keywords: Hepatitis C virus; NS5A Protein; Relapse; Ribavirin; Interferon; Peripheral Blood Mononuclear cells

Abbreviations: HCV: Hepatitis C Virus; PBMCS: Peripheral Blood Mononuclear Cells; IF: Immunofluorescence; ETR: End Treatment Response; SVR: Sustained Virological Response; NS5A: Nonstructural Protein 5A; ORF: Open Reading Frame; Peg: Polyethylene Glycol; IFN: Interferon; RBV: Ribavirin; DAA: Direct Acting Antiviral Agents; ISH: In Situ Hybridization; CD: Cluster of Differentiation; ELISA: Enzyme Linked Immunosorbent Assays; 5' UTR: Untranslated Region; SB-JFH-1: Splenoma B-Cell-Derived Japanese Fulminant Hepatitis-1; RPMI: Roseman Park Memorial Institute; SPSS: Statistical Package for Social Sciences

Introduction

Hepatitis C virus (HCV) is the main causative agent for liver disease and is a major health problem worldwide as more than 185 million people are chronically infected with HCV and about 70-80% of them suffer liver cirrhosis and hepatocellular carcinoma [1-4]. Genotype 1 of HCV is the most widespread genotype in the world which comprises of about 83.4 million (46.2%) of the total HCV patients [5,6]. Genotype 3 is the second most prevalent genotype globally and is predominant genotype in Pakistan with about 71% of the total HCV patients in the country [5,6].

HCV belongs to Flaviviridae family. It is a small enveloped virus having positive-sense RNA genome of about 9.6 kb consisting long open reading frame (ORF) that encodes ten structural and nonstructural proteins [7-9]. Till now, there is no effective and licensed vaccine available for the prevention of this infection [10]. Combination therapy of polyethylene glycol Interferon alpha (peg-IFN-α) plus ribavirin (RBV) is still used for the treatment of HCV infection but SVR rate of this therapy is about 40 to 80 %

depending on genotype [11]. The new and advanced standard of care treatment of HCV infection consists of direct acting antiviral agents (DAA) including HCV protease and polymerase inhibitors with or without the use of PEG IFN alfa and ribavirin [12-15]. Sofosbuvir, a DAA, is a new oral NS5B polymerase inhibitor approved by FDA for all genotypes of HCV [12,13]. Daclatasvir, an NS5A inhibitor, was approved by FDA in July 2015 to be used with sofosbuvir for the treatment of chronic HCV infection of genotype 3 in treatment-naive and treatment-experienced patients [14]. The combination of ledipasvir and sofosbuvir without INF and ribavirin is the first oral course of therapy approved by FDA for HCV infection in persons with severe liver disease. The use of these agents considerably improved sustained virologic response in patients with genotype 1 infection and can be used in shorter treatment durations [15]. These new drugs are more effective; however, they can create several serious side effects in treated patients due to which the treatment may be discontinued or even stopped [16].

The exact mechanism of HCV chronicity is still a question mark, it may be due to some mutations in viral genome which make HCV escape from immune system or it may be due to the ability of virus to infect the immune cells/tissues [17,18]. Although HCV is strongly hepatotropic as it mainly replicates in liver but in specific conditions, it can also replicate in lymph nodes, oral epithelial cells, adrenal glands, brain, pancreas, PBMCs and thyroid glands [19,20]. HCV genomic RNA has been detected and separated from PBMCs and other blood cells by in situ hybridization (ISH) [21]. Viral RNA has also been found in platelets from the patients having no viral RNA in serum samples at the end of treatment and its association has been found with relapse as reported previously [22]. It was also reported that cluster of differentiation 81 (CD81) or the classical LDL-R platelets do not express in platelets. Therefore, some other receptors may be responsible for the entry of HCV-RNA to the platelets [23,24].

The existence of positive strand of HCV RNA in PBMCs after end of treatment is linked with viral relapse and resistance to IFN/Ribavirin therapy [20,25]. It is now clear that the viral RNA enters and replicates in extrahepatic compartments like PBMCs [25-28]. Furthermore, studies have demonstrated that in individuals infected with HCV, core proteins have been detected in kidney samples and same protein has also been separated from glomerular and tubular epithelial cells of kidney tissues [29,30]. The nonstructural protein 5 A (NS5A) is a multifunctional phosphoprotein comprising of 447 amino acids. NS5A protein is limited to cytoplasm of cells [31]. The protein plays a critical role for replication of HCV and RNA-dependent RNA polymerase (NS5B). It has been described that NS5A modulates β-catenin signaling that plays an important role in HCV pathogenesis [32].

At present, a few studies are available on the expression of HCV NS5A protein in immune cells and their association with viral relapse. The present study was aimed to investigate the expression of NS5A protein in PBMCs of the responder patients at the end of therapy.

Methods

Patients and sampling

a) A total of 103 patients including 58 male and 45 females were enrolled in the study for treatment. All the enrolled patients were fulfilling following criteria:

b) Anti HCV antibodies positive confirmed by 3rd generation enzyme linked immunosorbent assays (ELISA); HCV RNA detection confirmed by PCR;

c) No co-infection;

d) No evidence of other liver related disease. A written informed consent was provided to all patients and the institutional ethical committee approved the study. All the patient was administered with a dose of 3MU interferon α-2b, three times per week subcutaneously along with an oral dose of 800-1200 mg Ribavirin daily according to body weight for a period of six months. After completion of therapy, patients were followed up for another six months. Patients with positive HCV RNA in plasma at the end of treatment were designated as non-responders and excluded from the study.

Extraction and detection of plasma HCV RNA

Quantitative detection of viral RNA in plasma was performed after six months of treatment and after six months of completion of treatment. Plasma HCV RNA was extracted and quantified using RoboGene® HCV RNA extraction and Quantification Kits (aj Roboscreen, Analytikjena, Germany) using real-time PCR (Corbett Research TM, Australia) by an internal RNA standard derived from the 5' untranslated region (5' UTR) as described previously [33,34].

PBMCs isolation and counting

PBMCs were separated on Ficoll-Histopaque density gradient (Sigma-Aldrich) from whole blood, following the previously described protocol [33].

In-vitro transcription of HCV RNA in B cell lines

A digested splenoma B-cell-derived Japanese Fulminant Hepatitis-1(SB-JFH-1) plasmid DNA was used for *in-vitro* transcription of HCV mRNA. The purified mRNA was electroporated into B cell line according to the previously published protocol [35]. Both uninfected healthy PBMCs (negative controls) and transfected Raji cells (positive controls) were used for comparison of patient's cells.

Detection of HCV NS5A proteins in PBMCs

The cell suspension volume was adjusted to 1×106 cells/ml with Roseman Park Memorial Institute (RPMI) medium. HCV nonstructural protein NS5A was detected in PBMCs by indirect immunofluorescence method according to the previously described procedure [36,37].

Statistical analysis

The data was analyzed using Statistical Package for Social Sciences (SPSS), version 16.0 (SPSS Inc. Chicago, IL). Chi-square

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and Fisher's Exact test were used for the association of HCV proteins in PBMCs with patient's relapse or response.

Results

Virological response at end of treatment

A total of 77 patients (74.8%) were found to be negative for viral RNA in plasma at the end of treatment which were designated as end treatment responders while the remaining 26 patients (25.2%) were detected with viral RNA in their plasma (non-responders). Among 58 male patients, 42 (72.4%) were responders and 16 (27.6%) were non-responders while in total of 45 females, 33 (77.8%) showed response to treatment and 10 (22.2%) were non-responders. These 26-end treatment non-responders were excluded from further study while all 77 responder patients were included and followed up for a period of another six months.

Sustained virological response and relapse

At the end of one year of treatment (six months after the end of treatment), samples were taken from all patients and tested for presence or absence of HCV RNA. Out of the 77 patients, 62 (80.5%) had undetectable HCV RNA in plasma at this stage (SVR), while 15 (19.5%) patients were detected with viral RNA in their blood (relapse). Among 42 male patients, 71.4% achieved SVR and 28.6% showed relapse while among 35 female patients, 91.4% achieved SVR and remaining 8.6% showed relapse.

HCV protein detection and relapse

At the end of six months treatment, purified PBMCs from the whole blood of 77 patients were tested for presence or absence of HCV NS5A proteins. Out of the total 77 responder patients, HCV NS5A protein was observed in 22 (28.6%) patients while it was not found in remaining 55 (71.4%) patients. Out of the 22 patients having NS5A protein in their PBMCs, 11 (50%) showed sustained virological response while other 11 (50%) showed relapse within 6 months. Among 55 patients with negative HCV proteins in PBMCs, 92.7% (51/55) achieved SVR while 7.3% (4/55) sowed relapse. The patients with positive NS5A protein in PBMCs at ETR, showed significantly higher relapse (P=0.000) rate as compared to those who did not have NS5A protein in PBMCS at the same stage (Table 1).

Table 1: Comparison of positive and negative patients for NS5A protein in PBMCs at end of the treatment stage for relapse at week 48.

	NS5A Positive in PBMCs (n=22)	NS5A Negative in PBMCs (n=55)	Odds Ratio	P
Relapse (n=15)	11 (50 %)	4 (7.3 %)	12.7	0
SVR (n=62)	11 (50 %)	51 (92.7 %)		

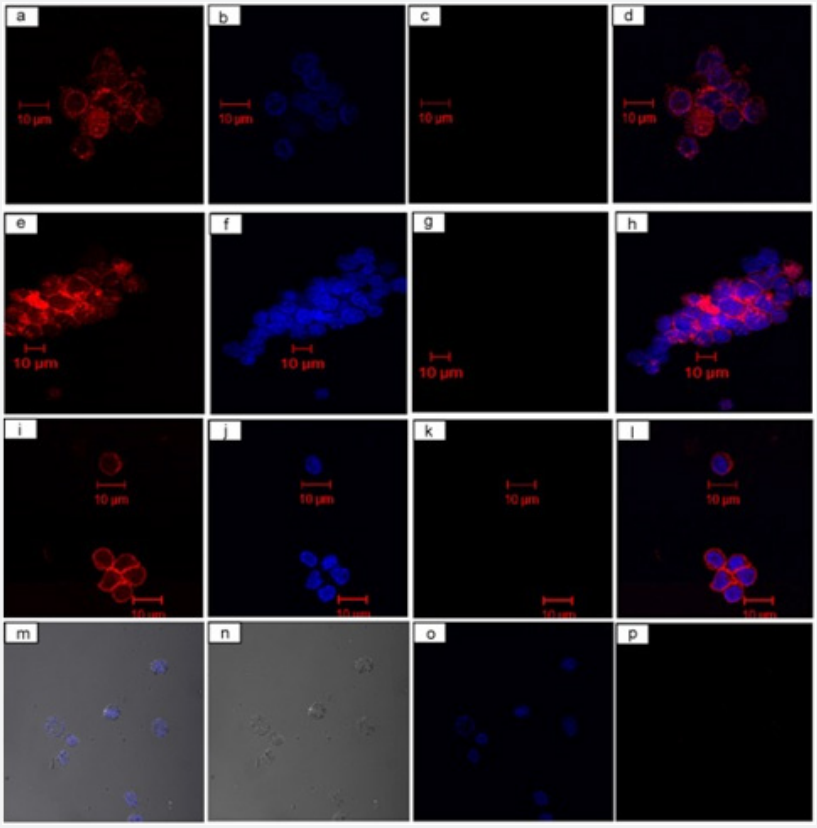

Figure 1: PBMCs sample used for HCV NS5A protein expression in the cell cytoplasm of responded patients a-d) positive control, e-h) post infection of healthy cells *in-vitro*, i-l) negative control, m-p) Immunofluorescence analysis; red signals indicating HCV proteins (a, e, i). Blue signal indicating nuclei of cells (b, f j, m), no signal, indicating back ground cells (c, g, k, o), red and blue signals indicating protein in cytoplasm and nuclei infected cells (d, h, l). The cells were observed under LCM-510 Confocal Microscope with 63X objective.

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NS5A protein was observed evenly in the cytoplasm of infected PBMCs (Figure 1: a-d). In positive control cells, HCV NS5A protein was observed in the cytoplasm of these cells (Figure 1: e-h). Uninfected PBMCs were used as negative control (Figure 1: m-p). For further authentication, uninfected PBMCs were isolated from healthy individuals and incubated with HCV in cell culture media and the cells were observed for HCV proteins (Figure 1: i-l).

The rate of HCV NS5A positivity in PBMCs population

Table 2: concentration of HCV NS5A protein in PBMCs and its association with relapse after 72 weeks.

	Less than 60% in PBMCs (n= 8)	More than 60% PBMCs (n= 11)	P
Relapse	2 (25%)	4 (36.4%)	0.49
SVR	6 (75%)	7 (63.6%)	

In this study, HCV protein was observed in cytoplasm of PBMCs. No sample was observed with 100 % infectivity of PBMCs. The patients were divided into two groups: one with more than 60 % infectivity and the other with less than 60 %. There was no significant difference of relapse between both of the groups (Table 2). These results indicate that HCV cannot infect all the cells and some cells have the ability to resist against HCV invasion. However, it was unclear whether the population consists of same cells or different number of cells.

Discussion

The SVR rate of the current study (80.5%) was slightly lower than the SVR rate reported in a previous study (88.2%) from Pakistan for same treatment regimen [34]. However, the SVR rate in the current study is quiet higher as compared to another previous study for same treatment [2]. Previously, we detected HCV RNA in PBMCs of patients at end of treatment stage and found a significant influence on HCV relapse [36]. Similar results were found in the current study with HCV NS5A protein in PBMCs. It means the presence of HCV in PBMCs at any stage after infection can affect the treatment response. Therefore, the present results are in agreement with the findings of our previous studies [33,34]. Although, the true mechanism behind the occurrence of HCV replication in PBMCs is not yet known, some studies have reported that the virus can infect and replicate in PBMCs [38].

The existence of HCV negative RNA in immune cells strongly supports the concept of HCV replication in immune cells of the body because minus strand RNA indicates active replicative intermediates in such cells. Although few researchers don't support this concept as they consider it as artifact, miss or self-priming in PCR reaction [39], or passive adsorption (contamination) of HCV from plasma [40]. Hanno et al. [41] conducted a study in HCV infected patients at the completion of treatment. They detected HCV RNA in PBMCs of patients (32%) with undetectable HCV RNA in their sera. The patients who were positive for HCV RNA in PBMCs showed 50% relapse compared to 6% relapse in those who have no HCV RNA in PBMCs. This study is very much similar to the current results. The relapse rate was reduced to 25% when these patients were treated with same treatment for six more months [42].

In the current study, HCV NS5A protein was checked by IF method in the PBMCs of end treatment responder patients. HCV NS5A protein was detected in 22 (28.6%) patients while it was not found in remaining 55 (71.4%) patients. Out of the 22 patients having NS5A protein in their PBMCs, 11 (50%) showed sustained virological response while other 11 (50%) showed relapse within 6 months. Among 55 patients with negative HCV proteins in PBMCs, 92.7% (51/55) achieved SVR while 7.3% (4/55) sowed relapse. Our findings are not in agreement with the findings of some previous studies who reported that the presence of HCV RNA in PBMCs at ETR stage could not predict the relapse [43,44]. However, Taliani et al. [43] described the association of SVR with absence and presence of HCV RNA in serum and PBMCs.

In the current study, the expression of NS5A proteins was recorded in cytoplasm of PBMCs. This finding is in accordance with the report of a previous study [36], that HCV NS5 protein expression was seen in cytoplasm of PBMCs from patients with chronic hepatitis C. Further, some HCV proteins such as core, NS3 and NS5 have also been detected in PBMCs. In another study by Chen et al. [42], the link between the expression of HCV proteins in PBMCs it was found that the core protein is expressed in the nucleus of PBMCs from extreme chronic infected patients than that from the modest patients. In the current study, we observed NS5A protein in cytoplasm of infected PBMCs. It can now be said that the NS5A is cytoplasmic protein and the core proteins are nuclear proteins.

The present results are in accordance to the findings of our previous study [33], in which ETR positive patients were tested for the presence of HCV RNA in PBMCs. Significantly high relapse rate was observed in those patients who had detectable HCV RNA in their PBMCs than those who did not have HCV RNA in PBMCs.

The present study is also similar to a previous study [45], which reported the presence of HCV core proteins in lymphocytes and monocytes which are the subclasses of PBMCs. HCV proteins were also reported from the monocytes/ macrophages as well as B and T cells which are the fraction of the PBMCs. Furthermore, it was assumed the deposition of HCV antigens and HCV RNA with the presence of hepatitis virus in these cells. Both minus and plus strands of HCV RNA genome were detected in macrophage/ monocyte and B cell fractions as well as in T cell fractions from either BMMC or PBMC. HCV core proteins, and NS3 protein expression was also reported in PBMCs previously [21]. The efficacy of antiviral regimen and risk of liver cirrhosis may be affected by presence of HCV RNA in extrahepatic tissues for long time. High level expression of HCV core proteins in PBMCs has been linked with complex type of HCV infection [42]. It is thought

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that the negative strand of HCV RNA may induce the expression of viral proteins, which subsequently can stimulate an anti-HCV immune response as well [46,47].

HCV proteins such as core NS3, NS4A, NS4B and NS5A were detected in PBMCs of patients in a previous study [48]. These results indicate translation of HCV RNA within PBMCs. Furthermore, they suggested that PBMCs are a major extrahepatic site for the dynamic replication of this virus.

The results of present study are also confirmed by the study of Pawelczyk et al. [49], who conducted their study separately on CD3 cells, CD14 cells and CD19 cells for detection of HCV RNA and proteins. They detected negative strand of HCV RNA in 27% of the cells and NS3 proteins in 57.6% of PBMCs. They also found a replication marker in 50% of CD3 cells and 30.8% of both CD14 and CD19 cells. Relatively high concentration (2.4%) of marker was observed in CD3 cells while low concentration (1.2%) was detected in CD14 cells and lowest concentration (0.4%) in CD19 cells. The results of our study show that HCV cannot infect all cells of a PBMCs population and some cells have the ability to resist against HCV invasion.

Conclusion

The present study supports the idea of HCV viral replication as well as translation in PBMCs even after clearance of viremia in plasma. The study concludes that PBMCs may act as secondary site for HCV replication. We suggest testing of replicative intermediates and proteins of HCV in PBMCs of patients treated with DAA.

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Availability of Data and Materials

The datasets used during the current study are available from corresponding author on reasonable request.

Authors' contributions NZ, MJA, and AR designed the study. NZ and MM performed the laboratory work. MM, NZ, MA, HK, and FA analyzed the data. MM, FZ, and AR prepared the initial draft of manuscript. AJM, MA, NZ and MM critically reviewed and revised the manuscript. HK, MJA and FA provided useful feedback and technical support. All authors read and approved the final manuscript.

Ethics Approval and Consent to Participate

The study was approved by institutional ethics committee of PMAS Arid Agriculture University Rawalpindi and each patient gave written consent before the start of study.

Competing Interests

The authors declared that there is no competing interest among them.

References

1. Davis GL, Alter MJ, El-Serag H, Poynard T, Jennings LW (2010) Aging of hepatitis C virus (HCV)-infected persons in the United States: a multiple cohort model of HCV prevalence and disease progression. Gastroenterology 138(2): 513-521.
2. Papatheodoridis G, Hatzakis A (2012) Public health issues of hepatitis C virus infection. Best Pract Res Clin Gastroenterol 26(4): 371-380.
3. Cooke G, Lemoine M, Thursz M, Gore C, Swan T, et al. (2013) Viral hepatitis and the Global Burden of Disease: a need to regroup. J Viral Hepatitis 20(9): 600-601.
4. Idrees M, Riazuddin S (2009) A study of best positive predictors for sustained virologic response to interferon alpha plus ribavirin therapy in naive chronic hepatitis C patients. BMC Gastroenterol 9(1): 5.
5. Messina JP, Humphreys I, Flaxman A, Brown A, Cooke GS, et al. (2015) Global distribution and prevalence of hepatitis C virus genotypes. Hepatology 61(1): 77-87.
6. Waqar M, Khan AU, Rehman HU, Idrees M, Wasim M, et al. (2014) Determination of hepatitis C virus genotypes circulating in different districts of Punjab (Pakistan). Europ J Gastroenterol Hepatol 26(1): 59-64.
7. Simmonds P, Becher P, Bukh J, Gould EA, Meyers G, et al. (2017) ICTV Virus Taxonomy Profile: Flaviviridae. J Gen Virol 98(1): 2-3.
8. Shirota Y, Luo H, Qin W, Kaneko S, Yamashita T, et al. (2002) Hepatitis C virus (HCV) NS5A binds RNA-dependent RNA polymerase (RdRP) NS5B and modulates RNA-dependent RNA polymerase activity. J Biol Chem 277(13): 11149-11155.
9. Choo Q-L, Kuo G, Weiner AJ, Overby LR, Bradley DW, et al (1989) Isolation of a cDNA Clone Derived from a Blood-Borne Non-A, Non-B Viral Hepititis Genome 244(4902): 359.
10. Abdelwahab SF (2016) Cellular immune response to hepatitis-C-virus in subjects without viremia or seroconversion: is it important? Infect Agent Cancer 11(1): 23.
11. Rodriguez-Torres M, Jeffers LJ, Sheikh MY, Rossaro L, Ankoma-Sey V, et al. (2009) Peginterferon alfa-2a and ribavirin in Latino and non-Latino whites with hepatitis C. N Engl J Med 360(3): 257-267.
12. Jacobson IM, Gordon SC, Kowdley KV, Yoshida EM, Rodriguez-Torres M, et al. (2013) Sofosbuvir for hepatitis C genotype 2 or 3 in patients without treatment options. N Engl J Med 368(20): 1867-1877.
13. Lam AM, Murakami E, Espiritu C, Steuer HMM, Niu C, et al. (2010) PSI-7851, a pronucleotide of β-D-2′-deoxy-2′-fluoro-2′-C-methyluridine monophosphate, is a potent and pan-genotype inhibitor of hepatitis C virus replication. Antimicrob Agent Chemother 54(8): 3187-3196.
14. Sulkowski MS, Gardiner DF, Rodriguez-Torres M, Reddy KR, Hassanein T, et al. (2014) Daclatasvir plus sofosbuvir for previously treated or untreated chronic HCV infection. N Engl J Med 370(3): 211-221.
15. Charlton M, Everson GT, Flamm SL, Kumar P, Landis C, et al. (2015) Ledipasvir and sofosbuvir plus ribavirin for treatment of HCV infection in patients with advanced liver disease. Gastroenterology 149(3): 649-659.
16. Schaefer M, Schmidt F, Folwaczny C, Lorenz R, Martin G, et al. (2003) Adherence and mental side effects during hepatitis C treatment with interferon alfa and ribavirin in psychiatric risk groups. Hepatology 37(2): 443-451.

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17. Oldstone MB (1997) How viruses escape from cytotoxic T lymphocytes: molecular parameters and players. Virology 234(2): 179-185.

18. Pileri P, Uematsu Y, Campagnoli S, Galli G, Falugi F, et al. (1998) Binding of hepatitis C virus to CD81. Science 282(5390): 938-941.

19. Radkowski M, Gallegos-Orozco JF, Jablonska J, Colby TV, Walewska-Zielecka B, et al. (2005) Persistence of hepatitis C virus in patients successfully treated for chronic hepatitis C. Hepatology 41(1): 106-114.

20. Amini S, Alavian SM, Mostafavi E, Vahabpour R, Bahramali G, et al. (2012) Presence of plus-strand HCV RNA in serum and PBMCs as an indicator for relapse and resistance to IFN therapy in patients infected by HCV. Future Virol 7(3): 323-330.

21. Sansonno D, Iacobelli A, Cornacchiulo V, Iodice G, Dammacco F (1996) Detection of hepatitis C virus (HCV) proteins by immunofluorescence and HCV RNA genomic sequences by non-isotopic in situ hybridization in bone marrow and peripheral blood mononuclear cells of chronically HCV infected patients. Clin Exp Immunol 103(3): 414-421.

22. Espírito-Santo EMP, Melo CEB, Marques VA, Lampe E, Almeida AJd (2013) Analysis of hepatitis C virus (HCV) RNA load in platelets of HCV-monoinfected patients receiving antiviral therapy. Ann Hepatol 12(3): 373-379.

23. Amer A, Madi AM, Shebl FM, Al Faridi D, Alkhinji M, et al. (2016) Platelets as a Possible Reservoir of HCV And Predictor of Response to Treatment. J Virol Antivir Res 5(3).

24. Padovani JL, Corvino SM, Drexler JF, Silva GF, Pardini Mid MC, et al. (2013) In vitro detection of hepatitis C virus in platelets from uninfected individuals exposed to the virus. Revista da Sociedade Brasileira de Medicina Tropical 46(2): 154-155.

25. Castillo I, Rodriguez-Inigo E, Bartolome J, De Lucas S, Ortiz-Movilla N, et al. (2005) Hepatitis C virus replicates in peripheral blood mononuclear cells of patients with occult hepatitis C virus infection. Gut 54(5): 682-685.

26. Januszkiewicz-Lewandowska D, Wysocki J, Pernak M, Nowicka K, Zawada M, et al. (2007) Presence of hepatitis C virus (HCV)-RNA in peripheral blood mononuclear cells in HCV serum negative patients during interferon and ribavirin therapy. Japan J Infect Dis 60(1) :29-32

27. Ariede JR, Pardini MIdMC, Silva GF, Grotto RMT (2015) Platelets can be a biological compartment for the Hepatitis C Virus. Brazil J Microbiol 46(2): 627-629.

28. Chary A, Winters MA, Eisen R, Knight TH, Asmuth DM, et al. (2012) Quantitation of hepatitis C virus RNA in peripheral blood mononuclear cells in HCV-monoinfection and HIV/HCV-coinfection. J Med Virol 84(3): 431-437.

29. Sansonno D, Lauletta G, Montrone M, Grandaliano G, Schena F, et al. (2005) Hepatitis C virus RNA and core protein in kidney glomerular and tubular structures isolated with laser capture microdissection. Clinic Exp Immunol 140(3): 498-506.

30. Cao Y, Zhang Y, Wang S, Zou W (2009) Detection of the hepatitis C virus antigen in kidney tissue from infected patients with various glomerulonephritis. Nephrol Dialysis Transplant 24(9): 2745-2751.

31. Gosert R, Egger D, Lohmann V, Bartenschlager R, Blum HE, et al. (2003) Identification of the hepatitis C virus RNA replication complex in Huh-7 cells harboring subgenomic replicons. J Virol 77(9): 5487-5492.

32. Park CY, Choi SH, Kang SM, Kang JI, Ahn BY, et al. (2009) Nonstructural 5A protein activates β-catenin signaling cascades: implication of hepatitis C virus-induced liver pathogenesis. J Hepatol 51(5): 853-864.

33. Zaman N, Asad MJ, Raza A, Raja GK, Akhter S, et al. (2014) Presence of HCV RNA in peripheral blood mononuclear cells may predict patients response to interferon and ribavirin therapy. Ann Saudi Med 34(5): 401-406.

34. Mehmood A, Asad MJ, Ovais M, Zaman N, Aziz H, et al. (2017) The Absence of HCV RNA and NS5A Protein in Peripheral Blood Mononuclear Cells Is a Prognostic Tool for Sustained Virological Response. Viral Immunoly 30(8): 568-575.

35. Gong GZ, Lai LY, Jiang YF, He Y, Su XS (2003) HCV replication in PBMC and its influence on interferon therapy. World J Gastroenterol 9(2): 291-294.

36. Zaman N, Asad MJ, Raza A, Raja GK, Akhter S, et al. (2015) Detection of HCV RNA and NS5A Protein in Peripheral Blood Mononuclear Cells After Sustained Virological Response May Cause Viral Relapse. Pak J Zool 47(4): 981-987

37. Kato T, Date T, Murayama A, Morikawa K, Akazawa D, et al. (2006) Cell culture and infection system for hepatitis C virus. Nat protoc 1(5): 2334-2339.

38. Cavalheiro NdP, Filgueiras TC, Melo CE, Morimitsu SR, Araújo ESAd, et al. (2007) Detection of HCV by PCR in serum and PBMC of patients with hepatitis C after treatment. Brazil J Infect Dis 11(5): 471-474.

39. Lerat H, Berby F, Trabaud MA, Vidalin O, Major M, et al. (1996) Specific detection of hepatitis C virus minus strand RNA in hematopoietic cells. J Clin Invest 97(3) :845-851.

40. Meier V, Mihm S, Braun PW, Ramadori G (2001) HCV-RNA positivity in peripheral blood mononuclear cells of patients with chronic HCV infection: does it really mean viral replication? World J Gastroenterol 7(2): 228-234.

41. Hanno AFF, Mohiedeen KM, Alshayeb AF, Deghedy A (2014) HCV RNA in peripheral blood mononuclear cells (PBMCs) as a predictor of the response to antiviral therapy in chronic hepatitis C. Alexandria J Med 50(4): 317-322.

42. Chen L, Chen P, Fan G, Li L, Liu C (2002) Localization of hepatitis C virus core protein in the nucleus of peripheral blood mononuclear cells of hepatitis C patients. Chin J Exp Clinic Virol 16(1): 37-39.

43. Taliani G, Badolato MC, Lecce R, Poliandri G, Bozza A, et al. (1995) Hepatitis C virus RNA in peripheral blood mononuclear cells: relation with response to interferon treatment. J Med Virol 47(1): 16-22.

44. Chen AY, Zeremski M, Chauhan R, Jacobson IM, Talal AH, et al. (2013) Persistence of hepatitis C virus during and after otherwise clinically successful treatment of chronic hepatitis C with standard pegylated interferon α-2b and ribavirin therapy. PloS one 8(11): e80078.

45. Bouffard P, Hayashi P, Acevedo R, Levy N, Zeldis J (1992) Hepatitis C virus is detected in a monocyte/macrophage subpopulation of peripheral blood mononuclear cells of infected patients. J Infect Dis 166(6): 1276-1280.

46. Di Liberto G, Roque-Afonso AM, Kara R, Ducoulombier D, Fallot G, et al. (2006) Clinical and therapeutic implications of hepatitis C virus compartmentalization. Gastroenterology 131(1): 76-84.

47. Zignego AL, De Carli M, Monti M, Careccia G, La Villa G, et al. (1995) Hepatitis C virus infection of mononuclear cells from peripheral blood and liver infiltrates in chronically infected patients. J Med Virol 47(1): 58-64.

48. Masalova O, Vishnevskaya T, Pichugin A, Alkhovsky S, Shkurko T, et al. (2012) Detection of hepatitis C virus proteins in peripheral blood mononuclear cells of patients with chronic hepatitis C: correlation with activity and stage of hepatitis. Clinic Microbiol Infect 18: 211.

49. Pawełczyk A, Kubisa N, Jabłońska J, Bukowska-Ośko I, Cortes KC, et al. (2013) Detection of hepatitis C virus (HCV) negative strand RNA and NS3 protein in peripheral blood mononuclear cells (PBMC): CD3+, CD14+ and CD19+. Virol J 10(1): 346.

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