http://www.pjbs.org

PJBS

ISSN 1028-8880

# Pakistan Journal of Biological Sciences

ANSInet
Asian Network for Scientific Information
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan

Pakistan Journal of Biological Sciences 17 (5): 601-607, 2014
ISSN 1028-8880 / DOI: 10.3923/pjbs.2014.601.607


# *In vitro* Evidence for Association Between Hepatitis C Virus Infection and Insulin Signaling Pathways: A Review

[1]Hossein Khedmat, [2]Shahram Agah and [3]Saeed Taheri
[1]Baqiyatallah Research Center for Gastroenterology and Liver Diseases,
Baqiyatallah University of Medical Sciences, Tehran, Iran
[2]Colorectal Research Center, Tehran University of Medical Sciences, Tehran, Iran
[3]Dr. Taheri Medical Research Group, Tehran, Iran

**Abstract:** Hepatitis C virus infection, besides its substantial unfavorable impact on liver health, has several other manifestations, from which we have focused on its associations with insulin resistance and diabetes mellitus in an *in vitro* setting in this review of the literature. Several epidemiologic studies have already proven the strong association between chronic HCV infection and development of diabetes mellitus. However, attempts to clarifying molecular mechanisms for this association have not made up until recent years. We made a comprehensive review of the literature but we only found 6 studies investigating HCV impact on insulin signaling pathway in an *in vitro* era. We tried to review and compare the findings of these studies to show what we already know on the issue and what we still need to know.

**Key words:** Hepatitis C virus, insulin signaling pathway, HCV, diabetes

## INTRODUCTION

Hepatitis C Virus (HCV) is a member of the Flaviviridae family and is the responsible agent in a considerable number of acute and chronic liver diseases in human populations (NIH, 2002). Infection with HCV is a common problem with an average prevalence of 3% in the world (Alter, 1995; Khedmat *et al*., 2009). Acute HCV infection remains in the host body and becomes chronic in about 85% of cases (Micallef *et al*., 2006) leading to several HCV-associated complications. HCV induces several pathways which can ultimately lead to steatosis, type II diabetes, fibrosis, inflammation, apoptosis and hepatocellular carcinoma (Fartoux *et al*., 2005; Pekow *et al*., 2007; Lau *et al*., 1998; Bieche *et al*., 2005; Khattab *et al*., 2010).

As mentioned, HCV infection is associated with insulin resistance and type 2 diabetes mellitus. Mechanisms through which HCV induces impaired glucose tolerance and type II diabetes are poorly understood. Recently emerged evidence suggests that the virus directly causes insulin resistance through interference with the insulin signaling pathway and dysregulation of important factors playing major roles in lipid metabolism (Negro, 2006; Mehta *et al*., 2000; Asselah *et al*., 2006). Moreover, insulin resistance has reportedly been associated with increased rates of fibrosis progression and altered response to pharmaceutical treatment (Romero-Gomez *et al*., 2005). Some epidemiological studies including some of very large populations have shown that type II diabetes is significantly more prevalent in patients with HCV infection (Caronia *et al*., 1999; Knobler *et al*., 2000). However the number of studies investigating molecular mechanisms leading to insulin resistance by HCV infection is very limited and all of which have been reported within the last decade. Despite a comprehensive review of the publish reports, we only found six studies surveying the impact of HCV on insulin resistance in an *in vitro* setting (Aytug *et al*., 2003; Pazienza *et al*., 2007; Christen *et al*., 2007; Kawaguchi *et al*., 2004; Banerjee *et al*., 2008; Bernsmeier *et al*., 2008). In the current study, we aimed to review these articles and to present their findings one by one and finally to conclude our knowledge on the cellular and molecular mechanisms of inducing insulin resistance and type II diabetes by HCV infection (Fig. 1). Insulin signaling pathway has been very perfectly reviewed in a review article by Pessin and Saltiel (2000).

**Corresponding Author:** Hossein Khedmat, Baqiyatallah Research Center for Gastroenterology and Liver Diseases, Baqiyatallah University of Medical Sciences, Mullasadra Street, Vanaq Square, Tehran, Iran

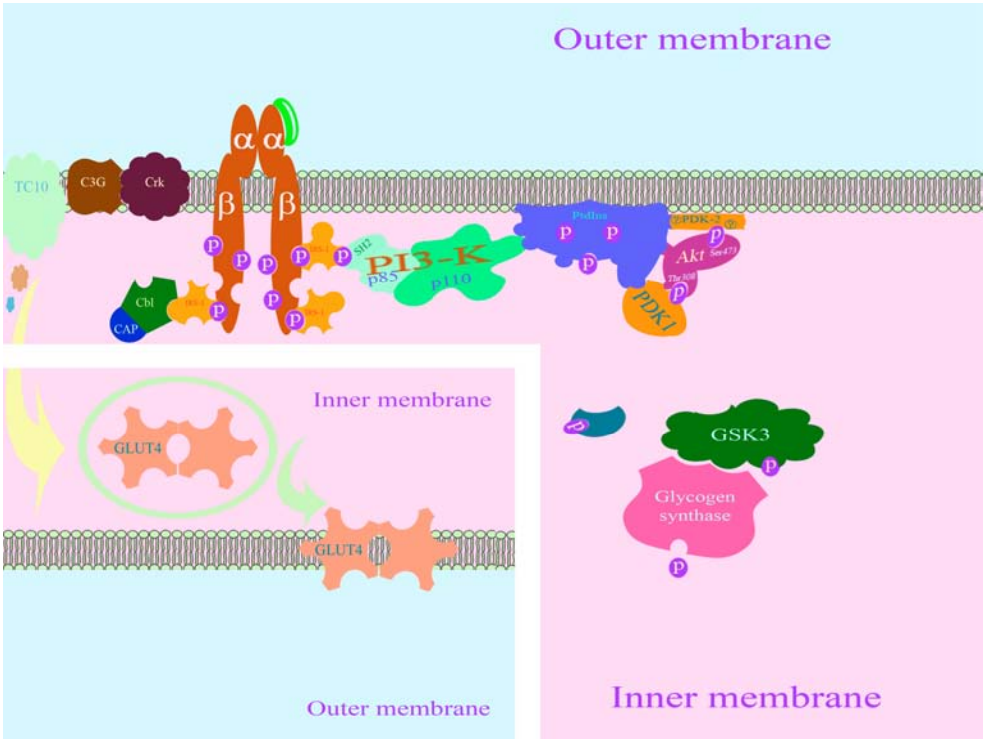


Fig. 1: Schematic view on a summarized description for mechanisms intervening in the insulin signaling pathway

## HCV IMPAIRS IRS-1/PI3-KINASE SIGNALING

Aytug *et al.* (2003) took liver biopsy specimens from 42 HCV-infected and 10 non-infected subjects who had other liver pathologies, matched by age and BMI and exposed them to insulin and then examined the contents for the upstream insulin signaling molecules by immunoprecipitation and Western blot analysis. Study results demonstrated that HCV infection is associated with a trend toward a 3-fold increase in Insulin Receptor (IR) protein levels. Analysis of insulin receptor substrate-1 (IRS-1) content also showed a trend toward a 2.6-fold increase in IRS-1 protein in liver specimens obtained from HCV-infected patients. Nevertheless, the level of hepatic α-actin was comparable between the two groups; suggesting that the elevated trend seen in IR content was specific and not due to generalized alterations in cellular protein levels.

Aytug *et al.* (2003) also demonstrated a 3-fold decrease in insulin-stimulated p85 PI3-kinase association with IRS-1 in patients with HCV infection. As well, basal IRS-1/p85 PI3-kinase association was decreased in HCV infected cells. Analysis of PI3-kinase enzymatic activity in IRS-1 immunoprecipitates also showed a 60% decrease in insulin stimulated PI3-kinase activity in liver tissue harvested from HCV-infected subjects. Nevertheless, cellular levels of the p85 regulatory subunit of PI3-kinase were comparable between HCV infected and non-infected cells.

Analysis of Akt activation status using phospho-Akt antibody showed severely blunted Akt phosphorylation in HCV-infected subjects. Using phosphorylation-specific MAPK antibodie, MAPK phosphorylation was shown to be increased by 3-fold in HCV-infected patients both in the basal state as well as in insulin-treated liver preparations. Insulin also increased MAPK phosphorylation in preparations from non–HCV-infected subjects by 2-fold.

Thus, HCV infection is accompanied by elevated IR and IRS-1 contents but normal levels of p85 subunit of PI3-kinase.

## DOWNREGULATION OF IRS1 BY THE HCV CORE PROTEIN IN SPECIFIC VIRUS GENOTYPES

Pazienza *et al.* (2007) examined the *in vitro* interactions between the HCV core protein of genotypes 3a and 1b with the insulin-signaling pathway. They

measured the expression levels of IRS-1, IRS-2 and other factors involved in the insulin signal pathway in human hepatoma cell line (Huh-7) transiently expressing the HCV core protein of genotypes 3a or 1b. They found both HCV core proteins 1b and 3a inhibit insulin induced phosphorylation of Akt. They also tested whether treatment with a PPARγ agonist, rosiglitazone, could reverse the downregulation of IRS-1 as well as the inhibition of Akt phosphorylation associated with genotype 3a. After administering rosiglitazone, in cells transfected with the core protein 3a, both IRS-1 and insulin stimulated-Akt phosphorylation levels increased, however cells transfected with the core protein 1b represented no such reaction.

SOCS-1 and SOCS-3 mRNA levels were unchanged following transfection with both core proteins. Nevertheless, SOCS-7 mRNA levels were significantly higher in cells expressing the core protein 3a no significant change has been observed in core protein 1b transfected cells. Investigating the role of SOCS-7 in IRS-1 downregulation in genotype 3a-transfected cells, it has been confirmed using 2 different siRNAs. Both siRNAs reduced SOCS-7 expression by 36 and 50%, respectively, SOCS-7 downregulation resulted in a recovery up to 80% of the total IRS-1 protein level. A parallel increase of Akt phosphorylation was also observed when SOCS-7 was knocked down by siRNA.

Inhibition of proteasomal activity by MG132 was associated with an increase inIRS-1 protein levels in cells transfected with any of the two core proteins. mTOR activity was increased in cells expressing the core protein 1b, as recently shown while it was not modified in cells transfected with 3a. The expression levels of mTOR protein remained unchanged.

## PROTEIN PHOSPHATASE 2A UP REGULATION BY HCV

Christen *et al.* (2007) employed human osteosarcoma-derived, tetracycline-regulated cell lines UHCV-57.3 which can express the entire HCV polyprotein upon induction. As well human hepatoma cells Huh-7 were also used. Total RNA was isolated from cells using a Perfect RNA Eukaryotic Mini Kit. RNA was produced using Moloney murine leukemia virus reverse transcriptase. Thapsigargin or tunicamycin was used for inducing ER stress and to decrease cytosolic calcium levels, cells were treated with BACT.-AM.

The expression of HCV proteins in UHCV57.3 cells induced the up-regulation of the ER chaperon BiP and the splicing of XBP-1 mRNA, demonstrating the activation of an ER stress response in these cells. HCV protein expression also induced the expression of PP2Ac. ER stress and PP2Ac induction were specific for HCV protein expression, because neither was found in the control cells UTA6 and UGFP which have no HCV DNA. Treatment of untransfected Huh7 cells for 8 hours with thapsigargin, an inhibitor of the ER Ca-ATPase, induced a classical ER stress response, documented by an up-regulation of BiP, a phosphorylation of P-eIF2α and splicing of the XBP1 mRNA. PP2Ac was also strongly up-regulated both at the protein and mRNA level.

For demonstrating pharmacological induction of ER stress could induce PP2Ac expression independent of HCV protein expression, investigators cultured UHCV57.3 cells in tetracycline-supplemented medium for repressing the expression of viral proteins and then thapsigargin was used for the next 8 h. The treatment activated the ER stress response pathways (BiP, XBP1 mRNA splicing) and caused a strong induction of PP2Ac. The same PP2Ac induction was observed in the parental cell line UTA6. HCV protein expression in UHCV57.3 cells significantly reduced the $Ca_2$ content in the ER.

A canonical CREB binding site sequence was found in the promoter site of the PP2Ac. CREB was phosphorylated (activated) in UHCV57.3 cells that were induced to express viral proteins and thereby had an activated ER stress response but not in control UTA6 or UGFP cells. Moreover, CREB was found to be necessary for PP2Ac up-regulation, because pretreatment of cells with a CREB-specific siRNA prevented the HCV protein induced PP2Ac overexpression. The phosphorylation of CREB in response to HCV protein expression in UHCV57.3 was prevented by treatment of the cells with the Ca-chelator BAPT.. In accordance with a direct role of CREB in the up-regulation of PP2Ac, $CaCl_2$ treatment of Huh7 cells also induced an overexpression of PP2Ac.

## HCV AND IRS 1 AND 2 DOWN REGULATION

Kawaguchi *et al.* (2004) investigated changes in glucose metabolism in noncirrhotic patients with various hepatobiliary disorders and the molecular mechanisms for HCV-associated glucose intolerance. The effects of HCV core on IRS1, IRS2, SOCS3 and insulin receptor expression were examined in HepG2 and Huh7 cells prepared by transient transfection with Myc tagged HCV core and HLF cells with stable transfection of Myc-tagged HCV core.

HCV core dose dependently decreased IRS1 and IRS2 expression in HepG2 cells. In contrast, SOCS3 expression was dose dependently increased by transient transfection with Myc-tagged HCV core in HepG2 cells. No changes in insulin receptor expression and STAT5 were detected.

The treatment with MG132 (a proteosomal proteolysis inhibitor) caused an increase in expression levels of IRS1 and IRS2. To investigate the involvement of ubiquitination in downregulation of IRS1 and IRS2 in HepG2 cells transfected with HCV core, whole-cell extracts were immunoprecipitated with anti-IRS1 or anti-IRS2 antibodies and immunoblotted with anti-ubiquitin monoclonal antibodies. HCV core caused an accumulation of ubiquitin-conjugated IRS1 and IRS2.

Authors also examined the relationship between SOCS3 and regulation of IRS1 and IRS2 by using SOCS3-/-MEF cells. HCV core down-regulated IRS1 and IRS2 in SOCS3+/+ MEF cells. On the other hand, HCV core did not cause downregulation of IRS1 and IRS2 in SOCS3-/-MEF cells.

HLF cells with stable transfection of HCV core were treated with 100 ng $mL^{-1}$ insulin from 0 to 60 min and phosphorylation of p85 subunit of PI3K and Akt were examind. Insulin-induced phosphorylation of p85 subunit of PI3K and Akt was observed in HLF cells transfected with empty vector. However, HCV core decreased phosphorylation of p85 subunit of PI3K and Akt at the base line and inhibited insulin induced phosphorylation of p85 subunit of PI3K and Akt.

## UP REGULATION OF SERINE PHOSPHORYLATION OF IRS-1 AND IMPAIRMENT OF THE DOWNSTREAM AKT/PROTEIN KINASE B SIGNALING PATHWAY BY HCV CORE PROTEIN

Banerjee *et al.* (2008) employed transfected HepG2 and Huh-7 cells with HCV core or HCV1-2962 plasmid DNA and immortalized human hepatocytes generated by stable transfection of an HCV core (genotype 1a) genomic region into primary hepatocytes, in their study. Cells were treated with or without TNF-α and IL-6. Huh-7 cells exhibited an increase in the $Ser^{312}$ phosphorylation level of IRS-1 upon treatment with TNF-α. On the other hand, IRS-1 $Ser^{312}$ phosphorylation was also increased in IL-6-treated Huh-7 cells compared to the basal level. HCV core or HCV polyprotein expression also led to an increase in $Ser^{312}$ phosphorylation of IRS-1 in Huh-7 cells. However, treatment with TNF-α or IL-6 did not significantly enhance the phosphorylation status of IRS-1 at $Ser^{312}$ site in Huh-7 cells stably transfected with HCV core or full-length genomic region. A higher level of $Ser^{312}$ phosphorylated IRS-1 in virus-infected Huh-7 cells was also detected compared to that in control Huh-7 cells using Western blot analysis.

Banerjee *et al.* (2008) also found a significant inhibition of the IRS-1 $Ser^{312}$ phosphorylation in Huh-7 core cells in the presence of A JNK inhibitor (SP600125). After incubating HCV core expressing Huh-7 cells with a PI-3K inhibitor (LY294002), authors observed an inhibition of IRS-1 $Ser^{312}$ in Huh-7 core expressing cells in the presence of LY294002. Putting together, their results demonstrated that both JNK and PI3-K inhibitors reduce HCV core-mediated upregulation of phospho-IRS-1.

To investigate $Ser^{473}$ and $Thr^{308}$ phosphorylation status of Akt, Banerjee *et al.* (2008) infected Huh-7 cells with HCV. The results suggested that $Ser^{473}$ phosphorylation of Akt is increased in HCV JFH1-infected Huh-7 hepatocytes, whereas the $Thr^{308}$ phosphorylation level was not significantly altered after infection with HCV.

Full activation of Akt by insulin appears to require phosphorylation of $Thr^{308}$ and $Ser^{473}$. Results of the study by Banerjee *et al.* (2008). indicated that HCV infection does not significantly modulate the $Thr^{308}$ phosphorylation status of Akt in human hepatocytes, as it does with $pSer^{473}$.

In HepG2 cells expressing or not-expressing HCV core protein, $Ser^{473}$ phosphorylation of Akt was increased after insulin treatment. Treatment of insulin and TNF-α together did not significantly alter $Ser^{473}$ phosphorylation status. On the other hand, $Thr^{308}$ phosphorylation of Akt was induced by insulin in HepG2 control cells and was inhibited by TNF-α. Nevertheless, insulin-induced $Thr^{308}$ phosphorylation was not altered in HepG2 cells expressing HCV core protein. The present observations suggested that HCV core protein-induced insulin resistance may occur via the downregulation of $Thr^{308}$ phosphorylation of Akt which may serve as a signature molecule in HCV coremediated insulin resistance.

Glucose uptake was substantially reduced by over a 5 fold rate in Huh-7 cells stably transfected with HCV core, FL, or infected with HCV genotype 2a compared to its basal level in the control cell line. Interestingly, exogenous usage of insulin or TNF-α did not alter glucose uptake in Huh-7 cells expressing HCV protein.

Hepatocytes expressing HCV core protein were treated with a JNK inhibitor (SP600125) and the inhibition of glucose uptake by insulin-stimulated hepatocytes was significantly relieved by using the JNK inhibitor. However, using a PI-3K inhibitor (LY294002) did not impair glucose uptake, as was expected due to its mechanism of action. Our findings suggest that insulin-induced glucose uptake is impaired in human hepatocytes expressing HCV core protein.

## OVER-EXPRESSION OF PROTEIN PHOSPHATASE 2A BY HCV INFECTION

Bernsmeier *et al.* (2008) examined the possibility of PP2A over-expression association with insulin resistance. So, insulin signaling pathway was studies in cell lines that allow the regulated over-expression of HCV proteins and of the PP2A catalytic subunit (PP2Ac). Their study showed that insulin-induced tyrosine phosphorylation of IRS-1 and IRS-1 association with PI3K was normal in cells expressing HCV proteins while serine phosphorylation of Akt and GSK3b were impaired. To test if PP2A could dephosphorylate Akt/PKB *in vitro* authors added various amounts of purified PP2A to cell extracts of insulin stimulated Huh7 cells and they observed Akt/PKB dephosphorylation. Pretreatment of the cells with an inhibitor of PP2A (okadaic acid), resulted in Akt/PKB phosphorylation on serine 473 and threonine 308. On the other hand, when cells were pre-incubated with the PI3K inhibitor (wortmannin), neither threonine 308 nor serine 473 phosphorylation of Akt/PKB was induced by okadaic acid.

An increase of serine 473 phosphorylation of Akt/PKB was also evident in cells where PP2Ac expression was reduced using a siRNA silencing vector. These findings support a model in which PP2A is necessary for the dephosphorylation and inactivation of Akt/PKB, thereby counteracting a constitutive phosphorylation through background activity levels of PI3K. The α subunit of AMPK (AMPKα) was found to be constitutively phosphorylated on threonine 172 in Huh7 cells. This phosphorylation was enhanced when cells were treated for 2 h with okadaic acid. Furthermore, siRNA silencing of PP2Ac increased the phosphorylation on threonine 172. Similar to Akt, AMPK seems to be continuously phosphorylated by upstream kinases and then dephosphorylated by PP2A either directly or indirectly. A role of PP2A in AMPK dephosphorylation is supported by the finding that addition of purified PP2A to whole cell extracts of Huh7 cells leads to a dephosphorylation of $pThr^{172}$AMPKa.

## DISCUSSION

Based on the findings of the reviewed articles, different studies had quite antonym findings regarding impact of HCV infection on IR and IRS-1 content as well as its phosphorylation. In this section, we try to conclude the confirmatory and/or contradictory findings of different studies reviewed in the current review article on different effects of HCV infection on insulin signaling pathway components.

According to Kawaguchi *et al.* (2004), HCV infection downregulates IRS-1 and IRS-2 but has no impact on IR expression. However, Aytug *et al.* (2003) have shown upregulation of IR and IRS-1 contents in infected hepatocytes. On the other hand, phosphorylation levels of IRS-1 has been reportedly decreased by Pazienza *et al.* (2007) but normal in insulin-induced HCV transfected cells by Bernsmeier *et al.* (2008).

Aytug *et al.* (2003) found normal levels of p85 subunit of PI3 Kinase in HCV infected cells and Kawaguchi *et al.* (2004) reported downregulation of phosphorylation of the p85 subyunit of PI3K in HCV transfected cells. Association of p85-PI3K to IRS-1 was decreased in the study by Aytug *et al.* (2003) but normal after insulin treatment in the study by Bernsmeier *et al.* (2008).

Akt phosphorylation in HCV infected cells was reported to be severely blunted by Aytug *et al.* (2003); Pazienza *et al.* (2007), Kawaguchi *et al.* (2004) and Bernsmeier *et al.* (2008). Banerjee *et al.* (2008) suggested that insulin cannot upregulate $Thr^{308}$ phosphorylation of Akt in HCV infected cells and it's the potential mechanism of insulin resistance induced by HCV.

Pazienza *et al.* (2007) reported that PPARγ agonist reversed IRS-1 and Akt phosphorylation downregulation in core protein 3a transfected cells. SOCS-7 (but not other SOCS) was also significantly higher in the mentioned cells and its role in downregulation of IRS-1 content and phosphorylation of Akt was confirmed using 2 siRNAs. On the other hand, Kawaguchi *et al.* (2004) reported an upregulation of SOCS-3 in HCV infected cells and they found that HCV have no impact on IRS-1 and IRS-2 when cells are SOCS-3 negative and vice versa.

According to a study by Christen *et al.* (2007), HCV protein expression can cause ER stress response and PP2Ac over expression. They found a CREB sequence in the promoter site of the PP2Ac which was phosphorylated in HCV transfected cells and non phosphorylated in the control cells; using siRNA and Ca-chelator, authors showed that downregulation of CREB results in PP2Ac downregulation while use of $CaCl_2$ induces PP2Ac overexpression. Bernsmeier *et al.* (2008) found that purified PP2A dephosphorylates Akt while both siRNA and PP2A inhibitor promote Akt phosphorylation on both sites. These data support a model where PP2A is necessary for the constant dephosphorylation and inactivation of Akt and insulin resistance as the result.

Banerjee *et al.* (2008) found that PI3-K inhibitor does not impair glucose uptake while JNK inhibitor relieves glucose uptake inhibition induced by HCV. They concluded that JNK pathway is the main rout of insulin resistance in HCV infection. Bernsmeier *et al.* (2008) found that HCV impairs GSK3 phosphorylation.

## CONCLUSION

In conclusion, this review showed that findings of different studies on the impact of HCV infection on IR or its substrates are quite contradictory and is subjected for future investigation. Overwhelming data confirms severe blunting of Akt phosphorylation in HCV infected cells. JNK pathway is suggested as the main rout of insulin resistance in HCV infection. On the other hand, SOCS-3 is also suggested as a key component of this process but there still a need for further studies is felt for confirmation.

## REFERENCES


Alter, M.J., 1995. Epidemiology of hepatitis C in the West. Semin. Liv. Dis., 15: 5-14.

Asselah, T., L. Rubbia-Brandt, P. Marcellin and F. Negro, 2006. Steatosis in chronic hepatitis C: Why does it really matter? Gut, 55: 123-130.

Aytug, S., D. Reich, L.E. Sapiro, D. Bernstein and N. Begum, 2003. Impaired IRS-1/PI3-kinase signaling in patients with HCV: A mechanism for increased prevalence of type 2 diabetes. Hepatology, 38: 1384-1392.

Banerjee, S., K. Saito, M. Ait-Goughoulte, K. Meyer, R.B. Ray and R. Ray, 2008. Hepatitis C virus core protein upregulates serine phosphorylation of insulin receptor substrate-1 and impairs the downstream akt/protein kinase B signaling pathway for insulin resistance. J. Virol., 82: 2606-2612.

Bernsmeier, C., F.H. Duong, V. Christen, P. Pugnale, F. Negro, L. Terracciano and M.H. Heim, 2008. Virus-induced over-expression of protein phosphatase 2A inhibits insulin signalling in chronic hepatitis C. J. Hepatol., 49: 429-440.

Bieche, I., T. Asselah, I. Laurendeau, D. Vidaud and C. Degot *et al.*, 2005. Molecular profiling of early stage liver fibrosis in patients with chronic hepatitis C virus infection. Virology, 332: 130-144.

Caronia, S., K. Taylor, L. Pagliaro, C. Carr and U. Palazzo *et al.*, 1999. Further evidence for an association between non-insulin-dependent diabetes mellitus and chronic hepatitis C virus infection. Hepatology, 30: 1059-1063.

Christen, V., S. Treves, F.H. Duong and M.H. Heim, 2007. Activation of endoplasmic reticulum stress response by hepatitis viruses up-regulates protein phosphatase 2A. Hepatology, 46: 558-565.

Fartoux, L., A. Poujol-Robert, J. Guechot, D. Wendum, R. Poupon and L. Serfaty, 2005. Insulin resistance is a cause of steatosis and fibrosis progression in chronic hepatitis C. Gut, 54: 1003-1008.

Kawaguchi, T., T. Yoshida, M. Harada, T. Hisamoto and Y. Nagao *et al.*, 2004. Hepatitis C virus down-regulates insulin receptor substrates 1 and 2 through up-regulation of suppressor of cytokine signaling 3. Am. J. Pathol., 165: 1499-1508.

Khattab, M.A., M. Eslam and S.M. Alavian, 2010. Hepatitis C virus as a multifaceted disease: A simple and updated approach for extrahepatic manifestations of hepatitis C virus infection. Hepat. Mon., 10: 258-269.

Khedmat, H., S.M. Alavian, S.M. Miri, M. Amini and H. Abolghasemi *et al.*, 2009. Trends in Seroprevalence of Hepatitis B, Hepatitis C, HIV and Syphilis infections in Iranian blood donors from 2003 to 2005. Hepat. Mon., 9: 24-28.

Knobler, H., R. Schihmanter, A. Zifroni, G. Fenakel and A. Schattner, 2000. Increased risk of type 2 diabetes in noncirrhotic patients with chronic hepatitis C virus infection. Mayo. Clin. Proc., 75: 355-359.

Lau, J.Y., X. Xie, M.M. Lai and P.C. Wu, 1998. Apoptosis and viral hepatitis. Semin. Liver Dis., 18: 169-176.

Mehta, S.H., F.L. Brancati, M.S. Sulkowski, S.A. Strathdee, M. Szklo and D.L. Thomas, 2000. Prevalence of type 2 diabetes mellitus among persons with hepatitis C virus infection in the United States. Ann. Intern. Med., 133: 592-599.

Micallef, J.M., J.M. Kaldor and G.J. Dore, 2006. Spontaneous viral clearance following acute hepatitis C infection: A systematic review of longitudinal studies. J. Viral. Hepat., 13: 34-41.

NIH, 2002. National Institutes of Health Consensus Development Conference Statement: Management of hepatitis C, In: Hepatology, NIH (Ed.). Vol. 36, Elsevier, USA., pp: s3-s20.

Negro, F., 2006. Insulin resistance and HCV: Will new knowledge modify clinical management? J. Hepatol., 45: 514-519.

Pazienza, V., S. Clement, P. Pugnale, S. Conzelman, M. Foti, A. Mangia and F. Negro, 2007. The hepatitis C virus core protein of genotypes 3a and 1b downregulates insulin receptor substrate 1 through genotype-specific mechanisms. Hepatology, 45: 1164-1171.

Pekow, J.R., A.K. Bhan, H. Zheng and R.T. Chung, 2007. Hepatic steatosis is associated with increased frequency of hepatocellular carcinoma in patients with hepatitis C-related cirrhosis. Cancer, 109: 2490-2496.

Pessin, J.E. and A.R. Saltiel, 2000. Signaling pathways in insulin action: Molecular targets of insulin resistance. J. Clin. Invest., 106: 165-169.

Romero-Gomez, M., M. Del Mar Viloria, R.J. Andrade, J. Salmeron and M. Diago *et al*., 2005. Insulin resistance impairs sustained response rate to peginterferon plus ribavirin in chronic hepatitis C patients. Gastroenterology, 128: 636-641.