Insulin Resistance in Liver Diseases

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Abstract

Present report gives a brief and consolidated review of insulin resistance developed in chronic liver diseases. Insulin resistance remains an important feature of chronic liver diseases and progresses disease towards fibrogenesis. Of hepatitis viral infections, hepatitis C virus (HCV) was reported to have a significant role in inducing insulin resistance. Both viral particles as such, as well its structural components induce insulin resistance. Hepatitis C virus core protein, specially, causes insulin resistance via its direct action on insulin signaling cascade as well as by inducing over expression of certain cytokines including TNF-α. Insulin resistance has a direct relation with liver steatosis and oxidative stress. Both steatosis and oxidative stress enhances insulin resistance and vice-a-versa. Insulin resistance has widespread implications on metabolism and needs its correction before planning therapeutic regimen in liver diseases.

Introduction

Insulin is an anabolic hormone secreted by pancreatic β-cells. Insulin affects a wide range of physiological processes. However, it is best known for the maintenance of glucose homeostasis. On elevation of plasma glucose, β cells secrete insulin that stimulates glucose uptake and glycogen synthesis and simultaneously inhibits hepatic glucose production, specially glycogenolysis and gluconeogenesis, thus maintaining normoglycemia. In addition, insulin is also involved in regulation of amino acid uptake, lipid metabolism in muscle and adipose tissue, and cell growth and survival [1-4]. A coordinated action and secretion of insulin maintains its normal metabolic actions. In type 2 diabetes (T2DM), both its action and secretion are impaired, though the exact mechanism is still not known.

The principal action pathway of insulin involves sequential activation of the insulin receptor, insulin receptor substrates (IRS), phosphatidylinositol-3-kinase (PI3K), Akt and protein kinase [5-7]. Akt is a serine-threonine-kinase that phosphorylates proteins in several pathways regulating aspects of metabolism, apoptosis, and proliferation. Akt signaling promotes proliferation and increased cell survival and is thought to play an important role in prostate cancer progression.
Akt is activated by its phosphorylation by phosphoinositide-dependant kinase (PDK1/2) and promotes glycogen synthesis simultaneously suppressing gluconeogenesis (Fig. 1). In striated muscles and adipose, activated Akt promotes translocation of glucose transporter, GLUT-4, to the plasma membrane, thus, facilitating glucose uptake [5]. Insulin regulates lipid metabolism via sterol regulatory element binding protein Ic (SREBP1c) [8]. Similarly, its action on protein metabolism and cell survival involve the mediation of mammalian target of rapamycin (mTOR) pathway [5]. Mammalian target of rapamycin (mTOR) is a protein kinase involved in translation control and long-lasting synaptic plasticity. mTOR functions as the central component of two multi-protein signaling complexes, mTORC1 and mTORC2. The studies in genetically modified mice also suggest that mTOR couples receptors to the translation machinery for establishing long-lasting synaptic changes that are the basis for higher order brain function, including long-term memory. Finally, perturbation of the mTOR signaling cascade appears to be a common pathophysiological feature of human neurological disorders, including mental retardation syndromes and autism spectrum disorders [9].

Insulin resistance (IR) results from defects at the level of ligand-receptor-response pathway. Such a defect may occur either at the receptor level or in IRS molecules. Further, these defects can result from either reduced levels of signaling proteins or modulation of their activity by phosphorylation. In case of IRS-1, it is activated by phosphorylation of tyrosine residue but inhibited by phosphorylation of serine residues [10]. Similarly, inhibition at PI3K level also changes or diminishes insulin action. IR plays not only an important role in T2DM pathogenesis but at the same time, causes, several metabolic disturbances during liver diseases.

Human liver has two important IRS proteins i.e. IRS-1 and IRS-2 [11]. Whereas IRS-1 plays role in growth and insulin resistance, IRS-2 is involved in β-cell failure and hepatic insulin resistance. Also it was reported that IRS-1 controls peripheral glucose uptake and IRS-2 regulates both peripheral and central insulin action [12]. This shows that IRS-2 is more important for insulin signaling and glucose homeostasis in the liver [13]. However, other reports indicate IRS-1 involvement in glucose homeostasis and IRS-2 involvement in lipid metabolism [14]. In fact, there is no definite line to demarcate their separate roles except their differential involvement in certain pathways of insulin action cascade.

Present article is aimed to give an overall view of the possible causes of insulin resistance (IR) in liver diseases with delineation of underlying mechanism. Further, it also describes the role of HCV and its structural components in causing insulin resistance and T2DM. This article presents a detailed description of HCV related proteins playing significant role in IR inducement during HCV infection, specially in chronic liver diseases. It also discusses the impact of IR in final outcome of liver diseases both without treatment and after treatment.

### Presence of IR in Various Liver Diseases

Insulin resistance (IR) has been reported in different conditions, such as, the metabolic syndrome, obesity, cirrhosis and diabetes. IR has been noticed quite often in liver diseases. It is an underlying mechanism involved in the development of non-alcoholic fatty liver diseases (NAFLD) and has recently been associated with chronic hepatitis C virus (HCV) infection also. IR in chronic HCV infection predicts faster [15, 16] progression of diseases to fibrosis and cirrhosis, leading to liver failure and hepatocellular carcinoma (HCC). Presence of insulin resistance also predicts a poor response to antiviral therapy in chronic HCV infection. Of liver dis-
eases, IR is present in a large number of patients with cirrhosis: 60-80% are glucose intolerant and 20% of them develop diabetes mellitus [17-18]. Plasma insulin and C-peptide levels were found 2-3 times higher in cirrhotic patients than in non-cirrhotic controls [17]. A lower muscle mass, an increased lipid oxidation and alcohol consumption usually induce insulin resistance. The chronic elevation of different insulin antagonists, such as, free fatty acids, cytokines, glucagon, growth hormone, catecholamines may contribute to IR in cirrhotic patients.

The association between HCV and T2DM was noted by Allison et al [15]. In their report, they demonstrated that patients with cirrhosis and HCV developed T2DM more frequently than those with non-HCV cirrhosis. Studies on comparative prevalence of T2DM in HCV and HBV related cirrhosis recorded it in 20% vs. 12% cases [16]. Also T2DM was noted in chronic HCV infection without cirrhosis [17, 18]. Subsequent studies found that incidence of T2DM in persons with HCV infection and in age group of more than 40 was quite high. At the same time, its association with HBV infection was very low. Some studies reported that HCV causes specially T2DM and not T1DM. It is worth to note here that T2DM was associated with HCV genotype 1b, 2a [19] and genotype 4 [20], though all HCV infected patients do not develop IR. Similarly, IR was not recorded in chronic HBV [20] patients thus, implying that HCV infected patients with sign of cirrhosis are more vulnerable to develop IR.

Although mechanism of IR inducement in HCV is not fully understood [21], it was found that HCV induces IR via different pathways including those responsible for oxidative stress. Steatosis, fibrosis, apoptosis, altered gene expression and hepatocellular carcinoma. In all these, there is interference of intracellular insulin signaling by HCV proteins, such as, inactivation of IRS-1 and Akt pathways [22].

Factors Causing IR in Liver Diseases

Steatosis:

Hepatic steatosis is defined as accumulation of fat in hepatocytes. Liver diseases including alcoholic liver diseases, metabolic disorders, hepatitis viral diseases and drug induced liver diseases develop hepatic steatosis [23-25]. Steatosis is one of major factor causing insulin resistance during liver diseases [26]. There is a vice-a-versa relation and in turn IR promotes the development of steatosis. Therefore, it is important to understand the occurrence and mechanism of hepatic steatosis in different liver diseases. Steatosis is frequently found in chronic HCV infection, usually with its prevalence in 40-80% patients. During HCV infection, two types of steatosis were noted: metabolic steatosis and viral steatosis. Hepatic steatosis is a common histological feature of chronic HCV infection. Furthermore, steatosis promotes inflammation and fibrosis in liver. Many studies have shown that HCV causes steatosis which affects progression of HCV related liver disease.

In fact, liver steatosis has been maximally studied in relation to HCV infection and involvement of HCV components. HCV core protein contributes to liver steatosis [27], hepatic fibrosis and hepatocellular carcinoma. Studies on mechanism of steatosis by HCV suggest that core protein inhibits microsomal triglyceride transfer protein (MTP) activity and very low density lipoprotein (VLDL) secretion. It also impairs the expression of Peroxisome Proliferator-Activated Receptor (PPAR) [28], PPAR, is a member of the nuclear hormone receptor superfamily that is essential for the development of adipocytes. It is the transcription factors which regulate adipocyte differentiation and genes involved in fatty acid and glucose metabolism [29]. PPAR, increases transcription of genes responsible for fatty acid synthesis and their uptake and thus, regulates lipid synthesis, transport and storage within hepatocytes. Similarly, SREBPIs, sterol regulatory element binding proteins, are transcription factors that regulate FA synthesis in liver. These factors increase transcription of genes involved in hepatic fatty acid synthesis. In order to study the effect of HCV infection on steatosis, Kim and workers [30] have shown that HCV core protein induces SREBP1 gene expression, causing increased fatty acid synthesis. Also core elevates PPAR, activity and increases FA uptake. Steatosis results both from viral and host factors. It has been mostly noted in patients with genotype 3a. Studies by Abid et al [31] demonstrated that genotype 3a derived core protein was about 3 fold more efficient than the corresponding protein from genotype 1b at inducing triglyceride accumulation. HCV 3a core protein also diminished PPAR, mRNA compared with 1b core. All these studies [32, 33] shown that HCV core leads to up regulation or down regulation of several genes involved in lipid transport and metabolism in genotype specific manner. And so, there is a direct link between virus infection and steatosis development.

Liver fibrosis is considered to be responsible for
IR and T2DM in patients with chronic liver diseases. During cirrhosis, there is diminished hepatic insulin extraction and thus, hyper-insulinemia. Both IR and insulin secretion contribute to glucose intolerance in chronic HCV [34]. IR was always found higher in patients with chronic HCV than patients with other causes of chronic hepatitis matched by sex, BMI and fibrosis staging [35]. HOMA-IR, Homeostasis Model Assessment—Insulin Resistance, correlated with HCV-RNA level. Similarly, diabetes was seen more often in HCV than other liver diseases [36]. In chronic HCV infection, steatosis upregulates hepatocyte CD95/FAS and thus increases apoptosis, inflammation and fibrosis [37].

Whereas IR is the underlying mechanism involved in steatosis (fatty liver) [38], at the same time, fat in liver also stimulates IR [39]. In case of non-alcoholic fatty liver, there is a role of several adipokines and pro-inflammatory cytokines, promoting steatosis and IR. Activated adipocytes release many adipokines [40, 41] including leptin, resistin and visfatin that have important role in steatotic hepatitis. Adiponectin [42], a hormone produced by adipocyte is present in plasma and enhances insulin sensitivity, decreases triglyceride level and improves glucose metabolism [40, 41]. Low adiponectin also causes IR and T2DM and hepatic fibrosis. Adiponectin also acts as anti-fibrogenic and carries anti-inflammatory properties. Like adiponectin, even leptin sustains IR and stimulates release of TNF-α, IL-6, IL-12 and profibrotic cytokines, thus causing liver fibrogenesis.

Oxidative Stress:

Oxidative stress (OS) is supposed to be an important part of liver pathogenesis during viral hepatitis. Second, oxidative stress has relation with steatosis and thus plays important part in causation of IR. Although, presence of oxidative stress has been noted in other hepatitis like hepatitis B also, however, there is a remarkable increase in oxidative stress in HCV infection. Several studies conducted at molecular level have shown that structural components of HCV induce an effective oxidative stress. It was noticed that HCV-core protein present within the outer membrane of mitochondria induces oxidation of mitochondrial glutathione and promotes Ca²⁺ uptake into mitochondria by sensitizing it to mitochondrial permeability transition. Clement et al [43] explained the molecular mechanism and demonstrated that following glutathione oxidation, there is increased reactive oxygen species (ROS) production by mitochondrial electron transport complex I and III. The HCV non-structural proteins, NS3, NS5A, etc. are associated with membrane of endoplasmic reticulum, (ER) and activate the release of Ca²⁺ from ER. This induces oxidative stress. NS3 also induces ROS production via activation of NADPH oxidase 2 (NoX2).

Various reports have shown the presence of increased markers of oxidative stress during HCV infection. These include the presence of 8-hydroxydeoxyguanosin and 4-hydroxy, 2-nonenal, a product of lipid peroxidation in blood of HCV infected patients [44, 45]. Oxidative stress gives rise to IR that plays important role in liver pathogenesis. Further, oxidative stress caused during HCV infection depends on HCV genotypes. It has been observed that serum level of thioredoxin, a marker of oxidative stress depends and varies with HCV genotypes [46]. That HCV causes oxidative stress by itself is indicated by its presence in HCV carriers without a sign of liver diseases or any other sign of inflammation [47]. HCV may directly cause the oxidative stress. Although oxidative stress has been noted in HBV infection also, however, HCV appears more effective to generate oxidative stress [44]. HCV induced oxidative stress contributes to the activation of pro-apoptotic Bax together with the prevention of antiapoptotic BcL-xL, thus sensitizing HCV infected cells to apoptosis [48].

Hepatitis viruses:

As described earlier, development of T2DM is frequently associated with chronic HCV infection than HBV infection. The informations about inducement of IR in other hepatitis viral infections including hepatitis viruses A, D, E and G infections, etc. are very meager. Whereas it has been studied in relation to HBV and HCV, most of the available informations indicate HCV and its components to have special role in causing IR. Presence of IR in chronic HCV infection predicts non-response to anti-viral therapy [49] for all genotypes including genotype 1, 2 and 3. At the same time, those who respond to therapy also show improved insulin sensitivity. IR usually relates with HCV viral load and thus, is increased with increase in viral load [50, 51]. HCV associated IR favours viral replication, though the exact mechanism how HCV induces IR is not clear.

The relationship among HCV, IR, steatosis and hepatic fibrosis is quite complex [52] and depends on HCV-genotypes. IR, which is caused both by metabolic factor [53] as well as virus itself, usually remains associated with steatosis. The available informations on IR inducement do not clearly mention whether all HCV-
genotypes including genotype 1, 3 and 4 etc. cause IR directly or via steatosis. Second, whether all of them act similarly or have differential mechanism of causing IR and steatosis, is not very well understood [54].

In HCV patients, both steatosis and IR remain predictive factors for later progression to hepatic fibrosis and cirrhosis [55-57]. Diabetes also increases the risk of HCC [58] in all these patients. Although mechanism of IR during HCV infection still needs several studies to confirm it yet, the available informations indicate that HCV infection causes hepatic inflammation that gives rise to IR. Chronic inflammation increases levels of interleukin IL-1, tumor necrosis factor (TNF-α), IL-6 and leptin. At the same time, level of adiponectin [59] get reduced. These changes stimulate inflammatory mediator IR kinase B (IRKB) [60] which induces proteosomal degradation of IRKB, translocates effective molecule NFKβ to nucleus and stimulate secretion of IL-6 [61]. Following another course, IKKB also induces IR by inhibiting phosphorylation of IRS-1 at serine 312 [62]. Such a mechanism gets a support that IR is reversed by administering antibodies against TNF-α. In contrast to this theory of IR during HCV infection, few other studies do not support the change in serum inflammatory cytokines or adipokines to be responsible for causation of IR [63]. These studies stress that direct effect of HCV on insulin signaling causes IR [64-66]. There is an important role of HCV core protein in causing IR by a direct effect on IRS-1 and IRS-2, where HCV core reduces their level or activity. However, whether it is IRS expression, degradation or change in its activity caused by HCV core, shows disagreement among different studies [21, 67-69].

HCV core protein is supposed to increase levels of the molecular molecule suppressor of cytokine signaling (SOCS) [51], leading to proteosomal degradation of IRS-1 and IRS-2 [69]. The patients who respond to antiviral therapy, usually have increased level of hepatic IRS-1 and IRS-2 and high insulin sensitivity [70]. This implies that level of SOCS-3 in peripheral lymphocytes is a good predictor of response to interferon therapy [71, 72].

The role of HCV genotypes in causing IR is a recent area with finding and reports on activities of different genotypes. One study in Huh7 hepatoma cell line demonstrated over expression of genotype 1b and 3a core proteins with no difference in SOCS-3 expression. In contrast, cells expressing genotype 1b core shown increased phosphorylation of IRS-1 at inhibitory serine residue and increased mTOR activity. These studies conclude that IR in HCV genotype 1 is due to core-induced induction of mTOR resulting in low IRS-1 signaling. Other studies ascribed the effect of HCV core to c-Jun N-terminal kinase (JNK) mediated inhibitory phosphorylation of IRS-1 at serine 312. JNK is a volume-sensitive protein kinase. This cytosolic kinase was activated by both hypertonic and isosmotic shrinkage, indicating regulation by cell volume rather than osmolarity.

Some other studies based on HCV protein expressing cell lines and liver biopsies from HCV infected patients, demonstrated the role of HCV non-structural protein NS5A in causing IR during HCV infection. NS5A directly upregulates [73] protein phosphatase 2A (PP2A) which can effect cell pathways leading to dephosphorylation and inactivation of Akt [74] which plays important role in insulin signaling. PP2A also inhibits interferon signaling where there may be a link between IR and response to treatment [75].

Peroxisome proliferators activated receptor (PPAR) are nuclear receptors and modulate lipid and glucose metabolism. PPARs (α and γ) react to lipid excess, differentiate adipocytes and affect FA oxidation and glucose metabolism. PPAR-α in liver stimulates. FA oxidation in mitochondria and peroxisomes [76]. PPARγ stimulates adipocyte differentiation and lipid storage [76]. Both PPARs have been reported to play a role in HCV-induced IR. Cells expressing HCV core protein in vitro had reduced levels of PPARγ mRNA, and thus reduced insulin signaling. PPARγ is involved in HCV core induced steatosis [77].

HCV core is involved in inducing oxidative stress and steatosis that lead to insulin resistance. Several studies have reported increased mitochondrial reactive oxygen stress in hepatoma cells by HCV-core [77-81]. Studies in transgenic mice expressing HCV core protein also indicate that during HCV replication, core protein induces not only mitochondrial permeability transition and ROS production, but also misfolding of HCV proteins in ER to cause inflammation and ER stress [82]. NS3 also activate NADPH oxidase 2 (NoX2) that generates ROS [36] and also causes fibrosis by nitrosylating proteins. NS3 inhibitor on the other hand improves insulin sensitivity [83]. Core also induces overproduction of TNF-α which phosphorylate IRS-1 and IRS-2 at serine and reduce glucose transport. HCV itself induces insulin resistance by several factors involved in interferon resistance, allowing the virus to resist antiviral treatment and promote fibrosis progression [84]. However, relation among IR, oxidative stress, metabolic syndrome and steatosis are complex. Oxidative stress
and IR cause steatosis, which in turn accelerates the progression of fibrosis.

**Impact of Insulin Resistance (IR)**

In most of studies IR has been supposed to increase the disease progression and reduced response to therapy. In HCV infected chronic liver diseases, patients have shown reduced response to interferon-α (IFN-α) therapy. Whether IR causes all these problems in association or independent of contributing factors like steatosis and oxidative stress, is not very clear. As most of the reports demonstrate the significance of IR in relation to HCV infection, all therapeutic measures versus steatosis or oxidative stress were studied in HCV patients. Use of anti-oxidants in HCV patients, both with and without IFN-α based therapy, produced controversial results. There was a reduction in transaminases level but no significant change in HCV viral load [85]. Similarly, the impact of steatosis on antiviral therapy is not clear. All studies have shown that steatosis shows an independent response to therapy that has HCV-genotype specific effect [23]. Similarly, steatosis causes fibrosis and the fibrosis stage depends on HCV-genotypes [57]. It appears as if IR indeed is a better predictor of Sustained virologic response (SVR) than metabolic steatosis [58]. Increasing IR reduces initial virological response and SVR independent of HCV genotypes. It is supposed that deregulation of SOCS-3 may be involved in both IR and lack of response to therapy [87]. One major concern of IR during HCV infection is the difficulty in framing the exact treatment model because of non-availability of well established mechanism. And therefore, the drugs targeting PPARγ, protein kinase or IRS-2 have been put to trial [78, 79]. Whereas in certain cases treated patients showed improvement in their HOMA-IR, non had satisfactory virological response. Reducing IR prior to antiviral therapy may improve outcome [80]. Several other studies are underway whose results are still awaited. In view of all these studies, it appears as if correcting IR in chronic HCV patients may be helpful to give effective treatment to the patients. However, many more studies are needed to divulge the mystery of complicated mechanism and the role of IR in producing final effect on treatment.

**Conclusion**

Based on all the informations compiled from literature, it is clear that insulin resistance is quite common in chronic liver diseases. Level of resistance is raised with severity of disease and is frequently noted in patients with liver cirrhosis. During hepatitis viral infections, it is noticed mainly in HCV infected patients. Both HCV as such, as well as its structural components, particularly, HCV core protein induces and increases insulin resistance. In all the situations, insulin resistance is closely associated with hepatic steatosis, oxidative stress, inflammation and liver fibrogenesis. All these conditions increase each other both in association as well as independently. Insulin resistance has a major effect on treatment of liver diseases and therefore, its presence and level decides the therapeutic model for liver diseases. Beyond this point, there is still a lot to be done in this area for more understanding of its mechanism and final solution.

**References**

11. Thirone AC, Huang C, Klip A. Tissue-specific roles of IRS


