

## The effect of hepatotropic virus B and C on the degree of hyperglycemia and iron overload and the correlation between hyperglycemia and iron overload in hepatitis patients

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### Abstract

**Aims:** This study was design to evaluate the effect of hepatotropic virus's types on the degree of hyperglycemia and iron overload and the correlation between hyperglycemia and iron overload in patients with hepatitis B and C.

**Methods:** Fifty patients infected with hepatitis B and C virus admitted in Al-Hussein teaching hospital /Thi-Qar governorate, along with fifty healthy individual as a control group for comparison purposes. The study period was from April 2011 to July 2012.

**Results:** Of these fifty patients 47 were male (94%) and 3 were female (6%), out of which 39 (78 %) had viral hepatitis B infection and 11 (22 %) had viral hepatitis C infection. The levels of blood glucose ( $209.52 \pm 55.11$  mg/dL) and iron ( $386.65 \pm 95.62$   $\mu$ g/dL) were significantly elevated in hepatitis patients as compare with the control group ( $73.22 \pm 8.30$  mg/dL) and ( $154.15 \pm 11.18$   $\mu$ g/dL) respectively. The concentration of blood glucose was  $210.39 \pm 52.37$  mg/dL and  $195.84 \pm 64.77$  mg/dL in hepatitis B and C respectively, there is no significant different in blood glucose between HBV and HCV patients. The concentration of iron was  $390.88 \pm 114.82$   $\mu$ g/dL and  $385.27 \pm 89.87$   $\mu$ g/dL in hepatitis B and C respectively, there is no significant different in blood iron between HBV and HCV patients. There is a positive correlation between blood glucose and iron in hepatitis patients.

**Conclusion:** The fifty hepatitis patients associated with severe hyperglycemia and iron overload and there is no effect of hepatotropic virus types in the severity degree of hyperglycemia and iron overload. There is a positive correlation between levels of glucose and iron.

Finally, this study suggests that the liver damaged in viral infection disease is not due to the virus directly but to metabolic abnormalities, which are associated with infection such as hyperglycemia and iron overload. On other hand, hyperglycemia had a role in the severity of iron overload that is a problem because its exacerbate oxidative stress and could be the underlying mechanism connecting low rate response to antiviral therapy. So screening for glucose and iron abnormalities should be indicate in hepatitis patients and patients must be use suitable drugs before starting the antiviral therapy.

**Keywords:** Hyperglycemia, Iron overload, Hepatitis B virus, Hepatitis C virus

## تأثير فيروس الكبد B و C على درجة فرط السكر والحديد والعلاقة بين فرط السكر وفرط الحديد لدى مرضى التهاب الكبد الفيروسي

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### الخلاصة

الأهداف: صممت الدراسة الحالية لتحديد تأثير نوع فيروس الكبد على درجة فرط السكر والحديد والعلاقة بين فرط السكر وفرط الحديد لدى مرضى التهاب الكبد الفيروسي من نوع B و C. طريقة العمل : تم تجميع خمسين عينة من أشخاص مصابين بفيروس الكبد B و C وافدين إلى مستشفى الحسين التعليمي / محافظة ذي قار , وأيضاً خمسين عينة من الأشخاص الأصحاء كمجموعة سيطرة لغرض المقارنة. كانت فترة الدراسة من نيسان ٢٠١١ ولغاية تموز ٢٠١٢ . النتائج: تضمن الخمسين شخص ٤٧ رجل (٩٤%) و ٣ نساء (٦%) , ٣٩ (٧٨%) مصابين بفيروس الكبد B و ١١ (٢٢%) مصابين بفيروس الكبد C . وجد إن مستويات السكر ( 209.52 ± 55.11 mg/dL ) والحديد ( ٣٨٦,٦٥ μg/dL ± 95.62 ) مرتفعة وبشكل ملحوظ لدى المرضى مقارنة بمجاميع السيطرة ( 73.22 ± 8.30 mg/dL ) و ( 154.15 ± 11.18 μg/dL ) على التوالي . كان تركيز السكر لدى مرضى التهاب الكبد الفيروسي B و C mg/dL 210.39 ± 52.37 و mg/dL 195.84 ± 64.77 على التوالي , عند المقارنة لوحظ عدم وجود فرق معنوي بين القيم للمرضى المصابين بالتهاب الكبد الفيروسي B و C. تركيز الحديد لدى مرضى التهاب الكبد الفيروسي B و C كان 390.88 ± 114.82 μg/dL و 385.27 ± 89.87 μg/dL على التوالي, عند المقارنة لوحظ عدم وجود فرق معنوي بين القيم للمرضى المصابين بالتهاب الكبد الفيروسي B و C. كما و لوحظ أيضاً وجود علاقة طردية بين التغير في مستوى السكر والحديد . الاستنتاجات: يعاني الخمسين شخص من فرط السكر والحديد بدرجة شديدة ولا يوجد هنالك تأثير لنوع الفيروس على درجة الشدة لفرط السكر والحديد.توجد علاقة موجبة بين التغير في مستوى السكر والحديد. تقترح هذه الدراسة أن تلف الكبد في حالة الإصابة الفيروسية لا يعود للفيروس مباشرة وإنما للتغيرات المرافقة مثل فرط السكر والحديد حيث أن شدة تغير مستوى السكر له دور في زيادة فرط الحديد الذي أيضاً يعتبر مشكلة في تحفيز الشد التأكسدي ضمن ميكانيكية مسببا في النهاية إلى تقليل سرعة الاستجابة للعلاج المضاد للفيروس لذلك يجب التوصية بفحص شدة تغير مستوى السكر والحديد ومعالجته بالأدوية المخفضة المطلوبة أضافتا للعلاج المضاد للفيروس.

**الكلمة المفتاح:** فرط السكر , فرط الحديد , التهاب الكبد الفيروسي B , التهاب الكبد الفيروسي C

## **Introduction**

Viral hepatitis is a major human health problem worldwide [1]. Viral infections considered to be hepatotropic include viral hepatitis caused by hepatitis viruses A, B, C, D, E, and F, while the non-hepatotropic are Cytomegalovirus, Herpes Virus, and Epstein–Barr Virus. Most produce an acute infection, and only hepatitis viruses B, C, D, and E cause chronic liver disease. Many agents are capable of causing liver damage, including drugs, toxins, infections, and metabolic alterations, any of which may lead to liver failure. Viral infection is a frequent cause of liver damage [2]. Several studies have shown that the damage or metabolic changes induced by these viruses have a significant impact on several organs of the body besides the liver [3].

Hepatitis B virus (HBV) is a member of the Hepadnavirus family [4]. Acute infection of HBV in most people can trigger natural immunity to this virus, patients with persistent infection are prone for developing chronic hepatitis, liver cirrhosis, and/or hepatocellular carcinoma (HCC) [5-7]. Generally, It is believed that HBV infection is non-cytopathic, with its disease pathogenesis mediated by host innate and adaptive immune responses, as well as other host-virus interactions [8]. The virus HBV spread by contact with an infected person's blood, semen, or other body fluid. [9, 10].

The hepatitis C virus (HCV) is a linear, single-stranded RNA virus of the Flaviviridae family that was identified in 1989 and is recognized as the major causal agent of non-A, non-B hepatitis[11]. It is one of the leading causes of chronic liver disease worldwide, affecting 3% of the world's population. Diagnosis and treatment of HCV-related autoimmune features has become a clinical challenge in HCV-infected patients, in whom chronic liver disease associated with severe autoimmune features may contribute to a very poor prognosis [12, 13].

The HCV characterized by a high replicative potential and a high mutation rate. Due to the diversity of HCV genomes, the virus is classified into 6 genotypes, with several subtypes within each genotype. Chronic hepatitis C develops in approximately 70-80% of HCV-infected patients.

HCV replicates mainly in hepatocytes, but its nucleic acids have also been found in peripheral blood mononuclear cells and in central nervous system cells.[14-16]

Hepatitis C is of concern to both industrialized and developing countries [17]. Hepatitis C liver disease ranges in severity from a mild illness lasting a few weeks to a serious, lifelong illness that attacks the liver. It is spread primarily through contact with the blood of an infected person [18-20].

Hyperglycemia can impair a wide range of functions in neutrophils and macrophages, including chemotaxis, adherence, phagocytosis, and intracellular killing of microorganisms [17].

Iron is a necessary nutrient; it is need only in small amounts. High levels of iron are associated with an increased risk for cancer, heart disease, and other illnesses such as endocrine problems, arthritis, diabetes, and liver disease [21]. Liver iron accumulation in patients with chronic hepatitis has received increasing attention in recent years [22].

Some authors have found glycemic abnormalities in HBV-infected patients similar to those associated with HCV infection [23] However, others have reported that IR and HBV infection are not related [24,25] and that HBV infection may be protective against the development of IR [26,27] These inconsistencies indicate the need for prospective studies.

This study was design to evaluate the effect of hepatotropic virus's types on the degree of iron overload and hyperglycemia in patients with hepatitis B and C .These data on the relevance of blood glucose and iron as a prognostic factors prompted us to ascertain whether HCV and HBV related liver damage. Also, evaluate the correlation between hyperglycemia and iron overload in both hepatitis patients.

## **Patients and methods**

Fifty patients with hepatitis B and C virus infection were admitted in Al-Hussein teaching hospital /Thi-Qar governorate, were evaluated retrospectively over a period of 16 months from April 2011 to July 2012, along with fifty healthy

individual as a control group for comparison purpose.

The diagnosis of HBV and HCV had assayed by enzyme linked immune sorbent assay (ELISA) method, which based on double antibody sandwich technique [28,29]. Assay process performed manually by automatic ELA micro plate immuno-analyzer in the virus’s lab at Al Hussein Teaching Hospital Laboratory.

Biochemical analysis of blood sugar and iron had done by using standard common methods [30, 31].

**Statistical analysis**

Data analyzed by using statistical package for social sciences (SPSS) software version 15. Categorical variables expressed as frequency and percentages. Continuous variables expressed as mean and standard deviation. Continuous variables compared by the t-test. P-values < 0.05 was considered statistically significant.

**Results**

Table 1 and figure 1 show the prevalence of hepatitis according to the gender and virus type. Of these fifty patients 47 were male (94%) and 3 were female (6%), out of which 39 (78 %) had viral hepatitis B infection and 11 (22 %) had viral hepatitis C infection.

Table 1: The prevalence of hepatitis according to the gender and virus type

Gender	Patients number (%)	Hepatitis type	
		HBV (%)	HCV (%)
Males	47 (94)	38 (76)	9 (18)
Females	3 (6)	1 (2)	2 (4)
Total	50(100)	39 (78)	11 (22)

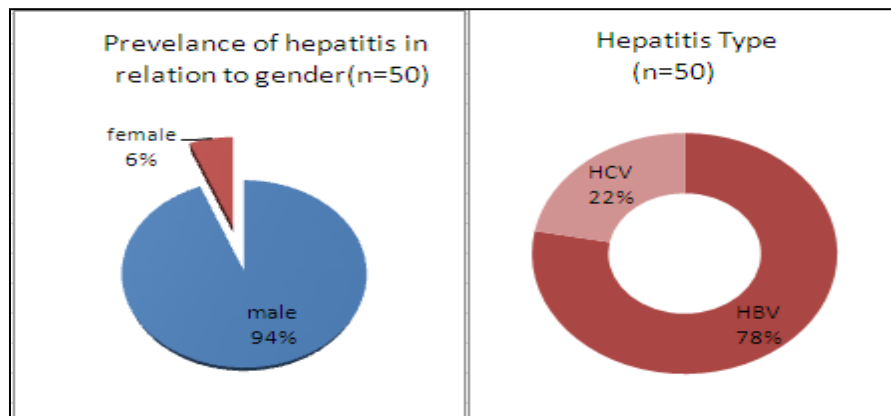


Figure 1: The prevalence of hepatitis in relation to gender of patients and hepatitis type

In table 2 and figure 2 we show the levels of blood glucose and iron in hepatitis patients and healthy controls group. Blood glucose  $209.52 \pm 55.11$  mg/dL and iron  $386.65 \pm 95.62$  µg/dL were significantly elevated in hepatitis patients as

compare with the control group ( $73.22 \pm 8.30$  mg/dL) and ( $154.15 \pm 11.18$  µg/dL) respectively.

Table 2: Changes in blood glucose and iron levels in fifty hepatitis patients and control group

Parameter	Control (n = 50)	Hepatitis (n= 50)
Glucose (mg/dL) mean ± SD *	73.22 ± 8.30 <sup>a</sup>	209.52 ± 55.11 <sup>b</sup>
Iron(µg/dL) mean ± SD	154.15 ± 11.18 <sup>a</sup>	386.65 ± 95.62 <sup>b</sup>

\*Each value represents mean ± SD values with non identical superscript (a, b or c ...etc.) were considered significantly different (P ≤ 0.05).

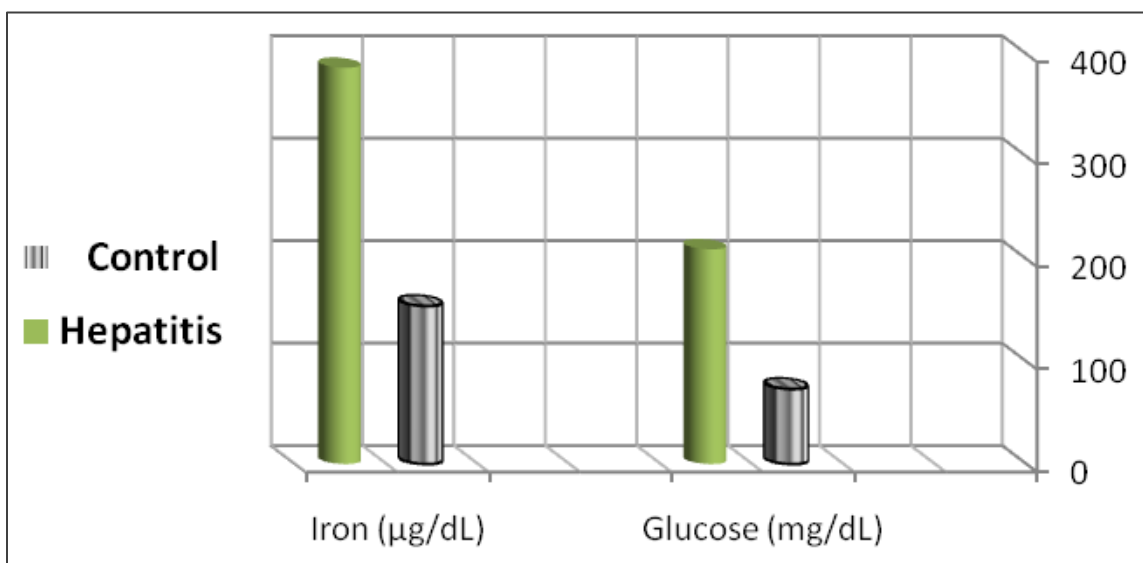


Figure 2: the levels of blood glucose (mg/dL) and iron (µg/dL) in hepatitis patients and healthy controls group

In table 3, figure 3 and figure 4, we can show the concentration of blood glucose and serum iron in both hepatitis patients group.

The concentrations of blood glucose were 210.39 ± 52.37 mg/dL and 195.84 ± 64.77 mg/dL in hepatitis B and C respectively, there is no

significant different in blood glucose between HBV and HCV patients. The concentrations of iron were 390.88 ± 114.82 µg/dL and 385.27 ± 89.87 µg/dL in hepatitis B and C respectively, there is no significant different in blood iron between HBV and HCV patients.

Table 3: Serum glucose and iron level of fifty hepatitis patients in relation to the type of hepatotropic viruses

Parameters	HBV	HCV
Glucose (mg/dL) mean ± SD	210.39 ± 52.37 <sup>a</sup>	195.84 ± 64.77 <sup>a</sup>
Iron(µg/dL) mean ± SD	390.88 ± 114.82 <sup>a</sup>	385.27 ± 89.87 <sup>a</sup>

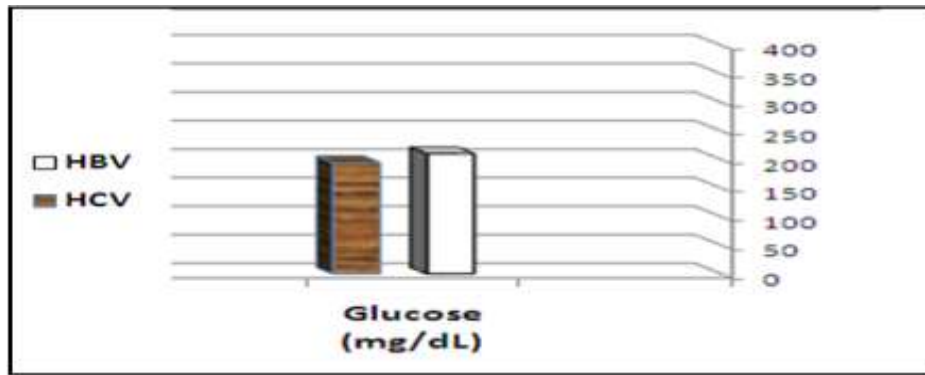


Figure 3: The concentration of blood glucose in both hepatitis patients group

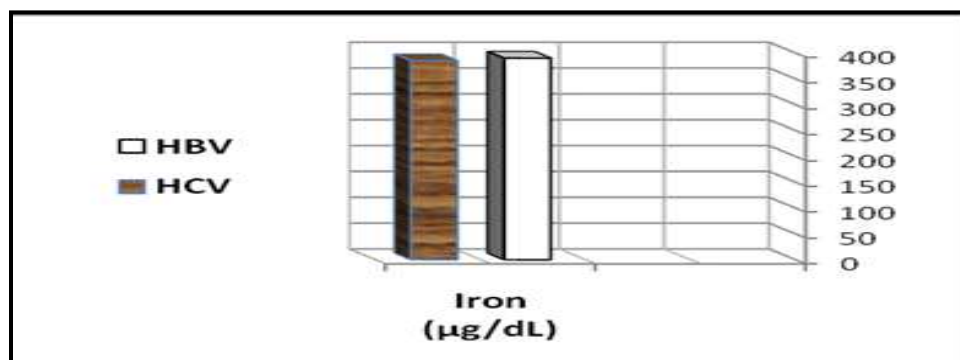


Figure 4: The concentration of serum iron in both hepatitis patients group

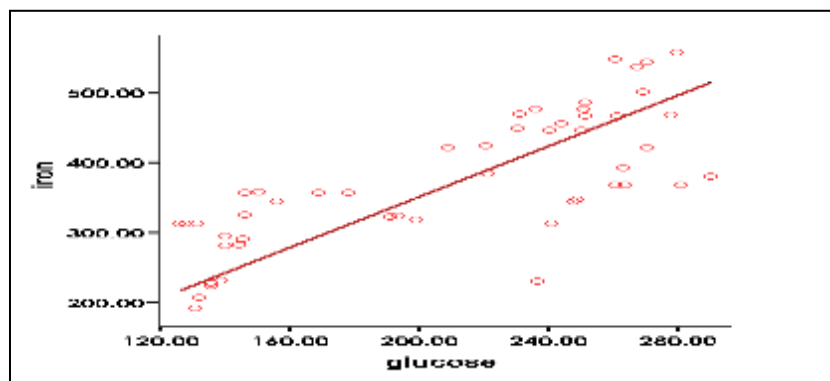


Figure 5: The correlation between hyperglycemia and iron overload

**Discussion**

Patients with chronic hepatitis complicated with severe degree of hyperglycemia and insulin resistance (IR). Recent studies have confirmed a close relationship between IR and other liver diseases. Hyperinsulinemia occurs in chronic viral hepatitis B and C associate with stage of fibrosis,

cirrhosis, liver failure, hepatocellular carcinoma, and a poor response to antiviral therapy against in hepatitis patients because chronic hepatitis is not a single disease, but rather a clinical and pathological syndrome [32-34].

The mechanisms by which hepatic virus produce IR and hyperglycemia are seem that core

protein increases the synthesis of TNF- $\alpha$ , which, at its turn, produces cytokines suppressor of cytokine signaling 3 (SOCS3) that inhibit the insulin receptors 1 and 2 (cytokines of phosphorylation), the result being a diminution of the intracellular signal at insulin with the blockage of enzyme GLUT4. GLUT4 is responsible for the access of glucose inside the cell and its blockage directly results into an increased glucose concentration in the blood [35-37].

In 1994, Allison et al described for the first time the correlation between HCV and type 2 diabetes mellitus (T2DM), [38] although this relationship was found only in cirrhotic patients with HCV. Later studies reported this association in cirrhotic patients with HBV. [39] Subsequent studies found that cirrhosis is not necessary for the development of T2DM because several HCV-infected patients without cirrhosis presented T2DM [40-43]

The association between HBV and the development of hepatic steatosis is also somewhat controversial. Wang and coworkers affirmed that HBV infection is not related to the development of liver steatosis, [44] whereas others have suggested that the accumulation of lipids in the liver occurs through several mechanisms that eventually activate sterol regulatory element binding protein 1 (SREBP-1) and peroxisome proliferator activated receptor- $\gamma$  (PPAR $\gamma$ )[45].

This last point is interesting because the pathogenesis of HCV associated IR occurs through the inhibition of PPAR $\gamma$  and, as noted, HBV infection produces the exact opposite effect on PPAR $\gamma$ . Although other mechanisms responsible for IR development have not been studied thoroughly, this last point may explain the purported protective effect of HBV infection [46]

Iron homeostasis in the human body maintained by mechanisms controlling iron absorption from the intestinal tract, iron recycling from macrophages and mobilization of hepatic iron stores [47].

In humans, carefully regulated system of iron absorption control accompanies by a lack of effective physiological mechanisms for the

excretion of excess iron accumulating in different tissues. This is the main reason for progressive iron loading [48].

The reasons for increased iron status in chronic hepatitis may be attributing to a variety of factors. First, hepatocellular damage may cause release of iron in hepatocytes [49], as supported by the finding of positive correlation between ferritin level and transaminase level and by increase level of serum iron as degree of hepatic inflammation increases. Second, chronic liver disease may often be associated with an increased iron absorption in the gastrointestinal tract with marginally elevated hepatic iron [50].

Hepcidin, exclusively synthesized in the liver, is thought to be a key regulator for iron homeostasis and is induced by infection and inflammation suggesting that hepcidin may play a pivotal role in the pathogenesis of iron overload and hyperferritinemia in patients with chronic hepatitis [51].

A new study demonstrates that Stat3 provides hepato protection against mediated apoptotic liver damage by two mechanisms: direct inactivation of caspases and reduction of reactive oxygen species [52], which is responsible for signaling in inflammation. The iron-induced production of bone morphogenetic proteins (BMPs); one of the multifunctional growth factors with its co-receptor hemojuvelin likely constitutes a key, endogenous signaling pathway for hepcidin activation also through its interaction with IL-6/STAT3 [53]. Hepcidin and its interaction with the transmembrane iron transporter ferroportin (FPN) play crucial roles in the systemic iron balance through down-regulation of iron release from enterocytes and phagocytes [48]. The expression of hepcidin is a complex process, strongly inhibited by hypoxia, anemia, while being activated by inflammation and iron overload. Molecular mechanisms of hepcidin regulation involve stimulation of hepcidin mRNA transcription through the interleukin-6 (IL-6)/STAT3 pathway. Stat3 is a vital transcription factor that is activated downstream of the gp130

receptor, primarily via IL-6 signaling in adult liver [52].

Excess iron increases the formation of reactive oxygen species leading to lipid peroxidation, damage to DNA, thereby to cell membranes and genomic damage. Reactive oxygen species, which include hydroxyl radicals, may cause hepatic stellate cell activation and proliferation and upregulate synthesis of smooth muscle actin and collagen, thus contributing to hepatic fibrogenesis [54-56]. Viral hepatitis and immune-mediated liver damage are believed to occur largely via activation of the Fas apoptotic death pathway. The link between Fas-mediated damage and the induction of reactive oxygen species (ROS) and oxidative damage has only recently been established [57, 58].

In vitro studies also suggest that iron deposition in hepatocytes enhances HCV replication, thus facilitating the viral infection in the liver [59]. Moreover, these hydroxyl radicals known to generate promutagenic bases, such as 8-hydroxy-2'-deoxyguanosine (8-OHdG), which have been implicated in spontaneous DNA mutagenesis and carcinogenesis [60].

However, such observation was also reported by Devrajani *et al* at in Pakistan at 2010 [46] there is no significant different in blood iron between HBV ( $390.88 \pm 114.82$ ) and HCV ( $385.27 \pm 89.87$ ) patients.

Although in our study, we had not take the management parameters but it has been reported that iron-restricted diet may be an important therapeutic modality for improving liver injury in patients with chronic hepatitis C [61]. It has been suggested that iron may promote HCV replication. [51]

Where as a study published in Journal of gastroenterology and hepatology shown similar findings [62]

The increasing in blood glucose is correlated with iron overload due to iron deposition in the interstitial pancreatic cells, with resultant excess collagen deposition and defective microcirculation [63] and insulin resistance [64], are the likely mechanisms for type 2 diabetes.

Mendler in 1999 shows Fe overload may be responsible for insulin resistance, or *vice versa* [65] also Pietrangelo A. in 2010 suggests an etiologic link between hepatic Fe, hepatic dysfunction, and insulin resistance [63].

Finally, this study suggests that the reason of liver damaged in viral infection disease is not due to the virus directly, but to the severe inflammatory responses associated with the immune response to it and imbalance in the metabolism as glucose and iron.

Hyperglycemia and iron overload could be the underlying mechanism connecting low response rate to antiviral therapy. So screening for glucose and iron abnormalities should be indicated in hepatitis patients and patients must be use suitable drugs in addition to the antiviral therapy.

The information accumulated by this study will help provide a better understanding of involved metabolic processes in order to design appropriate therapeutic approaches for treating these patients and starting antiviral therapy.

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