Diabetogenic effect of hepatitis C virus and insulin resistance among chronic hepatitis C patients

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Context
Hepatitis C virus (HCV) is a major cause of chronic liver disease worldwide. In addition to established liver injury, type 2 diabetes mellitus is one of the most important extrahepatic metabolic disorders that are attributed to HCV infection.
Aim
The aim of this study was to investigate the impact of HCV infection in insulin resistance (IR).
Patients and methods
Our study included 100 patients with HCV who were divided into two groups according to the presence and absence of diabetes and 25 diabetic patients who served as a control group. They were subjected to full medical history and examination and laboratory investigations including high-sensitivity C-reactive protein (CRP), serum tumor necrosis factor α (TNF-α), interleukin 6 (IL-6), fasting insulin, and fasting glucose.
Results
Our study showed increased IR among diabetic HCV-infected patients (group I) with a mean level of homeostasis model assessment of IR of 3.02, 1.457, and 1.064 in groups I, II, and III, respectively. There was also an increased level of proinflammatory cytokines (CRP, TNF-α, and IL-6) in this group, with mean levels of high-sensitivity CRP of 9.448, 7.7062, and 5.8229 mg/dl in groups I, II, and III, respectively. The mean level of IL-6 in group I was 215.63 pg/ml, in group II it was 167.62 pg/ml, and in group III it was 173.72 pg/ml. The mean level of TNF-α was 626.12, 618, and 422.76 pg/ml in groups I, II, and III, respectively, suggesting the role of proinflammatory cytokines in the pathogenesis of IR in chronic HCV.
Conclusion
HCV infection is associated with an increased level of proinflammatory cytokines that play a crucial role in the pathogenesis of IR in chronic HCV.

Keywords:
diabetes, hepatitis C virus, insulin resistance

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Introduction

Hepatitis C virus (HCV) is a major cause of acute and chronic liver disease worldwide. HCV is both hepatotropic and lymphotropic, which replicates in diseased extrahepatic organs and tissues and may trigger latent autoimmunity or induce autoimmune disorders [1].

In addition to established liver injury, type 2 diabetes mellitus (T2DM) is an important feature of extrahepatic metabolic disorders, which is attributed to HCV infection. This interaction between a common endocrine disorder and an infectious disease is an important issue to elucidate.

Insulin resistance (IR) plays an important role in the development of various complications associated with HCV infection. Recent evidence indicates that HCV-associated IR may result in accelerated hepatic fibrosis, steatosis, hepatocellular carcinoma, and resistance to antiviral treatment [2].

Thus, HCV-associated IR is a therapeutic target at any stage of HCV infection as there is an inverse correlation between the severity of IR and sustained virologic response in HCV patients [3].

Strikingly, Arase *et al.* [4] recently showed that HCV treatment may decrease the annual incidence of diabetes in HCV-infected patients, independent of other predisposing factors.

Aghemo *et al.* [5] confirmed these results, showing that viral eradication after treatment is also able to reduce the occurrence of IR significantly.

The aim of our study was to investigate the impact of HCV infection on glucose metabolism and to highlight

the role of inflammatory cytokines in IR development in HCV infection.

Patients and methods

Patients

Our case–control study included 100 patients with chronic HCV who were recruited from the inpatient ward and outpatient clinic of Internal Medicine at Cairo University Hospitals during the period from June 2013 to March 2014. The patients were divided into three groups. The protocol was approved by the ethical committee of Cairo University and an informed consent was obtained.

(1) Group I, which included 50 chronic HCV patients with T2DM.
(2) Group II, which included 50 nondiabetic chronic HCV patients.
(3) Group III, which included 25 patients with T2DM with matched age, sex, and disease duration who served as controls.

Exclusion criteria

(1) Concomitant hepatic viral infections (hepatitis B virus) or Child–Turcotte–Pugh stages B and C according to the modified Child–Turcotte–Pugh score.
(2) Presence of autoimmune hepatitis or hepatic focal lesions.
(3) Obesity (BMI>30).
(4) Presence of other coexisting medical illnesses.

All groups were carefully matched for age, sex, BMI, negative family history of T2DM, fasting blood glucose, and severity of liver disease, which was assessed according to modified Child–Pugh's classification.

They were subjected to the following.

(1) Full detailed medical history including age, occupation and residence, history of drug intake, any associated disease (e.g. hypertension), and any present complaint.
(2) Clinical examination including calculation of BMI as follows: BMI=Weight (kg)/height (m^2).
(3) The following laboratory investigations were carried out.
    (a) Evaluation of liver enzymes (alanine aminotransferase and aspartate aminotransferase), serum albumin, total proteins, serum bilirubin (total and direct), alkaline phosphatase, γ-glutamyltransferase, complete blood count, and prothrombin time.
    (b) Evaluation of HCV antibody total using enzyme-linked immunosorbent assay (ELISA) and evaluation of hepatitis B surface antigen, total hepatitis B core antibody, and Bilharsial antibody.
    (c) Evaluation of high-sensitivity C-reactive protein (CRP) using ELISA, serum tumor necrosis factor α (TNF-α) determination, and interleukin 6 (IL-6) evaluation using a sandwich ELISA kit (Koma Biotech Inc., Seoul, Korea).
    (d) Fasting insulin and fasting glucose evaluation; IR was determined using the homeostasis model assessment (HOMA) of IR according to the following formula:

$$\text{HOMA} - \text{IR} = \frac{\text{Fasting glucose (mmol/l)} \times \text{fasting insulin (mIU/l)}}{22.5 \text{ (glucose in mmol/l) or } 403 \text{ (glucose in mg/ml)}}.$$

A HOMA score close to 1 indicates normal insulin sensitivity. IR is associated with high HOMA scores.

Results

Age distribution among the studied groups

There was no statistically significant difference in age distribution between the three groups (P=0.648) (Table 1).

Sex distribution among the studied groups

There was no statistically significant difference in sex distribution between the three groups (P=0.593) (Fig. 1).

As regards the main laboratory parameters included in our study (Table 2), we found that the highest mean CRP (9.44±2.165), TNF-α (626.12±238.738), IL-6 (215.63±62.004), fasting insulin (20.850±5.092), and IR (3.020±0.719) was in group I, whereas the highest mean fasting blood glucose was found in group III

Table 1 Age distribution among the three groups

	Age (years)				Analysis of variance	
	Range	Minimum	Maximum	Mean±SD	*F*	*P*-value
Group I	23.00	32.00	55.00	46.6200±5.68884	0.813	0.648
Group II	28.00	27.00	55.00	44.5102±8.62439		
Group III	26.00	32.00	58.00	46.9200±6.87944		

(198.28±30.245) and the highest mean insulin secretion was seen in group II (97.476±25.847).

C-reactive protein level mean and SD and its statistical significance

There was a statistically highly significant difference in CRP level between groups I and II ($P<0.001$) and between groups I and III ($P<0.001$), and a statistically significant difference between groups II and III ($P<0.05$) (Fig. 2).

Interleukin 6 level mean and SD and its statistical significance

There was a statistically highly significant difference in IL-6 level between groups I and II ($P<0.001$) as well as between groups I and III ($P<0.001$), whereas there was no statistically significant difference between groups II and III ($P=0.649$) (Fig. 3).

Tumor necrosis factor α level mean and SD and its statistical significance

There was a significant statistical difference in TNF-α level between groups I and II patients ($P<0.05$), whereas a highly significant statistical difference between groups I and III ($P<0.001$) and between groups II and III ($P<0.001$) (Fig. 4).

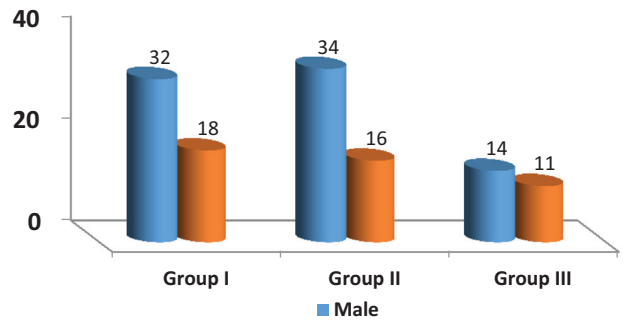
Fasting blood glucose level mean and SD and its statistical significance

There was a statistically highly significant difference in FBG level between groups I and II ($P<0.001$), between groups I and III ($P<0.001$), and between groups II and III ($P<0.001$) (Fig. 5).

Insulin resistance mean and SD and its statistical significance

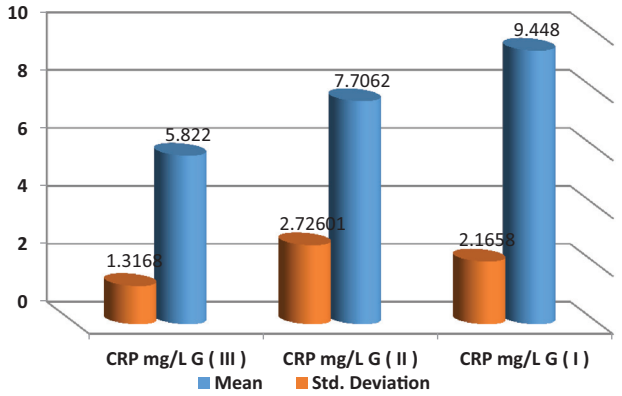
There was a statistically highly significant difference in IR between groups I and II ($P<0.001$) and between groups I and III ($P<0.001$), and a statistically significant difference was found between groups II and III ($P<0.05$) (Fig. 6).

Figure 1



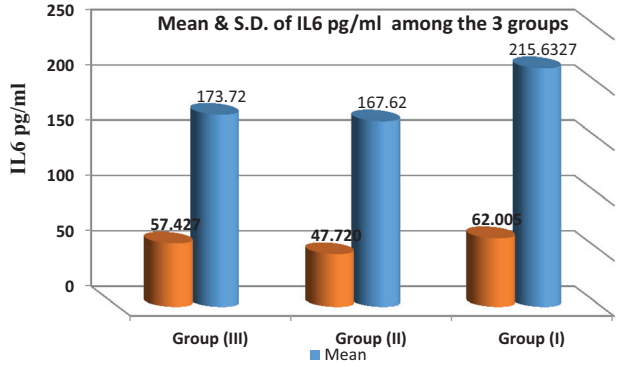
Sex distribution among the three groups.

Figure 2



The mean and SD of CRP level among the three groups. CRP, C-reactive protein.

Figure 3



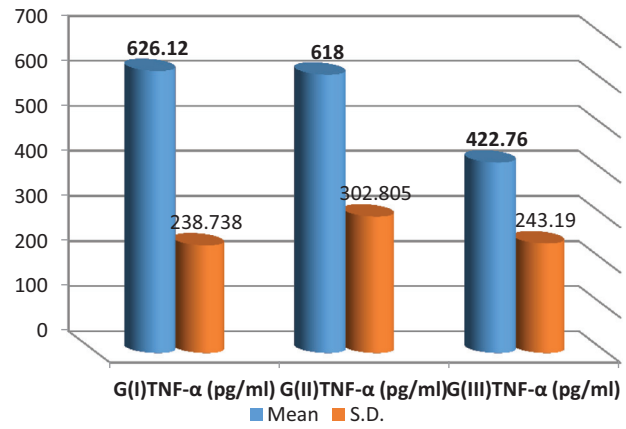
The mean and SD of IL-6 level among the three groups. IL-6, interleukin 6.

Table 2 Mean and SD of the main laboratory parameters among the three groups included in our study

	Group I (n=50) (mean±SD)	Group II (n=50) (mean±SD)	Group III (n=25) (mean±SD)
CRP (mg/l)	9.44±2.165	7.70±2.726	5.82±1.316
TNF-α (pg/ml)	626.12±238.738	618±302.805	422.76±243.190
IL-6 (pg/ml)	215.63±62.004	167.62 ±47.719	173.72±57.427
FBG (mg/dl)	168.3±52.234	89.95±11.898	198.28±30.245
Fasting insulin (μIU/ml)	20.850±5.092	11.357±2.302	6.846±1.088
Insulin resistance	3.020±0.719	1.457±0.287	1.064±0.160
Insulin secretion	36.552±18.855	97.476±25.847	72.93±26.17

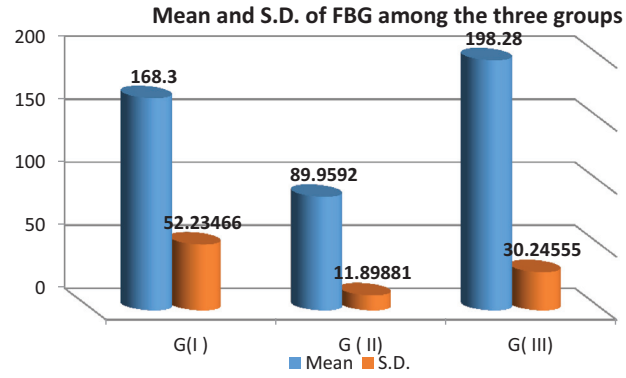
CRP, C-reactive protein; FBG, fasting blood glucose; IL, interleukin; TNF-α, tumor necrosis factor α.

Figure 4



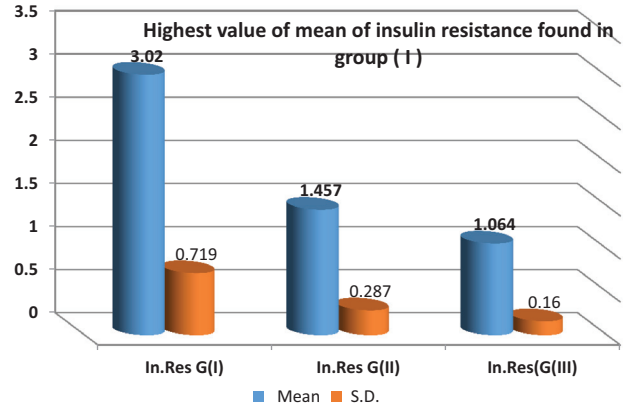
The mean and SD of TNF-α (pg/ml) in the three groups. TNF-α, tumor necrosis factor α.

Figure 5



The mean and SD of FBG level among the three groups. FBG, fasting blood glucose.

Figure 6



The mean and SD of insulin resistance among the three groups with highest mean in group I.

Insulin secretion mean and SD and its statistical significance

There was a statistically highly significant difference in insulin secretion between groups I and II ($P<0.001$), between groups I and III ($P<0.001$), and between groups II and III ($P<0.001$) (Fig. 7).

As inflammatory mediators (CRP, IL-6, and TNF-α) induced by HCV were the main features in IR syndrome, it was important to correlate each of them with IR.

Correlation between C-reactive protein and insulin resistance

A statistically highly significant positive direct correlation between IR and CRP ($r=0.592$, $P<0.001$) was documented (Fig. 8).

Correlation between interleukin 6 and insulin resistance

A statistically highly significant positive direct correlation between IR and IL-6 ($r=0.581$, $P<0.001$) was documented (Fig. 9).

Correlation between tumor necrosis factor α and insulin resistance

A statistically significant positive direct correlation between IR and TNF-α ($r=0.404$, $P<0.004$) was documented (Fig. 10).

Discussion

Our study included 100 patients with chronic HCV divided into two groups according to the presence and absence of diabetes, and 25 diabetic patients of matched age, sex, and disease duration were included as the control group.

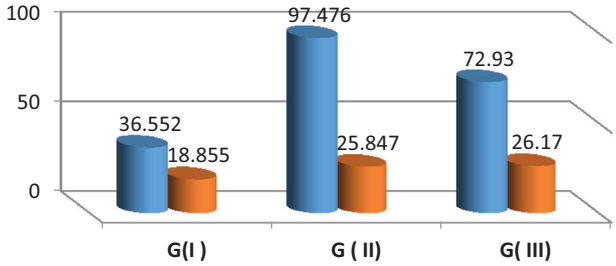
In our study, we compared the levels of CRP, TNF-α, and IL-6 in the three studied groups to establish a link between HCV and the occurrence of IR.

In our study, there was a statistically highly significant difference in CRP level among the three groups ($P<0.001$), with the highest mean level among HCV diabetic patients (group I), indicating that there was ongoing inflammation as confirmed by the elevated levels of CRP.

In our study, we found a statistically highly significant positive correlation between CRP level and IR ($r=0.592$, $P<0.001$), which means that CRP level increase among HCV patients share in the development of IR in patients with chronic liver disease.

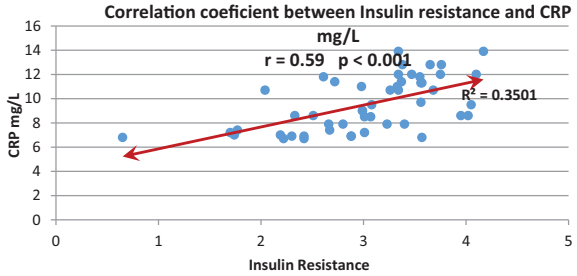
This finding is supported by Yudkin *et al.* [6], who demonstrated that, in 107 nondiabetic individuals, CRP levels were related to IR. Concentrations of CRP correlated both with those of IL-6 ($P<0.0005$) and of TNF-α ($P<0.0001$). These observations could

Figure 7



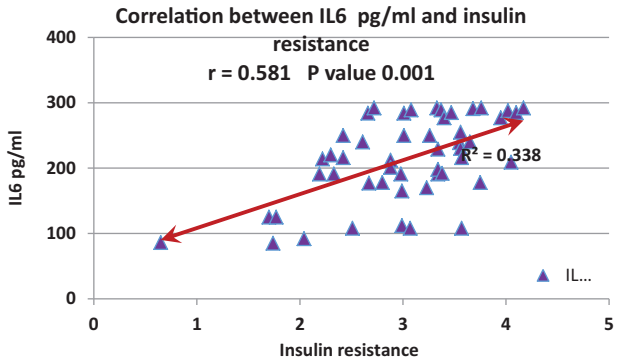
The mean and SD among the three groups showing that it is higher in group II as compared with groups I and III.

Figure 8



Correlation between CRP and insulin resistance. CRP, C-reactive protein.

Figure 9



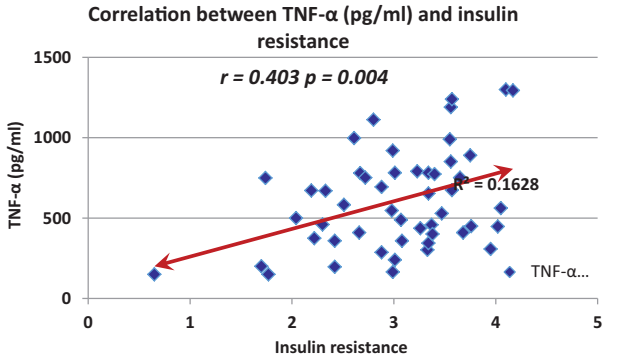
A statistically significant positive correlation coefficient between insulin resistance and IL-6 (r=0.581, P=0.001). IL-6, interleukin 6.

suggest that the cytokines, arising in part from adipose tissue, might themselves be partly responsible for the metabolic, hemodynamic, and hemostatic abnormalities that cluster with IR.

In our study we observed a statistically significant difference in the level of IL-6 in group I as compared with group II, as well as between groups I and III (P<0.001), whereas there was no statistical significance between level of IL-6 in groups II and III (P=0.649).

These findings are supported by Kasprzak *et al.* [7], who demonstrated an augmented expression of all

Figure 10



Statistically significant positive correlation coefficient between insulin resistance and TNF-α level (r=0.403, P=0.004). TNF-α, tumor necrosis factor α.

proinflammatory cytokines and of IL-1α and IL-6, particularly in the livers of chronic hepatitis C patients as compared with their expressions in the control group.

In our study, we found a statistically significant positive correlation between IL-6 level and IR (r=0.581, P<0.001). This finding is supported by Fernandez-Real *et al.* [8], who detected higher plasma IL-6 concentrations in patients with IR and T2DM. The study concluded that IR was a significant and an independent predictor of peripheral IL-6 levels.

Furthermore, Vozarova *et al.* [9] also showed a positive correlation between plasma IL-6 concentrations and fasting insulin concentrations, an index of IR.

HCV may induce a Th1 lymphocyte immune-mediated response, which leads to the activation of the TNF-α system and elevation of IL-6 levels. A high TNF-α level was considered to be one of the bases of IR [10].

In our study, there was a statistically significant difference in the level of TNF-α between groups I and II (P<0.05), between groups I and III (P<0.001), and between groups II and III (P<0.001).

Furthermore, a statistically significant positive correlation was observed between TNF-α and IR (r=0.403, P=0.004).

Our findings are supported by Lecube *et al.* [10], who provided the first clinical evidence of elevated levels of TNF-α and IL-6 in nondiabetic HCV-positive patients.

An earlier study of liver biopsy specimens from nondiabetic HCV patients revealed significant impairments in the insulin signaling pathway, which

are strikingly similar to the known effects of TNF-α and can lead to IR [11].

A study by Festa *et al.* [12] showed that chronic subclinical inflammation is a part of the IR syndrome. Moreover, in cross-sectional studies, levels of inflammatory biomarkers such as CRP and the proinflammatory cytokines IL-6 and TNF-α correlated with insulin sensitivity and with features of the IR syndrome [13].

Our study is one of the few studies that measured serum levels of TNF-α in patients with chronic HCV either diabetic or nondiabetic and confirmed the previous study by denoting the highly significant positive correlation between level of CRP, IL-6, TNF-α, and IR.In our study, the mean level of HOMA-IR was higher in diabetic HCV patients compared with the other groups. This finding was supported by Delgado *et al.* [14], who showed that 62% of patients with HCV had IR.

In our current study, nondiabetic patients with HCV (group II) had higher levels of insulin secreted from the β cells of the pancreas compared with other groups. There was a statistically highly significant difference ($P<0.001$) in insulin secretion between groups I and II, between groups I and III, and between groups II and III.

Although there are a limited number of studies that assess insulin secretion from the β cells of the pancreas, a study by Lecube *et al.* [10] was able to hypothesize that a deficit in insulin secretion is another potential mechanism involved in diabetes associated with HCV infection. This in part explains why in our study there were lower levels of insulin secretion in HCV diabetic patients compared with the other studied groups.

Conclusion

HCV is a major cause of acute and chronic liver disease worldwide. In addition to established liver injury, T2DM is an important feature of extrahepatic metabolic disorders, which is attributed to HCV infection. High levels of proinflammatory cytokines (e.g. CRP, TNF-α, and IL-6) as markers of the innate immunity have been found in HCV-infected patients and thereby they could be involved in the pathogenesis of IR associated with HCV.

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Nil.

Conflicts of interest
There are no conflicts of interest.

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