Interplay Between Tumor Necrosis Factor-α, Insulin Resistance and Type 2 Diabetes Mellitus in Chronic Hepatitis C Egyptian Patients

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Background and study aim : Hepatitis C is a disease with significant global impact, it is the most common cause of chronic liver diseases, and in addition it causes insulin resistance (IR) leading to increase the risk of type 2 diabetes mellitus (DM). This current study aimed to assess the relationship between serum tumor necrosis factor-α (TNF-α), insulin resistance (IR) and type 2 DM in patients HCV.

Patients and Methods: The study cohort consisted of 91 subjects stratified into 4 groups; Group (I): Included 25 HCV patients without DM, Group (II): Included 25 HCV diabetic patients, Group (III): Included 25 diabetic patients without HCV infection and group (IV): Included 16 healthy subjects serving as a control group. All patients were subjected to full history taking, thorough clinical examination and estimation of body mass index (BMI). Anti-HCV Ab was detected by the 3rd generation (ELISA) test and was confirmed by PCR. Assessment of fasting plasma insulin level (FBI) and TNF-α were done by ELISA test, while assessment of the insulin resistance was estimated by Homeostatic Model Assessment (HOMA-IR).

Results: Higher mean levels of FBS, 2 hr (2HPP) and fasting plasma insulin (FSI) were detected in group II (HCV+DM) compared to other groups with statistically significant differences between all the studied groups (P value <0.001), consequently HCV diabetic patients were found to have significant higher IR than HCV patients without DM, diabetic patients alone and control group (P value <0.001). Furthermore, there was highly statistically significant differences between all studied groups as regard level of TNF-α (P value <0.001) with higher mean level in group I (HCV group). Insignificant difference in level of TNF-α in HCV patients with or without IR (P value =0.072). Insignificant positive correlation between HOMA-IR and TNF-α (P value = 0.63).

Conclusion: Chronic HCV patients have significantly elevated fasting plasma insulin level, TNF-α and significant IR and there was insignificant correlation between HOMA-IR and TNF-α.

INTRODUCTION

Hepatitis C virus (HCV) infection is a major cause of chronic liver disease, affecting 3% of the world's population, both HCV liver disease and type 2 diabetes are two already prevalent diseases that will probably continue to increase in the next decades **[1]**. Chronic hepatitis C comprises extra hepatic features as, thyroiditis, arthritis, essential mixed cryoglobulinemia and other immunological diseases **[2]**. During the last decade, it has been hypothesized that diabetes could be one more of these extra hepatic conditions attributable to HCV infection. This raises the intriguing question of whether the rise in HCV infection is contributing to the increasing prevalence of type 2 diabetes **[3]**.

The specific mechanisms involved in the pathogenesis of diabetes associated with HCV infection remain to be elucidated; it seems that insulin resistance (IR) may play an essential role **[4]**. Two types of insulin resistance could be defined in patients with chronic hepatitis C: "metabolic" insulin resistance and "viral" insulin resistance **[5]**. Insulin secretion increases when insulin sensitivity decreases until a threshold in which insulin secretion

did not induce improvement in insulin sensitivity, and diabetic state emerges **[5,6]**. The development of IR in chronic hepatitis C infected patients is due to virus-specific alteration in host metabolism as chronic low-grade activation of the immune system may play a role in the pathogenesis of IR and DM. In chronic hepatitis C infection, markers of inflammation, like, tumor necrosis factor-α (TNF-α) may play an essential role in the pathogenesis of IR as TNF-α represents an integral component of the inflammatory response to HCV infection **[7].** So, HCV, insulin resistance and type 2 diabetes mellitus are associated to an extent that cannot be merely explained by chance, which suggests that HCV interferes with glucose metabolism, directly (through one or more of its proteins) and/or indirectly (by modulating the production of specific cytokines, like TNF-α **[8]**. In this study we aimed to assess the link between serum level of TNF-α, IR and DM in chronic HCV patients.

PATIENTS AND METHODS

This prospective study was conducted on 91 subjects (54 males and 37 females attending the New General Mansoura Hospital during the period from February 2014 to August 2014.They were divided into 4 groups: Group I; included 25 HCV patients without Diabetes mellitus. Group II; included 25 HCV diabetic patients. Group III; included 25 diabetic patients without HCV infection. Group IV; Included 16 healthy subjects serving as a control group. The inclusion criteria was adult patients tested positive for HCV antibody and HCV RNA by PCR, whereas patients with other etiologies of liver disease, patients with decompensated cirrhosis, hepatocellular carcinoma or type I diabetes mellitus, patients previously treated with pegylated interferon/ribavirin therapy were excluded.

Methodology:

All patients were subjected to the following: Full History taking and Clinical examination, laboratory assessment (ALT, AST, total and direct bilirubin, albumin, prothrombin time and creatinine) and imaging (ultrasound), data during the previous 3 months were revised, check for diabetes mellitus (Fasting and postprandial blood sugar to diagnose diabetic and non-diabetic groups according WHO guidelines (FBS 126 mg/dl, 2HPP 200mg/dl and/ or other co-morbid illness while assessment of fasting plasma insulin level and TNF-α were done by ELISA method. Detection of anti HCV was done by the 3rd generation ELISA test and confirmed by quantitative polymerase chain reaction (PCR). Assessment of the insulin resistance state was done by Homeostatic Model Assessment (HOMA-IR). Which calculated as follow: FPG mg/dl x FSI uU/÷ ml 405 .Where FPG is fasting plasma glucose in mg/dl, while FSI is fasting serum insulin in µU/ml. The homeostatic model assessment (HOMA) is a method used to quantify insulin resistance and beta-cell function from fasting insulin and insulin concentration **[9]**. A value greater than 3 indicates insulin resistance [10].

Statistical Analysis:

The collected data were tabulated and analyzed using SPSS (Statistical package for social science) program version 17 software. All data are expressed as the mean ± SD. For statistical analysis, we used analysis of variance (ANOVA) for repeated measurements followed by post hoc comparison with the Dennett procedure. The differences between the two groups were analyzed by Student's unpaired t-test. Pearson's correlation study was done between two quantitative variables. A probability value (p-value) less than 0.05 was considered statistically significant.

RESULTS

There was no statistically significant differences between all the studied groups as regard age, sex and BMI, while the majority of studied groups were males with higher mean age was in (HCV diabetic patients) group II (45.52 ± 8.77 years) (Table 1). Regarding laboratory finding there was highly statistically significant differences between all studied groups as regard platelet count (p-value <0.001), ALT level (p-value <0.001), AST level (p-value <0.001), Serum total bilirubin (p-value =0.006), Serum albumin (p-value =0.005) PT (p- value <0.001) and INR (p- value =0.001), In contrast there is no statistically significant differences between group I (HCV Patients) and group II (HCV diabetic patients) as regard viral load level (Table 2). Level of serum fasting insulin was significantly lower in patients with DM alone compared to HCV patients alone and patients with HCV and DM (8.08±12.46, 15.29 ± 26.76, 32.46 ± 26.34 respectively) with P value <0.001 and higher mean levels of fasting blood glucose, 2 hours post prandial blood glucose were in Group II (HCV diabetic patients) than other studied groups, with highly statistically significant differences between all studied groups

(p value <0.001) for each studied parameter (Table 3). Regarding the level of serum TNF-α among the studied groups it was higher in HCV patients alone followed by patients with HCV and DM then patients with DM alone then control group (59.41±13.61, 51.39± 16.54, 43.49 ± 15.32, 28.64 ± 14.94 respectively) with highly statistically significant differences between them (P value < 0.001) (Fig. 1).

IR was significantly higher among HCV patients with DM compared to patients with DM alone and HCV patients alone and control group (13.00±12.40, 6.58±12.52, 1.77±2.99, 1.19 ±1.20 respectively) P value <0.001 (Fig. 2). The majority of patients of group II had HOMA-IR > 3 [18 patients (72%)] (total number of HOMA-IR >3 was 31 in studied groups) with highly statistically significant differences between studied groups (p- value <0.001). FSI level was significantly higher in patients with IR than in patients without IR (38.84 ± 28.11, 4.79 ± 3.40 respectively) P value < 0.001. Also level of both FBS and 2 HPP was statistically higher in patients with IR than in patients without IR(P value <0.001). Mean level of HOMA-IR was significantly higher in patients with IR than in patients without IR (15.54±13.52, 1.18 ± 0.84 respectively) P value <0.001. While there was no significant differences in level of TNF-α in two groups (P value = 0.336) (Table 4). In comparison between HCV patients with IR and HCV patients without IR (total No = 50). Higher mean levels of FBS, 2 HPP, FSI and HOMA-IR was in HCV patients with IR (P value <0.001). While level of TNF-α was higher in HCV patients without IR than HCV patients with IR with no significant differences (P value = 0.072). So in this study TNF-α appears not to play role in IR in HCV patients (Table 5). IR in HCV patients showed insignificant positive correlation with age, BMI, TNF-α (P value = 0.096, 0.477, 0.639 respectively), but there was significant positive correlation between HOMA-IR in HCV patients and both FBS and FSI (P value = 0.004, <0.001 respectively) (Table 6).

Table (1): Demographic Criteria of studied groups.

Variable	Group (I) HCV (n=25)	Group (II) HCV&DM (n=25)	Group (III) DM (n=25)	Group (IV) Control (n=16)	Test	P-value
Age(Mean ± SD)	42.88±11.44	45.52±8.77	43.28±10.35	40.69±10.35	0.749	0.526
Sex:(No%)						
Male	15(60%)	14(56%)	14(56%)	9(56.3%)	0.115#	0.990
Female	10(40%)	11(44%)	11(44%)	7(43.8%)		
BMI(Kg/m²)	27.42±4.95	28.45±4.69	28.32±5.92	27.19±4.61	0.330	0.804

Table (2): Comparison between studied groups as regard laboratory findings (CBC, Liver profile tests, serum creatinine and PCR for HCVRNA).

Variable	Group (I) HCV (n=25)	Group (II) HCV&DM (n=25)	Group (III) DM (n=25)	Group (IV) Control (n=16)	Test	P-value
Hb(gm/dl)	12.21±1.99	12.35±1.84	13.22±2.63	12.97±2.27	1.163	0.329
WBCS(c/mm3)	6.34±2.25	7.27±2.06	6.90±2.10	6.83±2.41	0.759	0.520
PLT(c/mm3)	174.60±80.61	156.84±56.18	220.12±46.26	266.50±75.46	11.28	<0.001*
ALT(U/L)	44.04±26.05	48.32±22.04	30.76±15.20	22.50±10.53	7.198	<0.001*
AST(U/L)	47.88±29.38	44.04±22.16	29.44±16.87	18.31±7.80	8.067	<0.001*
Total Bilirubin (mg/dl)	1.12±0.49	1.08±0.35	0.86±0.17	0.81±0.21	4.456	0.006*
Direct Bilirubin (mg/dl)	0.24±0.14	0.25±0.12	0.21±0.07	0.20±0.08	1.181	0.322
S. Albumin (gm/dl)	3.95±0.73	4.04±0.54	4.45±0.40	4.34±0.40	4.511	0.005*
PT (sec)	14.81±2.22	14.36±1.57	13.22±.29	13.14±0.23	7.483	<0.001*
INR	1.24±0.29	1.16±0.19	1.05±0.08	1.03±0.05	6.451	0.001*
S. Creatinine (mg/dl)	0.79±0.24	0.82±0.22	0.87±0.27	0.78±0.17	0.662	0.577
Viral load PCR (IU/ml)	1176385.20 ± 1256664.41	1532572.40 ± 2378520.68	0	0	311.5	0.985

Table (3): Comparison between studied groups as regard fasting blood glucose level, 2hours postprandial blood glucose level and serum fasting insulin level

Variable	Group (I) HCV (n=25)	Group (II) HCV&DM (n=25)	Group (III) DM (n=25)	Group (IV) Control (n=16)	Test	P-value
FBS N= (70 to110 mg/dl)	85.24±9.83	153.08±31.97	143.20±39.36	83.88±10.14	40.633	<0.001*
2HPP N= up to (140mg/dl)	121.96±14.40	230.88±50.87	196.96±57.53	123.25±11.61	39.598	<0.001*
FSI N= (8.4–8.8uU/m1)	15.29±26.76	32.46±26.34	8.08±12.46	5.98±6.21	7.562	<0.001*

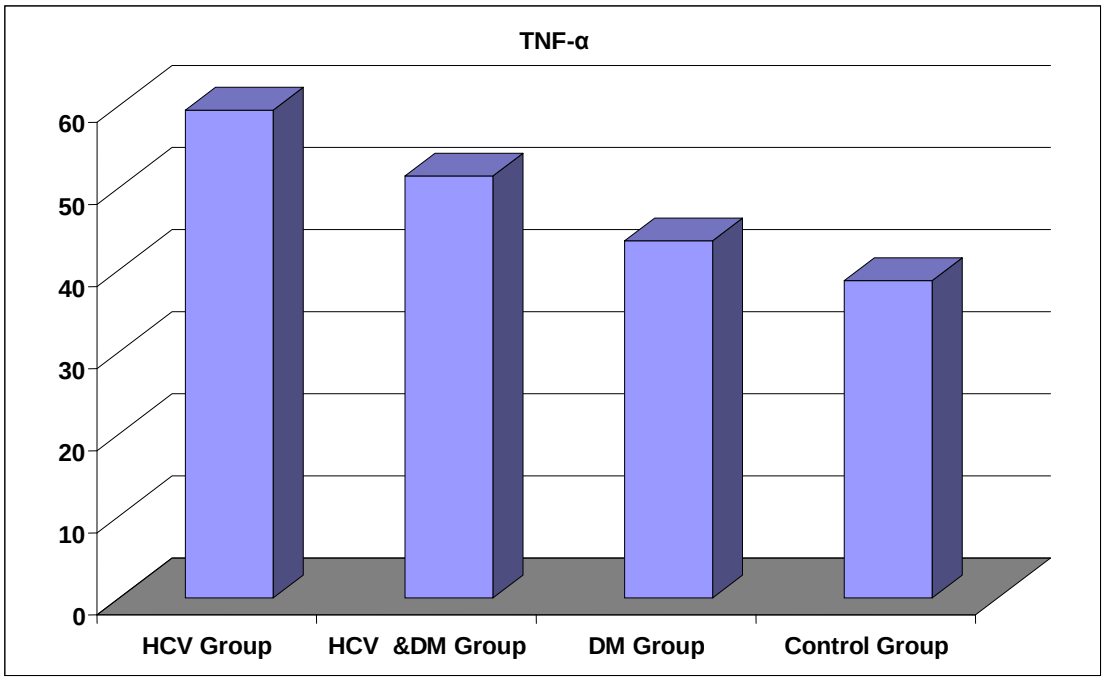

Fig. (1): Comparison between studied groups as regard Serum level of TNF-α

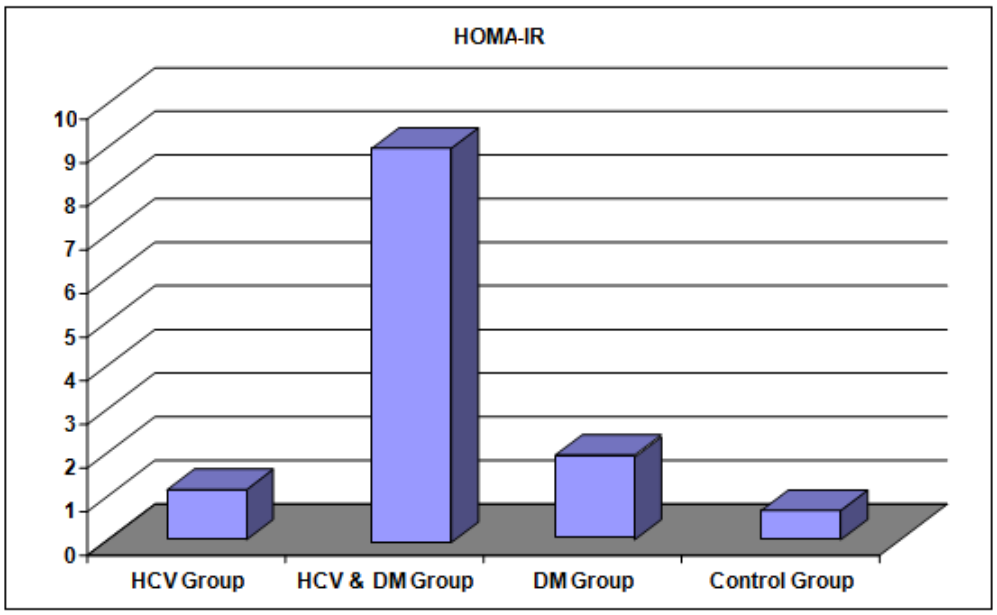

Fig. (2): Comparison between studied groups as regard HOMA-IR levels

Table (4): Comparison of patients with insulin resistance and patients without insulin resistance in studied groups as regard laboratory findings

Variable	HOMA<3 (n=60)	HOMA>3 (n=31)	test	P-value
Age (Mean ± SD)	40.92±10.13	48.00±8.80	3.301	0.001*
BMI(Kg/m^2)	26.62±4.45	30.40±5.30	3.600	0.001*
Hb(gm/dl)	12.81±2.03	12.37±2.52	0.907	0.367
WBCS(c/mm3)	6.50±2.07	7.48±2.28	2.065	0.042*
PLT(c/mm3)	208.95±70.73	177.94±81.03	1.886	0.063
ALT(IU/dl).	34.57±19.65	44.00±25.36	1.962	0.053
AST(IU/dl).	33.37±21.98	42.74±26.56	1.794	0.076
Total Bilirubin (mg/dl)	0.96±0.35	1.03±0.37	0.791	0.431
Direct Bilirubin (mg/dl)	0.22±0.11	0.25±0.11	1.409	0.162
S. Albumin (gm/dl)	4.24±0.59	4.07±0.55	1.356	0.178
PT(sec.)	13.87±1.61	14.12±1.55	0.719	0.474
INR	1.12±0.21	1.15±0.19	0.773	0.442
S. Creatinine (mg/dl)	0.77±0.20	0.92±0.25	3.200	0.002*
FBS(mg/dl)	102.67±31.41	152.26±40.41	5.965	<0.001*
2HPP(mg/dl)	148.93±44.23	218.74±67.42	5.215	<0.001*
FSI(uU/m1)	4.79±3.40	38.84±28.11	6.719	<0.001*
TNF-α(pg/ml)	50.40±17.68	46.81±14.84	0.968	0.336
HOMA_IR	1.18±0.84	15.54±13.52	5.905	<0.001*

Table (5): Comparison of HCV patients with insulin resistance and HCV patients without insulin resistance

Variable	HOMA < 3 (n =29)	HOMA > 3 (n = 21)	test	P- value
Age (M± SD)	40.79 ± 11.02	47.24 ± 9.88	2.089	0.048*
BMI (Kg/m²)	26.20 ± 5.05	30.17 ± 5.17	2.711	0.009*
HG (gm/dl)	12.67 ± 2.08	12.78 ± 2.77	0.161	0.873
WBCS (c/ mm3)	6.14 ± 1.93	7.28 ± 2.35	1.866	0.068
PLT (c/ mm3)	196.72 ± 78.35	198.24 ± 55.37	0.080	0.937
ALT (IU/dl).	37.17 ± 23.03	37.71 ± 21.43	0.085	0.933
AST (IU/dl).	38.86 ± 25.93	38.38 ± 25.47	0.065	0.948
Total Bilirubin (mg/dl)	1.04 ± 0.42	0.92 ± 0.32	1.121	0.268
Direct Bilirubin (mg/dl)	0.23 ± 0.13	0.22 ± 0.09	0.180	0.858
S. Albumin (gm/dl)	4.16 ± 0.69	4.25 ± 0.55	0.469	0.641
PT (sec.)	14.25 ± 2.03	13.69 ± 1.27	1.211	0.232
INR	1.17 ± 0.27	1.11 ± 0.17	1.042	0.303
S. Creatinine (mg/dl)	0.77 ± 0.23	0.91 ± 0.27	2.040	0.047*
FBS (mg/dl)	88.03 ± 11.57	150.38 ± 38.99	7.105	<0.001*
2HPP (mg/dl)	125.52 ± 17.18	206.33 ± 57.84	6.208	<0.001*
FSI (uU/m1)	5.41 ± 3.24	40.81 ± 24.75	6.515	<0.001*
TNF-α (pg/ml)	55.45 ± 16.45	45.93 ± 15.12	1.800	0.072
HOMA_IR	1.19 ± 0.76	15.95 ± 11.85	5.698	<0.001*

Table (6): Correlation of HOMA>3 in HCV patients with some studied parameters

Variable	HOMA_IR > 3 No (21)	
	R (range)	P - value
Age (M± SD)	0.382	0.096
BMI (Kg/m²)	0.164	0.477
FBS (mg/dl)	0.601	0.004*
FSI (uU/m1)	0.933	<0.001*
TNFα (pg/ml)	0.109	0.639

DISCUSSION

Metabolic abnormalities are common in patients with hepatitis C virus (HCV) infection, there is considerable evidence that patients with chronic HCV infection are at a greater risk of developing insulin resistance (IR) and ultimately, diabetes mellitus (DM) compared with non-infected individuals or patients with hepatitis B virus (HBV) infection **[11]**. The pathogenic mechanisms causing DM in patients with HCV infection are still not well understood, although both insulin resistance and impaired insulin secretion have been considered to play an important role in the development of DM **[12]**. More recently, the role of tumor necrosis factor (TNF-α) in the pathogenesis of DM in chronic hepatitis C patients has gained extensive interest **[13].** TNF-α has been shown to inhibit insulin-stimulated tyrosine phosphorylation of insulin receptor and insulin receptor substrate 1 in adipocytes, stimulate lipolysis, and increase serum-free fatty acids, leading to insulin resistance in muscle and liver, mediate hepatic insulin resistance to increase hepatic glucose production, and down-regulate genes in adipocytes encoding proteins such as insulin receptor substrate 1, glucose transporter-4, peroxisome proliferator-activated

receptors, and adiponectin. In addition, TNF- α may reduce beta-cell function by direct toxic effects, further contributing to the development of DM. Some studies have shown significantly higher levels of soluble TNF- α receptors in diabetic HCV patients than in non-diabetic HCV patients and controls **[14].** A link between chronic HCV infection, TNF- α, and type 2 DM is an attractive hypothesis. In the present study there was no statistical significant differences between all studied groups as regard age, sex and BMI. While the majority of studied groups were males and higher mean age was in (HCV diabetic patients) group II (45.52±8.77 years). These data come in agreement with Shintani et al, **[4].** Who found that the development of IR in patients with chronic HCV infection can occur early in the course of the disease and this effect appears to be independent of body weight.On other hand Petit et al, **[15]** reported that older age and obesity are correlated with genesis of diabetes in patients with HCV due to liver fat deposition which may contribute to insulin resistance, which in turn lead to loss of the restraining effect of insulin on hepatocyte production of glucose, leading to appear of diabetes mellitus. In the present work the mean FSI was significantly higher in HCV patients with DM compared to HCV patients without DM and patients with DM alone and control group (P value <0.001). This results are in agreement with the results of Hassan et al, Mansour et al and Ragab et al, **[16,17,18]**. Who found the mean fasting insulin was significantly higher in patients with HCV and DM compared to patients with HCV alone and patients with DM alone (p<0.01, p<0.05 and p<0.019) respectively. In this study the mean level of HOMA-IR was significantly higher among HCV patients with DM compared to patients with DM alone, HCV patients without DM and control group (P <0.001). Insulin resistance HOMA-IR>3 was most frequent among HCV patients with DM, followed by patients with DM alone then HCV patients alone and control group (P <0.001). And overall IR was found in 31 patients of study population (34%) and in 21 of HCV patients (42%). This agreed with Mansour et al, **[17],** who found the same significantly higher HOMA-IR among diabetic chronic HCV patients than non-diabetic chronic HCV patients and diabetic HCV -ve patients (p<0.05). While they found IR in HCV patients alone is higher than patients with DM alone. On the same hand Angelica et al, **[19]**, in the study had been carried out on 3 groups of patients: The 1st group- 17 patients with normal glucose tolerance and with chronic hepatitis C, the 2nd group: 15 healthy patients (control group) and the 3rd group: 13 patients with chronic hepatitis C and type 2 diabetes.His results agreed with our results as Insulin resistance HOMA-IR >3 was most frequent among HCV patients with DM, followed by patients with DM alone and HCV patients (P=0.0147). Also the study by Hassan et al, and Ragheb et al**, [16,18]** showed that HOMA-IR was higher HCV patients with DM compared to HCV patients without DM and DM alone and healthy control group(P value <0.001) for each in the formal study and (P = 0.0003) in latter study. In the present study FBS, 2HPP were higher in HCV patients with DM compared to DM alone, HCV patients without DM and control group (P < 0.001) for each of them. Also their levels in HCV patients with IR were higher than HCV patients without IR (P <0.001) for each. This was in agreement with Souza et al, **[20].** Who showed that Patients with chronic HCV and IR had higher levels of blood glucose (p=0.004), compared with patients without IR. As regarded to TNF-α levels among the studied group of patients, highest level of TNF-α found among HCV patients followed by patients with HCV with DM and the lowest level was among patients with DM alone and control group, with significant difference between studied groups (P <0.001), this finding not fully matched with most of the previous studies **[5,19]** that found significant elevation of level of TNF among HCV patients with DM and have IR in comparing with HCV and DM followed with HCV patients without DM followed by diabetic patients, in some studies they found levels of TNF-α were significantly higher in HCV with or without DM groups compared with DM alone. (p<0.001, <0.001) respectively **[21,22]**. Also Ragheb et al. **[18]**, found the same results but without significant difference between the studied groups (P value = 0.17). In this study there was no significant difference between HCV patients with IR and without IR regarding TNF-α level (P = 0.07), and there was no significant positive correlation between TNF-α and IR in HCV patients (P = 0.63), this finding was not matched with previous studies that found significant elevation of TNF among HCV patients with IR. There is an abundance of literature to suggest that adipose tissue derived cytokines (adipo-cytokines) may play a key role in the development of obesity-related insulin resistance, TNF, IL6 and leptin are proinflammatory cytokines that can

directly alter glucose and lipid metabolism. Other studies found that IR seems to be the over production of TNF-α **[5,6]**. This cytokine phosphorylates serine residues of insulin-receptor substrates 1 and 2 and enhances the production of suppressor of cytokines (SOC3). The SOC-3 substance inhibits the phosphorylation of Akt and phosphatidyl inositol 3 kinase (PI3K) **[23]**. This finding may explained by : 1- Type 2 diabetes is associated with both insulin resistance and a deficit of insulin secretion, as well as with high levels of other proinflammatory cytokines like IL6 and leptin, this condition should be excluded when the specific effect of HCV is evaluated. 2- study of adiponectin, the most abundant of all the adipocytokines has an opposite effect, increasing insulin sensitivity and having anti-steatotic, antiinflammatory and antifibrotic effects must evaluated **[24]**, 3- study of virological causes of IR (virological type of IR). The nonstructural protein 5A (NS5A) of HCV plays a significant role in virus-driven IR. HCV NS5A and increased endoplasmic reticulum stress in HCV infection contributes to overexpression of protein phosphatase 2A (PP2A) **[25,26]**. HCV core protein has been reported to induce over-expression of tumor necrosis factor (TNF-α) in the liver of transgenic mice and human hepatoma cell lines **[20].** These factors may have direct effect in the results of this study and also small number of studied patients can lead to limitation of this study results.

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Conflicts of interest: None.

Ethical approval: The study protocol was proven by ethical committee of Benha University and informed consent was obtained from all patients before participation in this study.

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