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Association between HCV infection and the incidence of Diabetes Mellitus in Egyptians

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▮▮▮ ABSTRACT

The present work was planned to find a correlation between HCV infection and diabetes mellitus (DM) incidence in Egyptian patients by finding a possible relationship between HCV antibodies and both anti-insulin antibodies (AIAs) and pancreatic islet cell antibodies (PICAs). The screening results showed that the percentage of HCV patients out of 200 subjects were 35.5 and 14.5% in males and females, respectively. Also, the association between DM and HCV were 13.21 and 13.64% in men and women, respectively. The titers of antibodies in diabetic or non-diabetic patients ranged from 30 - 58 for AIAs and from 30 - 40 for PICAs, The percentage of patients with raised serum transaminases was significantly more in DM patients with positive HCV antibody (52.3%) than DM patients with negative HCV antibody (16.7%). Conclusion, it can be concluded that diabetic or non-diabetic patients with HCV might have a rise titer of the antibodies toward insulin or its secretory cells in pancreas or toward both. where are more susceptible to both HCV-infection and antibodies than women, indicating to here is a significant association between HCV infection and induction of diabetes in Egyptians.

Keywords: HCV, Anti-insulin antibodies**, D**iabetes mellitus

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INTRODUCTION

Viral hepatitis can be caused by a variety of infections as well as toxic and metabolic injuries leading to a wide spectrum of systemic manifestations with different immunologic responses. The pathophysiologic findings of hepatitis A, B, C, D, E, F, and G viruses vary in their immunologic responses as well as in seriousness of effect and prognosis (Su-Hyung and Rehermann, 2014, Xu and Zhong, (2016). Hepatitis CV is one of RNA viruses in family flaviviridae, with at least 6 different genotypes (Dietz et al., 2015; Patiño-Galindo et al., 2016). Prior to 1989, HCV was considered of non-A, non-B hepatitis; now it is well established that 85-90% diagnosed hepatitis cases of non-A, non-B, were in fact HCV (Maunoury et al., 2018; Di Poto et al., 2018). The prevalence of this infection is higher in developing countries, approaching 4-6 % in the Middle East and Africa.

Prenatal exposure to the enteroviruses increases the risk of ID-DM. It was emphasized by Hitmen et al. (1997) and Viskari et al. (2012) that enterovirus infections of non-diabetics converted pancreatic islet cell antibodies (PICAs) from seronegativity to PICAs-seropositivity (Darius et al., 2014). They added that children who converted to PICAs-positivity during an enterovirus infection had a high-risk genotype than did children of constantly PICAs-negativity.

Enteroviruses have been examined for their possible role in the etiology of ID-DM and the evidence remains inconclusive. Acute enteroviral damage to pancreatic β-cells is proposed to be incompatible with the initiation of autoimmune response preceding IDDM (Petzold et al., 2015; de Beeck and Eizirik, 2016; Hyöty, 2016). Current studies are still continued to determine whether acute enteroviral infections can promote DM progression from autoimmunity and to identify the diabetogenic strains of enteroviruses (Alvaro-Benito et al., 2016; Meghan et al., 2017). The susceptible individuals incite an autoimmune response that results in progressive loss of cells at a rate exceeding the replacement capacity after 90% of β-cells destruction (Liusong et al., 2014).

The HCV infection evokes the generation of auto-antibodies and auto-immune responses as hypergammaglobulin (Paroli et al., 2012). These responses induce hepatitis, Hashimoto's thyroiditis and hypothyroidism in female humans. In addition, there is a significant assumption that an association between HCV infection and DM may be existed (Cuadros et al., 2013). They proposed that HCV probably acts either indirectly by triggering autoimmune insulitis or directly by β-cell damage leading to an ID-DM. Other investigators reported that type-1 DM in HCV may be developed after medication of patients with interferon-α therapy, which is involved in immunological events that lead to β-cells destruction and development of insulin autoantibodies of type-1 DM (Chedin et al., 1998; Antonelli et al., 2014.

In Egypt, HCV infection represents a significant health problem, its prevalence varies in different studies but in most of them it was persistent (El-Zayadi et al., 1992; de Vos et al., 2012). Recently, it has been suggested that an association between DM and HCV infection represent a case of research interest for two reasons. First, diabetes is more common in HCV- than HBV-liver cirrhosis and this may suggest a role of HCV infection in the pathogenesis of DM (Gimberts et al., 1999; Chen et al., 2016; Crux and Elahi, 2017).

Second, chronic HCV patients are more likely to develop diabetes than HCV seronegative patients and this may also lead to a suggestion that the pancreatic β-cells may be an extra-hepatic target for the virus (El-Zayadi et al., 1998; Hammerstad et al., 2015). The present study was undertaken to correlate between HCV infection and DM incidence in Egyptian patients by finding a possible relationship between HCV antibody seropositivity and both anti-insulin antibodies (AIAs) and pancreatic islet cell antibodies (PICAs).

Materials AND METHODS
Subjects
The present study was randomly performed upon 200 patients in the hospitals of Ain Shams University, Egypt. All cases under investigation either normal or patients were subjected to the clinical examination to diagnose hepatitis and other liver diseases. Diagnosis of Diabetes mellitus (DM) was carried out by measurements of plasma glucose level (Fasting, random and 2 hours post-prandial). Serological tests for secretion of both anti-insulin antibodies (AIAs) and Pancreatic Islet cell antibodies (PICAs) by using ELISA technique were also carried out on the same patients. After laboratory investigation, follow up was continued on 96 subjects, (66 males; 30 females; aged from 17 to 70 years) with hepatitis or diabetes complications or both, and they were divided into 5 groups as follows:

The 1st group included 10 diabetic patients with anti-HCV seropositivity; 7 males (70%) and 3 females (30%) whose age ranged 18-65 years. Three out 10 patients were treated with insulin while the rest patients received oral hypoglycemic agents. The 2nd group included 50 non-diabetic patients with anti-HCV seropositivity; 36 males (72%) and 14 females (28%) and aged from 17-67 years. Only five cases in this group was a part history interferon-α therapy, which had been discontinued due to severe thrombocytopenia. The 3rd group included 5 diabetic patients with anti-HCV seronegative; 3 males (60%) and 2 females (40%) and their age ranged from 18 to 70 years. Four patients out of all were treated with hypoglycemic oral drugs while the last

one was treated with insulin. The 4th group included 10 non-diabetic patients with anti-HCV seronegative; 7 males (70%) and 3 females (30%) with range of 19-68 years. The 5th group included 21 healthy subjects (hepatitis seronegative antibodies); 13 males (62.40%) and 8 females (37.60%) and their age ranged from 20 to 69 years.

ELISA

Reagents of ELISA kits which are used in AIA and PICAs were purchased from Biomedica, INC, California, USA and they were stored in the refrigerator at 2-8°C. Serum samples were also stored at 2-8°C during the time of analysis, they were frozen at - 20°C. The procedure is carried out according to the manufacturer's instructions and was repeated three times. The washing of microwells was performed automatically and then gently dried on a paper towel. The microwell reader was adjusted to read absorbance at 405 nm giving the spectrophotometric optical density (OD) which was directly proportional to the concentration of either AIAs or PICAs in the sample. The cut-off point = average OD of the negative control × 2.5 = 0.95 and 0.42 for AIAs and PICAs, respectively. If the average OD of tested serum was equal to or greater than cut off point, it was considered positive for AIAs and was considered seronegative or normal for PICAs.
Statistics: All results obtained are expressed as means + SD.

statistical analysis

Data were subjected to full using both Chi-square test and t-test. In all cases, the difference between means was considered significant at $p < 0.05$ at least.

RESULTS

Distribution of diabetes mellitus and HCV-antibodies:

As shown in table (1) and according to the results of the laboratory investigation, patients in the 1st group were diagnosed as positive for both DM and HCV antibodies with a mean of 47 + 5.26 and 53 + 8.33, respectively. The 2nd group subjects were negative for DM and positive for HCV antibodies by the third generation of ELISA. The 3rd group included only 5 subjects who were positive for DM and negative for HCV-antibodies, they were 3 males (60%) and 2 females (40%). Ten subjects in the 4th group were negative for both DM and HCV antibodies, but they have other hepatic complications as diagnosed by clinical examination. On the other hand, the laboratory examination revealed that 21 subjects in the 5th group were normal without clinical and laboratory or sonographic evidence of liver complications.

Figure (1) shows the laboratory percentages of diabetic incidence with or without HCV infection. Results revealed that the percentages of DM incidence in patients with hepatic complications were 65% and 35% for males and females, respectively.

Also, the percentages of HCV incidence alone were 71 and 29% for both sexes, respectively. The combination between both DM and HCV-antibodies were found to be 70 and 30% for diabetic males and females with hepatitis, respectively.

Table 1: Distribution of DM and HCV-antibodies in all investigated subjects.

Group / Diagnosis	Sex / (n)	Age (X + SD)	Range	Diseases			
				DM		HCV	
				No.	%	No.	%
Group I (+ve DM and +ve HCV)	M (7/10)	47.00 + 5.26	18 -95	7	70	7	70
	F (3/10)	53.00 + 8.33	37 − 65	3	30	3	30
Group II (-ve DM and +ve HCV)	M (36/50)	45.81 + 1.54	30 − 67	0	0	36	72
	F (14/50)	45.93 + 2.95	17 − 60	0	0	14	28
Group III (+ve DM and −ve HCV)	M (3/5)	44.33 + 15.01	18 − 70	3	3	0	0
	F (2/5)	55.00 + 13.00	42 − 48	2	2	0	0
Group IV (-ve DM and −ve HCV)	M (7/10)	51.43 + 3.37	41 − 65	0	0	0	0
	F (3/10)	37.67 + 10.97	19 − 57	0	0	0	0
Group V (Normal Subjects)	M (13/21)	49.31 + 2.90	37 − 69	0	0	0	0
	F (8/21)	43.00 + 3.59	20 − 52	0	0	0	0

Data are shown as means + SD, and (n) point to the number of examined subjects.

As compared to control group, the percentages of diabetic incidence in males and females were 32.5% and 17.5% respectively.

Also, the percentages of HCV incidence were 35.5% and 14.5% in both sexes, respectively. Taken all together, the percentages of DM in males and females with HCV were 13.21% and 13.64%, respectively. Also, the total percentages of diabetic patients whether males or females with hepatitis was 13.33% regardless of gender.
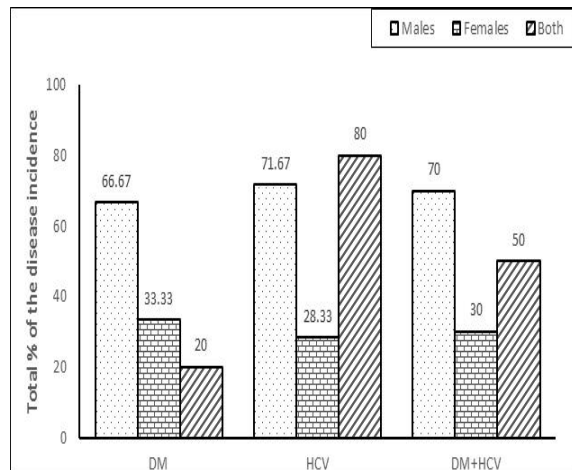

Figure (1): The incidence % of diabetes and hepatitis in all investigated subjects.

HBV-antibodies as seromarkers for diagnosis

Table (2) shows data obtained from the immune diagnosis of sera of all studied patients in all groups. From immune indices; hepatitis B surface antigen (HB-sAg), hepatitis B endogenous antigen (HB-eAg), and antibodies of the hepatitis B core HBc, HBe, and anti-hepatitis B core (HBc) are measured. The percentage of HB-sAg ranged from 13.89%-14.29% in male, and form 7.14%-66.67% in females, respectively. Taken males and females together, the total percentage of this antigen was 13.33%. Also, the percentage of HB-eAg was ranged from 2.78%-33.33% in males and females of group 5 with a total percentage 5.33 in both sexes. On the other hand, the percentage of anti-HBs ranged from 14.29%-42.86% in males and 21.43% in females of group (2), respectively, with a total percentage 25.33 in the investigated subjects. Likely, the percentage of anti-HBe ranged from 13.89 and 28.57% in males of groups 1 and 2, and form 14.29 - 33.33% in females of groups 2

and 5. Taken all together, the total percentage for both sexes was 13.33.

Determination of AIAs and PICAs:

Both AIAs and PICAs concentrations (OD) in anti-HCV seropositive patients in the 1st and 2nd groups are represented in Table (3). Means of AIAs in the 1st group was 0.8058 + 0.096 (0.387 - 1.261), and the percentage of seropositivity of this antibody titer was 30%. Regarding PICAs, the resulted mean of patients with anti-HCV seropositive in groups 1 and 2 was 0.379 + 0.045 (0.216 - 0.679), and the percentage of the concentration of these antibodies was also 30%. The concentration of AIAs in patients of the 2nd group was determined as 2.242 + 0.71 (0.275 − 0.229) with a total percentage 58 of these antibodies. Likewise, the mean of PICAs in the patients in the 2nd group was 0.3833 + 0.0189 (0.190 - 0.842) with a total percentage 40 seropositvity of such antibodies titer.

In Table (3), patients in the 3rd group have not AIAs, except one patient with 1.64 with a percentage of 20%. Taken seropositive and seronegative together, the mean concentration of AIAs titer was 0.726 + 0.125 (0.362 - 1.64). Regarding patients in the 3rd group, the analysis of sera showed that there is nobody gave PICAs seropositive results, and thus the total percentage was 0.00%. In this case, the mean titer of PICAs was found to be 0.2024 + 0.0196 (0.157 - 0.272).

Table (3) shows the concentration of both AIAs and PICAs in patients of the 4th group. For AIAs, it was found that two out of ten patients showed positive results (20%) with the mean at 2.73 + 0.14 (0.3 - 0.22). In the anti-HCV seronegative patients in the 4th group, there is patient gave PICAs seropositive, and thus the percentage becomes 0.00%. In this case, the mean of the obtained data was calculated as 0.1875 + 0.009 (0.164 - 0.25). The subjects in the 5th group, 3 out of 21 patients with other abdominal diseases have AIAs seropositive (14.3%).

The plasma glucose level of HCV-patients:

The obtained data in Table (4) showed that FPG is significantly increased in diabetic patients of the 1st group with hepatitis; either male (1.1%) or females (2.07%) compared to the value of FPG in the healthy persons of 5th group. Also, the level of FPG increased

significantly in diabetic patients of the 3rd group without hepatitis. The percentage of change in FPG levels reached 1.23 and 0.84% in males and females, respectively. This result showed that the FPG level was higher in males than in females.

The random plasma glucose (RPG) level was significantly increased in diabetic patients of the 1st group with hepatitis. In this case, the percentages of change in RPG levels versus group 5 were 0.56 and 1.50% in males and females, respectively. Likewise, RPG was increased significantly in diabetic male and female patients of group 3 without hepatitis by 1.076 and 0.79%, respectively. It is recognized that this increase in RPG was also found to be more marked in men than in women.

The level of post prandial plasma glucose (PPPG) was significantly increased in diabetic patients of groups 1 and 3 with and without hepatitis, respectively, and it was more marked in men than in women. These percentages of change in PPPG level among diabetic patients of group I with hepatitis reached 1.49 and 1.25% for male and female patients, respectively. Also, these percentages were 1.87 and 1.08% for male and female diabetic patients without hepatitis in group 3, respectively.

DISCUSSION

Regarding distribution and transmission of HCV-infection, the obtained results showed that increase in the percentage of HCV males than females by 2 fold. In addition, the association between DM and HCV were found in men and women the total percentage reached 13.33% regardless the type of sex.

The transmission of HCV is elusive where the exposure to contaminated blood and to a less extent other human secretions are known modes of infection (Pacheco and Horowitz, 1998; Miller et al., 2015). In the present study, pathological examination of the patients revealed that hepatitis seemed to be the most pronounced disease that was usually accompanied to hepatitis C-infection. Its percentages in both men and women were 24.53 and 27.27%, respectively, and showed a total percentage of 25.33%. Medical examination showed also that hepatic and

renal failure, and immune-mediated diseases with various percentages were diagnosed, and associated with HCV, DM or both. Moreover, abdominal ultrasonograhy showed that liver cirrhosis is the most conspicuous disease that associated with DM, HCV or with the combination between them. The percentages of this disease were higher in men than in women and of a total percentage of 61.33% in both sexes.

On immune diagnosis of sera in patients under investigation, it was observed that diabetic women with or without HCV-infection have not any antigen/antibodies titer, males seemed to be more susceptible to HCV-infection and antibodies formation than females. These results are in agreement with Stephen et al. (2006) who concluded that 85% of patients were infected at least with acute and chronic HCV. However, the mechanism of HCV high rate persistence is unknown, although some data suggested a relationship to the genetic diversity and mutation of the virus (Farcie and Purcell, 2000; Sanjuán et al., 2016).

Present results indicated that the infection with HCV followed by DM-incidence might induce further complications by increasing blood glucose levels in both human sexes. Also, men are found to be more susceptible to HCV-infection and DM-incidence than women. They are suffering from increased leakage of the liver enzymes and from disturbance of biochemical parameters, impairing the liver functions. For epidemiological studies, the estimation of diabetes prevalence and incidence should be based on a FPG > 126 mg/dL (7.0 mmol/L). This will identify patients as having impaired glucose homeostasis than will the fasting cutoff 110 mg/dL (601 mmol/L), it is essential that investigators always report which test was used (McCanc et al., 1997). Allison et al. (1994) and Hyöty (2016) reported that adult patients with chronic HCV infection are significantly more likely to be diabetics compared to patients with liver disease of other etiologies. El-Zayadi et al. (1996) who reported that etiologies of chronic hepatitis C patients in Egypt are three times more likely to develop DM than HCV seronegative patients also supported this suggestion.

Table (2): Antibodies of HBV infection as seromarkers in all investigated patient groups.

Group / Diagnosis	Sex/(n)	HBs Ag		HBe Ag		Anti-HBs		Anti-HBe		Anti-HBc	
		No.	%	No.	%	No.	%	No.	%	No.	%
Group I (+ve DM and +ve HCV)	M (7/10) F (3/10)	1 0	14.29 0.00	0 0	0.00 0.00	3 0	42.86 0.00	2 0	28.57 0.00	3 0	42.86 0.00
Group II (-ve DM and +ve HCV)	M (36/50) F (14/50)	5 1	13.89 7.14	1 0	2.78 0.00	11 3	30.56 21.43	5 2	13.89 14.29	18 4	50.00 28.57
Group III (+ve DM and –ve HCV)	M (3/5) F (2/5)	0 0	0.00 0.00	1 0	33.3 0.00	1 0	33.33 0.00	0 0	0.00 0.00	2 0	66.67 0.00
Group IV (-ve DM and –ve HCV)	M (7/10) F (3/10)	1 2	14.29 66.67	1 1	14.29 3.33	1 0	14.29 0.00	0 1	0.00 33.33	1 2	14.29 66.67

M and F means male and female patients, respectively. HBs Ag is Hepatitis – B-surface antigen, HBe Ag is Hepatitis –B-e antigen, Anti-HBC is Anti-Hepatitis-B-core, and HBV is Hepatitis B virus.

Table 3: Concentrations of both AIAs and PICAs in anti-HCV seropositive gorups.

Group / Diagnosis	Sex (n)	AIAs (X ± SD)	Range	%	PICAs (X ± SD)	Range	%
Group I (+ve DM and +ve HCV)	10	0.806 ± 0.096	0.387-1.261	30	0.379 ± 0.045	0.216-0.679	30
Group II (-ve DM and +ve HCV)	25	2.242 ± 0.71	0.275-22(over)	58	0.383 ± 0.019	0.190-0.842	40
Group III (+ve DM and –ve HCV)	5	0.873 ± 0.125	0.362-1.64	20	0.202 ± 0.02	0.157-0.272	0
Group IV (-ve DM and –ve HCV)	10	2.727 ± 2.14	0.3-22 (over)	20	0.188 ± 0.009	0.164-0.25	0
Group V (normal)	21	0.726 ± 0.043	0.41-1.098	14.3	0.224 ± 0.011	0.167-0.345	0

Data are shown in means + SD, and (n) point to the number of examined patients. Seropositivity = AIAs > 0.95 or PICAs > 0.42. AIAs: Anti-insulin antibodies, AICAs: Pancreatic islet cell antibodies, OD: Optical density.

Table (4): Fasting, random and post-prandial plasma glucose levels (mg/dl) in patients with anti-HCV seropositivity

Group / Diagnosis	Sex /(n)	Plasma Glucose Levels (mg / dL)					
		FPG		RPG (< 200 mg/dL)		2h PPPG (mg/dL)	
		(X ± SD)	%	(X ± SD)	%	(X ± SD)	%
Group I (+ve DM and +ve HCV)	M (7/10) F (3/10)	181.00 ± 19.52 260.67 ± 31.42	101 2.07	197.71 ± 33.34* 294.33 ± 55.97*	0.56 1.50	249.00 ± 27.38* 244.33 ± 56.70*	1.49 1.25
Group II (-ve DM and +ve HCV)	M (36/50) F (14/50)	88.76 ± 2.33 85.36 ± 6.134	- 0.02 - 0.07	12.18 ± 4.11 120.36 ± 4.87	0.008 0.078	109.59 ± 3.56 116.29 ± 3.62	0.09 0.098
Group III (+ve DM and –ve HCV)	M (3/5) F (2/5)	191.00 ± 64.78 161.500 ± 54.50	1.023 0.84	242.67 ± 83.03* 208.00 ± 68.00	1.076 0.79	287.00 ± 107.11* 225.00 ± 75.00*	1.87 1.08
Group IV (-ve DM and –ve HCV)	M (7/10) F (3/10)	92.71 + 3.95 106.67 + 3.33	0.03 0.19	103.43 + 15.92 97.00 + 13.32	0.11 0.112	107.00 + 4.2 114.33 + 8.09	0.07 0.08
Group V (normal)	M (13/21) F (8/21)	90.38 ± 6.39 91.13 ± 2.49		119.00 ± 4.52 110.75 ± 6.76		100.00 ± 7.04 105.38 ± 2.91	

Data are shown as means ± SD, and (n) point to the number of examined patients. FPG, RPG and PPPG are fasting, random and post-prandial plasma glucose levels respectively. * is statistically significant mean at P 0.05 at least.

In the present study, the 2-hour postprandial glucose in anti-HCV seropositive non-diabetic group was significantly higher than in the anti-HCV seronegative groups. So, in diabetic groups, anti-HCV positivity was associated with a significantly higher level of PICAs and hence a higher specificity of prediction of type-1 DM, more beta cells damage and insulin dependence. Petzold et al. (2015) revealed the critical role of PICAs especially surface PICAs, which preferentially bind to and lyse beta cells in presence of complement, so, impairing islet cells function.

In anti-HCV seropositive groups, AIAs were present in 32 cases (53.3%) while in anti-HCV seronegative groups, AIAs were present in 6 cases (16.7%). The anti-HCV seropositivity as a risk factor for AIAs seropositivity had a sensitivity of 84.2% and a specificity of 51.7%. This supports the frequent association of AIAs positivity with viral infections and liver dysfunction (Uchigata, 1994). In the same groups, PICAs were present in 23 cases (38.3%) while were 0.00% in the anti-HCV seronegative groups. As a risk factor for PICAs seropositivity, the anti-HCV positivity had a sensitivity of 100 and a specificity of 49.3%. These results supported other studies searching for a relationship between viral infections and seroconversion to PICAs positivity.

In addition, our results revealed that PICAs seronegativtiy in all patients of anti-HCV seropositive (group 2) who had a history of incomplete α-interferon therapy and since PICAs might play a critical role in pathogenesis of type-I DM. Hence, current results are in agreement with those of Ahmed (1994) who reported the control of DM in some interferon responders among anti-HCV seropositive patients. The interferon-α therapy in chronic viral hepatitis-B or –C was shown by Di-Cesare et al. (1996) to be not capable of inducing the development of PICAs. However, Imagawa et al. (1996) found that antibodies to glutamic acid decarboxylase could be induced by interferon-α therapy in chronic viral hepatitis .

In this study, combined AIAs and PICAs were present in 18 cases (30%) while were absent in anti-HCV seronegative groups. The anti-HCV positivity as a define risk factor for combined seropositivity of AIAs and PICAs, had a sensitivity of 100% and a specificity of 46.2%. This combined positivity strongly predicated the diagnosis of a pre-diabetic state in known non-diabetic patients (15 patients in group 2=30%). Also, it predicted the development of insulin-dependence in the three known diabetic patients of group 1 (30%). This again supported the autoimmune and diabetogenic potential of HCV-infection. Results also revealed significant independent risk factors for a higher percentage of seropositivity for AIAs and/or PICAs and /or a higher mean of PICAs to be mainly the anti-HCV seropositivity. A correlation exists between AIAs positivity and lowered serum albumin and lowered A/G ratio, which was present also in the anti-HCV seronegative groups but to a lesser extent. This is pointing to a link between AIAs and autoimmunity and supporting a previous studie by Uchigata, (1994) who showed a frequent association between AIAs seropositivity and diffuse poly immunity especially Grave's disease, rheumatoid arthritis and systemic lupus erythematous .

Other risk factors acted only in anti-HCV seropositive patients and included presence of HBV infection, which was a risk cofactor for higher percentage of positivity for PICAs. who revealed a higher prevalence of HBV-infection (in form of seropositivity for ant-HBC) among diabetics (44%) than among non-diabetic controls (11%). However, Fraser et al. (1996) revealed an association between DM and chronic hepatitis-C that was not present in patients with chronic hepatitis-B (DM was present in 39.1% and 2.5% of patients with chronic hepatitis-C and –B respectively.

It has been observed that HCV infection is associated with development of DM (Mason et al., (1999; (Zein, 1998). The mechanisms of this positive association need to be verified. The prevalence rise of type II diabetes among chronic HCV patients is independent of liver cirrhosis (Knobler et al., 2000). The intriguing pathogenesis appears to be unique of HCV. However, liver cirrhosis was a risk factor in

case of higher percentages for seropositivity of AIAs, PICAs and for combined positivity of them. Also, Splenomegaly and liver cirrhosis was a risk factor for a higher level of PICAs, and hence a higher specificity of prediction of type I DM (Di Poto et al., 2018).

This association between HCV-related liver cirrhosis and AIAs seropositivity again supported a previous study pointing to the spontaneous development of AIAs in non-diabetic patients (Uchigata, 1994). It was suggested that impaired glucose tolerance in cirrhotic may be due to peripheral insulin resistance of circulating AIAs (Ayman, 1991). Also, Podolsky and Issalbacher (1994) reported that there is a fourfold increase of risk cirrhosis in diabetics but mostly cirrhosis is diagnosed first before impaired glucose tolerance is detected. The latter may be due to a decrease in hepatocellular function mass leading to a decrease in hepatic glucose uptake, glycogenesis and portal-systemic shunting of glucose, insulin and glucagon. Also, insulin resistance may be due to both receptor and postreceptor defects in hepatocytes in patients with cirrhosis (Di Poto et al., 2018).

Several possible mechanisms can be postulated to link HCV infection to diabetes. It may be possible that HCV could infect the pancreatic β-cells, thereby induce their damage, or HCV may trigger an autoimmune destruction of endocrine pancreatic tissue. The role of the direct cytopathic effect of HCV on β-cell in the development of DM was primary supported by Zhang et al. (1996) who conducted an autopsy study and documented the widespread distribution of HCV within many-hepatic tissues. Autoimmunity directed to β-cell is usually present long before the clinical onset of DM. Either acute or persistent viral infection could trigger the autoimmune process, render the target cells vulnerable to autoimmune process, and render the target cells vulnerable to autoimmune attack without necessarily causing acute cell damage.

The previous studies showed possible link between HCV and DM that could be classified into two major categories, both of which are necessary to establish a definitive link. Studies in the first category reported an increase prevalence of HCV antibodies in patients with type 2 DM (Simo et al., 1996). This prevalence of HCV antibodies ranged between 4% and 28% in Afro-Caribbean and Caucasians diabetics (Gray et al., 1995). A similar study demonstrated a high frequency of HCV antibody (47.3%) among Egyptian patients with type 2 DM compared to a significantly lower frequency of HbsAg (1.1%) as shown by Sobh et al. (1997). The prevalence of HCV antibodies among Egyptian patients with type 2 DM (74%) is higher than its prevalence (11.22%) among Egyptian population in general (El-Zayadi et al., 1992; de Vos et al., 2012). Under this diabetes with increased risk factors for HCV acquisition (i.e. blood transfusion and intravenous needle exposure) were excluded, but the rate of HCV positively remained high suggesting a direct link between the two disorders (Simo et al., 1996).

The percentage of patients with raised serum transaminases was significantly more in diabetic patients with positive HCV antibody (52.3%) than those diabetic patients with negative HCV antibody (16.7%). It is probably incorrect to assume mild elevations of serum transaminases in type 2 diabetic patients are simply attributable to the metabolic disturbances of diabetes. This is particularly in subjects living in areas with a high prevalence of HCV such as Egypt (Gray et al., 1995). The high prevalence of HCV infection among diabetics suggests that HCV could have a direct role in the development of diabetes (Durand, 1997; Maunoury et al., 2018).

It can be concluded that diabetic or non-diabetic patients with hepatitis C might have a rise titer of the antibodies toward insulin or its secretory cells in pancreas or toward both. These antibodies can attack β-cells inducing insulin deficiency, arising DM incidence. It was also found that men are more susceptible to both HCV-infection and antibodies formation than women. Therefore, the results of this work indicated that there is a significant association between hepatitis C-virus (HCV) infection and Diabetes mellitus; also there are

autoantibodies and autoimmunity especially the development of a prediabetic state of autoimmune Diabetes mellitus and the development of insulin dependence in already diabetic patients.

Obtained results indicated that the infection with HCV followed by DM-incidence might induce further complications by increasing blood glucose levels in both human sexes; men are more susceptible to HCV-infection and DM-incidence than women. They are suffering from increased leakage of the liver enzymes and form disturbance of biochemical parameters, impairing the liver functions.

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