Is chronic hepatitis C infection a risk factor for developing diabetes mellitus before and after interferon therapy

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ABSTRACT

Viruses have been implicated as a possible agent in the development of diabetes mellitus (DM). The present work aimed to estimate the level of islet cell autoantibodies in the course of HCV infection; and to assess the relationship between those levels and liver pathology as detected by ultrasonography (US). In addition , we attempted to assess the effect of interferon therapy on the predisposition to diabetes and the severity of hepatitis and cirrhosis.The study was conducted on forty patients with chronic hepatitis C (HCV). Patients were divided into two groups: HCV-seropositive diabetics and HCV-seropositive non-diabetics. Twenty age-sex-matched normoglycemic healthy individuals served as a control group. Liver functions, abdominal US, and hepatitis B and C markers were assessed in all subjects. Predictive and diagnostic markers of insulin depedent diabetes mellitus (IDDM) viz: glutamic acid decarboxylase antibodies (GAD-Ab) and second islet cell autoantibodies (IA2-Ab) were estimated in the whole study population before interferon therapy and in HCV-seropositive non-diabetics after interferon (IFN) therapy. Abdominal US was also performed after IFN therapy. The results showed that HCV-positive diabetics had significantly higher values of ALT, AST and prothrombin time than HCV-positive non-diabetics ($p<0.05$) while results of liver biopsy showed no significant difference between the two groups regarding disease activity. In additon, HCV-positive diabetics had highly significant values of GAD-Ab and IA2-Ab when compared either to HCV-positive non-diabetics or controls ($p<0.001$). However, HCV-positive non-diabetics showed significantly higher levels of GAD-Ab and IA2-Ab when compared to controls ($p<0.05$). Levels of GAD-Ab and IA2-Ab were significantly higher in HCV-positive non-diabetics after IFN therapy compared to before IFN therapy ($P<0.05$). Statistically positive correlations were found between GAD and IA2 antibodies before and after interferon therapy ($r=0.49$ and $r=0.58$ respectively $p<0.05$). No significant correlations were detected between US findings and each of GAD-Ab and IA2-Ab in both HCV diabetics and non diabetics before and after IFN therapy. In conclusion, HCV may be a predisposing factor in development of diabetes mellitus, as detected by the increased levels of GAD and anti IA2 antibodies. HCV patients most probably become more prone to develop DM after interferon therapy. Thus, GAD and anti IA2 antibodies assessment is very important to detect patients at risk of developing DM before and after interferon therapy. In addition, it seems most probable that the severity of cirrhosis, as detected by US, is not associated with the predisposition to diabetes.

INTRODUCTION

Viral antigens may play an important role during the development of the various types of autoimmune diseases via hosts immune responses forming antibodies directed to some organs or may be generalized, which are named organ- and/or non organ – autoimmune diseases respectively (1).

Hepatitis C virus (HCV) infection may affect not only the liver but also various nonhepatic tissues causing many extrahepatic manifestations as cryoglobulinaemia, membranoproliferative glomerulonephritis, polyarthritis, porphyria cutanea tarda, and Sjogren syndrome (2,3,4). The mechanism for the extrahepatic manifestations associated with HCV infection is unclear, but it could be related to a direct cytotoxic pathological effect on the cells or autoimmune processes against target antigens (2,3,4).

Viruses have been implicated as a possible agent in the development of diabetes mellitus (DM)(5). The mechanism of viral infection leading to beta cell destruction involves the induction of the cytokine-interferon alpha (IFN-α) (6). Activation of Toll receptors by double-stranded RNA or poly-IC (viral mimic) through induction of IFN-α may activate or accelerate immune-mediated beta cell destruction (7). Treatment with Toll-like receptor ligands elicited overt autoimmune disease (8).

Type 1 diabetes is regarded as a chronic autoimmune disease caused by selective destruction of the insulin producing β cells. The disease is mediated by T cells but autoantibodies are well established markers for an ongoing autoimmune process within the islets. Distinct islet cell-specific auto-antibodies, ICA (islet cell antibodies) as well as autoantibodies to insulin; glutamic acid decarboxylase (GAD)65 or the second islet cell autoantibodies (tyrosine phosphatase) IA2, are markers for this autoimmune process. The GAD- and IA2 are the main autoantibodies found in classic type 1 diabetes with onset in childhood and adolescence. As these autoantibodies usually appear prior to the clinical onset of the disease, they may be used to predict type 1 diabetes in

predisposed individuals (5,9). However, GAD antibodies also occur in adults with type 2 diabetes. These patients are thought to have slowly evolving form of type 1 diabetes which is called latent autoimmune diabetes in adults (LADA) (5,9). It has also been shown that only those individuals in whom more than one diabetes-related autoantibody could be determined are at considerable risk of developing type 1 diabetes (10). The combination of antibodies to GAD and IA-2, is highly relevant for risk assessment of type 1 diabetes, they are more sensitive and predictive than ICAs (11). Therefore, combined screening for diabetes related autoantibodies is suggested to increase the specificity and the positive predictive value of the autoantibody tests.

Studies of DM and HCV include two categories; studies that screened for HCV antibodies in diabetic patients, and those that screened for DM in patients with HCV infection. Studies in the first category reported increased incidence of HCV antibodies among patients with DM (12). Studies in the second category focused mainly on patients with HCV-induced cirrhosis, and suggested an increased prevalence of DM in patients with liver cirrhosis caused by HCV as compared with those due to hepatitis B virus, alcoholic liver disease, cholestatic liver disease or autoimmune hepatitis (13, 14).

As a drug, interferon-alpha (IFN-α) has been shown to have different biologic effects (antiviral, antiproliferative, and immunomodulatory), and it is used for treatment of chronic viral and neoplastic diseases. Different side effects have been reported in patients treated with IFN-α, but their incidence and prognosis in the case of adverse reactions remain largely unknown (15). In particular, an increase in the production of thyroid autoantibodies with subsequent development of clinical thyroid diseases has been described in patients undergoing IFN-α for chronic hepatitis caused by HCV (5, 16). Furthermore, a high prevalence of other autoimmune diseases was found after IFN-α therapy. Repetitive treatment seems to facilitate these complication (17, 18, 19).

Cirrhosis can be predicted well by ultrasonnography (US), especially in patients with HCV infection (20). US is rarely normal in chronic hepatitis; increase echogenicity is encountered more frequently. Increased echoes in the liver is most probably due to increased collagen rather than fat although, some workers believe that fat itself is responsible (21,22). The hepatic texture varies from coarse to heterogeneous and the surface becomes irregular and the margins lose their smooth and sharp contour, becoming blunted and lobulated (23). The hepatic heterogeneity detected is due to necrosis and regenerating nodules. An ultrasound evaluation of the liver fibrosis stage based on the scoring system was found to be a reliable and effective alternative to the histological staging in chronic liver diseases (24).

The aim of our study was to estimate the level of GAD and IA2 antibodies in the course of HCV infection; to assess the relationship between those levels and liver pathology as detected by US. In addition, we attempted to assess the effect of interferon therapy on the predisposition to diabetes (as reflected by the levels of GAD and IA2 antibodies) and the severity of hepatitis and cirrhosis detected by US.

SUBJECTS AND METHODS

Subjects

1) Patients: the study was conducted on 40 patients (29 males and 11 females) with HCV-positive chronic active hepatitis as diagnosed by biochemical, serological tests and liver biopsy.
Their ages ranged between 31 and 52 years.

They were classified into two groups:

- Group I included 20 HCV-positive diabetics.
- Group II consisted of 20 HCV-positive non-diabetics.
- Patients having fasting plasma glucose (FPG) > 126 mg/dL or 2hr postprandial (2hr PP) > 200mg/dL at more than one estimation are considered diabetics (18).

Inclusion criteria: conditions known to adversely affect glucose metabolism e.g. obesity, corticosteroids therapy, previous interferon therapy were ruled out. Patients having a clinical, or ultrasonographic evidence for chronic pancreatitis and patients below 20 years old were excluded. Patients with ascites, encephalopathy, autoimmune diseases or HB-positive markers were also excluded.

2) Controls: this group included 20 healthy age and sex-matched subjects who were negative for biochemical function tests of liver disease, had normal fasting and 2 hours post prandial plasma glucose levels and had normal percentage of hemoglobin A1c (normoglycemic).

Methods

All individuals were subjected to:

1. **History taking and physical examination**: all HCV +ve diabetic patients had dibetes onset less than 5 years.
2. **Laboratory investigations including**:
   - Liver function tests: Serum albumin, total serum bilirubin and serum transaminases: alanine transaminase (ALT) and aspartate transaminase (AST).
   - Fasting, 2-hours postprandial plasma glucose levels and hemoglobin A1c.These were measured using commercially available kits.
   - HBsAg., Anti-HBc antibodies (IgG and IgM), HCV antibodies estimated using Recombinant Immuno-Blotting Assay (RIBA) and HCV RNA estimation per for med by PCR technique.

- Serum Anti-Glutamic Acid Decarboxylase (GAD65-antibodies): by direct radioligand assay (5), Medipan Diagnostica, Entwicklungs- und Vertiebs GmbH.Germany. These were measured in all sub jects as well as in HCV non-diabetics before and after IFN therapy.
- Serum second islet cell antibodies (IA-2 antibodies): measured by direct radioligand assay (9) ,DRG International Inc. USA. These were measured in all subjects (controls, all HCV patients before INF alpha therapy). In addition they were measured in HCV non-diabetics before and after IFN therapy.

3. **Abdominal ultrasonography**: US with 2–5 MHz fre quency probes, using either Toshiba power vision 7000 or Siemens Sonoline. An US grading system was performed (Mild, moderate and severe) by eva luating the margins, surface and parenchymal texture of the liver.
4. **Liver biopsy** was performed only in patients afterob taining an informed consent by sonographic guidance.

Interferon therapy:

- The HCV patients were subjected to optimal regimen of HCV therapy which is 24 weeks course of the com bination of alpha interferon and ribavirin. The dose of ribavirin was 1200 mg daily (given orally in two divi ded doses). Alpha interferon was given in doses of 3 mil lion units three times weekly by subcutaneous (SC) injection. (25).
- Serum samples were taken from HCV non diabetic patients group for determination of GAD-Ab and IA2-Ab after 24 weeks of antiviral therapy to be compared to their values before therapy. Abdominal US was also performed after therapy to be compared to US before therapy.

Statistical Analysis

Values were expressed as means + standard deviation and analyzed using student`s t-test (paired and unpaired). To study the relation between two variables, Pearson`s correlation coefficient (r) was calculated. Qualitative data (liver US findings) were determined, correlated to studied chemical markers using Spearman Rank order correlations (R). Analysis of variance (ANOVA test) followed by post hoc test was performed for comparison of serum GAD and IA2 antibodies in more than two groups (mild, moderate and severe determined by US) (26).

RESULTS

The two groups (HCV-positive diabetics and HCV-positive non diabetics) showed no significant difference regarding mean age (40.38+11.47 vs. 41.85+9.31 year, p>0.05), sex and residence distribution while HCV-positive diabetics gave significantly higher percentage of family history of DM (69.23% vs. 33.33%, p<0.05).

Table (1) shows that HCV-positive diabetics had significantly higher values of ALT, AST and than HCV-positive non-diabetics and controls. Fasting, 2-hours postprandial plasma glucose levels and hemoglobin A1C

Table 1
Liver function tests and plasma glucose level (fasting and 2hr post prandial) in Control group, Hepatitis C positive (HCV +ve) diabetic and HCV +ve non diabetic patients

Analyte	Control group (20 cases)	HCV Diab. (20 cases)	HCV non-Diab. (20 cases)
Total bilirubin (mg/dL)	0.7 ± 0.2	1.5 ±0.7 a	1.3 ± 0.6 a
ALT (U/L)	23.3 ± 11.7	93.4 ± 23.3 ab	75.7 ± 37.9 a
AST (U/L)	20.9 ± 12.8	105.5 ± 46.4 ab	62.2 ± 17.8 a
Albumin (mg/dL)	4.6 ± 1.4	3.6 ± 1.1 a	3.8 ± 0.9 a
Fasting plasma glucose (mg/dL)	78.2±20.4	130.5±50.2 ab	82.1±16.3
2hr. Postprandial plasma glucose (mg/dL)	86.3±26.1	180.3±60.6 ab	93.4±19.6
Hb A1c %	6.0±1.0	9.4±1.9 ab	6.3±1.3

Values are represented as means ± S.D. HCV Diab: (hepatitis C and diabetic patients); HCV non-Diab:(hepatitis C and non-diabetic patients). (a) denotes that the difference between the corresponding means of the present group and the mean of the control group is statistically significant i.e. p < 0.05(b) denotes that the difference between the corresponding means of the two patient groups is statistically significant i.e. p < 0.05.

were significantly high in HCV diabetics compared to each of control group HCV non diabetic group. No significant differences were found between HCV non diabetic group and control group p> 0.05.

Table (2): shows that HCV-positive diabetics showed significantly higher values of GAD -Ab and IA2- Ab than HCV-positive non-diabetics and controls (p<0.001) HCV-positive non-diabetics showed significantly higher values of GAD -Ab and IA2- Ab compared to controls (p<0.001).

Table (3): shows that the levels of both anti GAD and anti IA2 are significantly higher after interferon therapy compared to the levels before therapy (p < 0.05).

-Statistically positive correlations were found between GAD and IA2 antibodies before and after interferon therapy (r=0.49 and r=0.58 respectively (p<0.05) [Fig. 1,2]. No significant correlation was detected between anti-GAD antibodies and either fasting or postprandial blood sugar in both HCV-positive diabetics and non-diabetics (p>0.05).

Table 2
Levels of glutamic acid decarboxylase (GAD-Ab) and second islet cell antibodies (IA2-Ab) in Control group, HCV diabetics, and in HCV non diabetics before interferon therapy

Analyte	Controls (20 cases)	HCV Diab. (20 cases)	HCV non-Diab. (20 cases)
Anti-GAD (U/mL)	0.48 ± 0.09	1.31 ± 0.24 ab	0.76 ± 0.24 a
Anti-IA2 (U/mL)	0.49 ± 0.10	0.93± 0.13 ab	0.65 ± 0.10 a

Values are represented as means ± S.D. HCV Diab and HCV non-Diab. (hepatitis C and diabetic patients and hepatitis C and non-diabetic patients).
(a) denotes that the difference between the corresponding means of the present group and the mean of the control group is statistically significant i.e. p < 0.05.
(b) denotes that the difference between the corresponding means of the two patient groups is statistically significant i.e. p < 0.05

Table 3
Levels of glutamic acid decarboxylase (GAD-Ab) and second islet cell antibodies (IA2-Ab) in Hepatitis C positive (HCV +ve) non diabetics before and after interferon therapy

Analyte	HCV non diabetics before IFN therapy	HCV non diabetics after IFN therapy
Anti GAD (U/mL)	0.76 ± 0.24	0.84 ± 0.33 *
Anti IA2 (U/mL)	0.65 ± 0.15	0.72 ± 0.18 *

Values are represented as means ± S.D.
IFN : interferon.(*) denotes that the difference between the corresponding means of the two studied groups is statistically significant i.e. p < 0.05

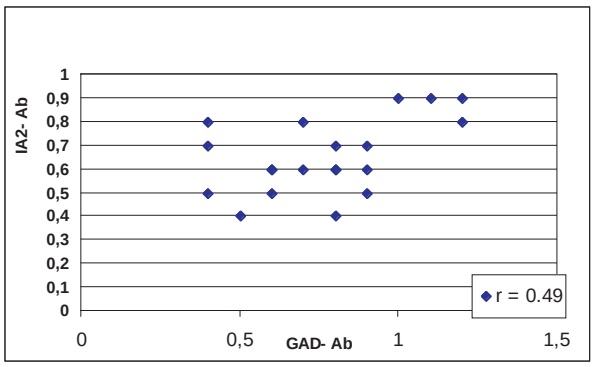

Figure 1
Correlation between serum glutamic acid decarboxylase antibodies (GAD-Ab) and second islet cell antibodies (IA2-Ab) before Interferon therapy

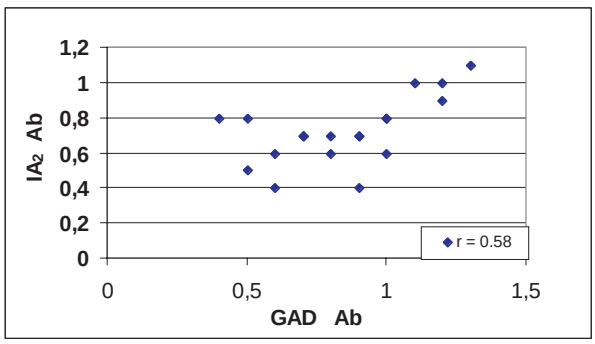

Figure 2
Correlation between serum glutamic acid decarboxylase antibodies (GAD-Ab) and second islet cell antibodies (IA2-Ab) after Interferon therapy

-Incidence of previous history of blood components transfusion, IV drug abuse or surgical procedures was non significant between the two groups (p>0.05).

-No significant correlations were detected between US findings and both GAD-Ab and IA2-Ab in both HCV diabetics and non diabetics before IFN therapy. Also, US findings did not correlate to both markers in HCV non diabetics after therapy (p>0.05).

-Assessment of liver biopsy for activity of chronic hepatitis did not differ significantly between both groups (p>0.05).

-According to US findings before IFN therapy the patients were classified into three groups (Table 4); mild, moderate and severe. Comparing the mean serum values of GAD-Ab in the three groups of HCV diabetic group, significant difference between moderate and severe groups (mean values were 1.50 + 0.26 and 1.12 + 0.155 respectively) (p<0.05) was observed. In non diabetics, significant difference between moderate and severe groups (mean values were 0.900 + 0.224 and 0.563 + 0.160 respectively) (p<0.05) was observed.

-No significant difference was detected in mean values of GAD-Ab in each of the HCV diabetics and non dibetics when comparing the mild group to the moderate and severe groups (mean values were 1.50 + 0.32 and 0.90+ 0.19 respectively, p>0.05).

-No significant difference was detected in mean values of IA2-Ab comparing the three groups (mild, moderate and severe) in both HCV diabetics and non diabetics (p>0.05).

-According to US findings after IFN therapy in non diabetic group, the patients were classified into 4 groups; normal, mild, moderate and severe (Table 5). Comparing the mean serum values of GAD-Abs in the 4 groups, significant difference was observed between moderate and severe groups (mean values were 1.00 + 0.255 and 0.625 + 0.167 respectively) (p<0.05).

-No significant difference was detected when comparing the mild group to the moderate and severe groups (mean values were 0.92 + 0.14, p>0.05).

-No significant difference was detected in mean values of IA2-Ab in the 4 groups (p>0.05).

DISCUSSION

Diabetes mellitus has been observed as one of the associated disorders among HCV chronic liver disease patients (2, 3, 7). In results of the present study, the mean serum levels of GAD and IA2- Abs were significantly higher in HCV-positive diabetics when compared either to HCV-positive non-diabetics or control group (p<0.001 for each). HCV-positive non-diabetics showed significant difference of both GAD and IA2- antibodies when compared to control group (p<0.05 for each). This may indicate that HCV-non diabetic patients may be liable to develop DM.

Although we cannot exclude latent autoimmune diabetes in adults (LADA) as a cause of development of DM (in the HCV diabetic group) or the prediabetic potential (in the HCV non- diabetic group), as our subjects were

Table 4
Ultrasound findings in Control group, Hepatitis C positive (HCV +ve) diabetic and HCV +ve non diabetic patients before interferon alpha therapy

Ultrasound finding	Control (N=20)	HCV diabetics (N=20)	HCV non diabetics (N=20)
Normal	20	-	-
Mild	-	2	5
Moderate	-	8	7
Severe	-	10	8

N= number of cases

Table 5
Ultrasound findings in HCV non diabetic group (ND) before and after interferon therapy

Ultrasound finding	Normal	Mild	Moderate	Severe
HCV (ND) before IFN	-	5	7	8
HCV (ND) after IFN	2	3	7	8

not investigated before the HCV infection, yet, the results of the present study suggest that HCV may predispose to the development of diabetes mellitus and that this predisposition is aggravated by INF-α therapy.

In HCV-positive non-diabetics, levels of GAD and IA2- antibodies were significantly higher after IFN therapy compared to before therapy which suggests that IFN therapy might render HCV patients to be more prone to develop DM. These results are in accordance to previous reported results. Diabetes mellitus was found to be more prevalent in patients with chronic hepatitis C than in patients with other liver diseases and occurred in the absence of predisposing factors of diabetes (13,14). El-Zayadi et al. (27) reported that chronic hepatitis C patients in Egypt were three times more likely to develop DM than HCV- seronegative patients. Montalo et al.(28) reported that HCV infection may influence some of the metabolic processes in the liver and it is known that chronic hepatitis C patients have steatosis and this might indicate an intrahepatic interference by HCV on lipid metabolism. The possibility of a disturbed carbohydrate metabolism in HCV patients should be considered (29) and, if proved to be true, would provide another explanation of development of impaired glucose tolerance and DM in HCV patients. Eibl et al. (5) reported the presence of GAD- Ab, IA2-Ab and islet cell antibodies [ICAs] which are considered a predictive and diagnostic markers for IDDM, in chronic hepatitis C patients. Chen et al. (18) reported that 34.6 % of Chinese patients with chronic hepatitis C had glucose intolerance. They added that chronic hepatitis C patients who were older in age, obese, had previous IFN treatment history or had family history of diabetes were prone to develop glucose intolerance.

Exciting new data are expanding our understanding of the mechanisms of steatogenesis in HCV infection and providing potential links between insulin resistance or hyperglycemic states and liver fibrogenesis (30). Also, Delgado et al. (31) reported that HCV is independently associated with increased insulin resistance (IR) after liver transplantation. These findings provide a possible pathogenetic basis for the association of DM with HCV. Also, Knobler and Schattner (32) reported that even non-diabetic HCV patients have insulin resistance, specific defects in the insulin-signaling pathway and elevated intrahepatic tumor necrosis factor (TNF-alpha) mRNA. TNF-alpha is known to cause insulin resistance, with similar defects in the insulin-signaling pathway. They concluded that TNF-alpha might be the link between HCV infection and diabetes, suggesting an additional mechanism of diabetes with important implications for prognosis and therapy.

On the contrary, Hoofnagle (33) failed to show any increase in the prevalence of GAD antibodies in chronic HCV-infected patients before interferon therapy. They suggested that HCV is not involved in the pathogenesis of DM. Mangia et al. (34) reported that the prevalence of DM was not different among patients with HCV, HBV infection, or alcohol abuse. Piquer et al. (35) suggested that beta cell autoimmunity is not associated with HCV.. Also, Betterle et al. (17) reported no association between the occurrence of organ- specific/non organ – specific autoimmune diseases and HCV infection. In addition, Okan et al. (36) disagreed that HCV infection might act as a trigger for DM, and they stated that diabetes should not be listed among the extrahepatic manifestations of this infection.

Liver cirrhosis, especially in its advanced stages, was described to be implicated in the etiology of diabetes probably due to development of peripheral insulin resistance and reduced number of insulin receptors (14). They added that cirrhosis appears to be a more important predictor of glucose intolerance than HCV infection, and the combination of both factors increases the risk of DM.

In the present study according to US findings before IFN therapy, patients were classified into 3 groups; mild, moderate and severe. Moderate and severe groups in both diabetic and non diabetic groups showed significant difference in serum GAD-Ab ($p<0.05$) (mean values were higher in moderate compared to severe group). No significant difference was detected in mean values of IA2-Ab in both HCV diabetics and non diabetics comparing the three groups. These results suggest that other factors as the virus itself and not the severity of liver affection detected by the US determine the incidence of diabetes in HCV infection. hese findings are supported by Allison et al. (37) reported that the severity of cirrhosis, therapy, sex and body mass index were not significantly associated. Knobler et al. (38) reported that prevalence of DM in HCV infection is independent on cirrhosis. Yazicioglu et al. (39) suggested that insulin resistance present in chronic hepatitis C patients is not directly related to hepatic injury, moreover, it may be associated with some component(s) inherent to hepatitis C virus. Knobler and Schattner (32) reported that patients with chronic hepatitis C virus (HCV) infection have a significantly increased prevalence of DM compared to controls or HBV-infected patients, independent of the presence of cirrhosis.

Induction or augmentation of autoimmunity during the treatment of chronic hepatitis C with interferon alpha is a well known phenomenon and a matter of great concern to physicians involved in the field of viral hepatitis. These autoimmune effects can be globally divided into appearance or increase in titers of autoantibodies and/or manifestation of overt autoimmune pathologies. Whereas the former may concern more than 50% of treated subjects, the latter is reported in only 1-2% of patients under therapy (40). Among the less common side effects of IFN therapy; it can lead to the develop-

ment of insulin-dependent diabetes mellitus (5,40). Chen et al. (18) confirmed interferon treatment to be an independent risk factor to develop glucose intolerance. Devendra and Eisenbarth (7) reported that IFN-alpha therapy is associated with autoimmune diseases and that elevated serum IFN-alpha levels have been associated with type 1 diabetes.

Sometimes, during, as well as after IFN treatment, the appearance of anti-islet cell antibodies has been shown, but its interrelationship with the development of disease is uncertain (41) However, clinically overt immune-mediated diseases are rare. Repetitive treatment seems to facilitate this complication (19). Betterle et al. (17) suggested that treatment with IFN-α might amplify an already existing autoimmune response against β-cells. However, they could not confirm that the HCV infection can induce an increase in the frequency of pancreatic autoimmunity. Wasmuth et al. (42) reported that different diabetes related autoantibodies can be induced during IFN therapy for chronic HCV infection. However, they proposed that only those patients with more than one autoantibody are at a considerable risk of progressing to clinically overt disease. They concluded that if one autoantibody appears during antiviral therapy, follow up should include screening for all other diabetes related autoantibodies.

Actual concepts concerning the pathogenesis of IFN-associated autoimmunity include induction of major histocompatibility complex (MHC) and other molecules as well as the modulation of lymphocyte functions. Another possible explanation is that cytokines might act as mediators of immune-endocrine regulation circuits that can interfere with the endocrine system on all levels of the hypotalamic-pituitary-adrenal axis (43, 44)

On the contrary, Tai et al. (45) reported that no case developed autoantibodies during the treatment in patients who were successfully treated with IFN-α, they added that insulin sensitivity improved and their plasma glucose stayed at the same level without secreting as much insulin from islet beta-cells (45). Also, Piquer et al. (35) reported that interferon treatment induces a transient increase in thyroid autoantibodies but does not influence the appearance of beta-cell autoantibodies.

Concerning US findings after IFN therapy in non diabetic group, the patients were classified into 4 groups; normal, mild, moderate and severe. Significant difference in serum GAD-Ab was observed between moderate and severe groups (mean values were higher in moderate compared to severe group) ($p<0.05$). No significant difference was detected in mean values of serum IA2-Ab in HCV non diabetics. These results show that IFN therapy improves the liver changes as 2 patients out of 5 of the mild group (detected before therapy) changed into normal regardless the development of DM. No significant correlations were detected btween liver US findings (severity of the disease) and both GAD and IA2-Ab in both HCV diabetics and non diabetics before and after IFN therapy ($p>0.05$).

In conclusion, HCV may predispose to the development of diabetes mellitus, as detected by the increased levels of GAD and anti IA2 antibodies. HCV patients become more prone to develop diabetes mellitus after interferon therapy. Thus, GAD and anti IA2 antibodies assessment is very important to detect patients at risk of developing DM before and after interferon therapy. In addition, it seems most probable that the severity of cirrhosis, as detected by US, is not associated with the predisposition to diabetes.

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