Serum levels of cytokines (TNF-α, IFN-γ& IL-10) in Type-2 diabetic patients with HCV infection

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Summary:

Back ground: Type 2 Diabetes mellitus (T2D) is a common complication of all liver diseases. However clinical and experimental data suggest a direct role of HCV in the perturbation of glucose metabolism. The aim of this study is to investigate the role of HCV infection as a risk factor to develop type 2 diabetes mellitus, and to study the immunopathogenicity of HCV in diabetes mellitus patients, through the assessment of IFN-γ, TNF- α and IL-10 serum levels.

Objectives: Is to investigate the role of HCV infection as a risk factor to develop type 2 diabetes mellitus, and to study the immunopathogenecity of HCV in diabetes mellitus patients, through the assessment of IFN-γ, TNF- α and IL-10 serum levels.

Patients and Methods: Thirty six known T2D patients attending the endocrine and diabetes center in Baghdad for check-up were enrolled in this study. Their age ranged from 15- 70 year. Twenty one patients have T2D only while the other Fifteen were have diabetes with HCV infection. Thirteen healthy individuals without any signs or symptoms of disease were also included in this study as healthy controls**.** Serum levels of cytokines including IFN- γ, TNF-α and IL-10 were analyzed by ELISA immunoassay.

Results: Higher serum levels of IFN-γ and TNF-α were observed in the investigated HCV patients with diabetes (43.87pg/ml, 68.1pg/ml respectively) compared to T2D patients (19.75 and 55.10 pg/ml respectively) and controls (8.08 and 31.4 pg/ml respectively). The statistical analysis revealed a significant difference between these three groups (P= 0.01). The mean serum levels of IL-10 were significantly elevated in T2D group as compared to HCV patients with T2D and control groups (28.7, 10.32, 15.78 pg/ml respectively, p=0.05).

Conclusion: The over production of proinflammatory cytokines (IFN-γ, TNF- α) and the low IL-10 level could play a crucial role in pathogenesis of HCV that leads to T2D via increasing the insulin resistance.

Key words: T2D, HCV, IFN-γ, TNF-α, IL-10.

Introduction:

Hepatitis C virus (HCV) infection and type 2 diabetes (T2D) are two common disorders with a high impact on health worldwide. Hepatitis C virus mainly affects the liver, but also several tissues outside the liver have been reported to be involved, resulting in a wide spectrum of extrahepatic manifestations (1).

The most frequent and clinically important endocrine disease- associated HCV are thyroid disorders and type 2 diabetes mellitus (2). A link between hepatitis C virus infection and development of diabetes mellitus remains controversial. Most controlled studies have suggested a significant association, the proportion of HCV- positive persons among diabetics being two- to seven- folds compared to controls (3, 4). Seropositivity rates of HbsAg and anti- HCV were 5.1% and 3.2% respectively in diabetic patients, and were 3.8% and 1.3% respectively in control group respectively (5). Mason *et al.,* 1999 found that the prevalence of HCV antibodies in T2D population ranges between 1.78 and 12.1% (6).The same authors found that 52% of persons with both HCV and T2D had risk factors for HCV infection before the onset of diabetes, whereas none had risk factors for HCV infection after the onset of diabetes. In other study, the prevalence rate of HCV infection was 11% among Nigerian type 2 diabetic patients (7).

Cross sectional and case- control studies support a role of hepatitis C as a factor implies in the development of T2D in high risk patients (male patients, older than 40 years and overweight) (8). The high prevalence of both diabetes and impaired fasting glucose (IFG) found in HCV- infected patients suggests that they should be considered a high risk group for diabetes development (9).

Cytokines such as tumor necrosis factor (TNF)-α and interleukin (IL-6) have been related with inflammation. Several reports have shown an increase in serum levels of TNF- α and its receptors in addition to IL-6 and IL-10 levels in HCV-infected patients (10, 11).

Subjects, Materials and Methods:

Our study was carried out according to the ethical standards for human experimentation. After explaining the aim of the study and the possible need

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for blood tests, written informed consent were obtained and ethical clearance granted. The subjects included in the research project were 36 known type 2 diabetic patients attending the endocrine and diabetes center in Baghdad for check-up from January to May 2009. Their age ranged from 15- 70 years. Twenty one patients have diabetes type 2 only while the other fifteen were have diabetes with HCV infection. Oral communication with those patients in the later group showed that about seven of them were experienced HCV before have diabetes. Thirteen healthy individuals without any signs or symptoms of disease were also included in this study as healthy controls.

Sample collection: Five ml of venous blood was collected from each patient and healthy individual. The blood was allowed to clot, and aliquot of sera samples were dispensed into tubes and stored at -20ċ prior use.

Methods of Assay:

The diagnostic rapid test kit was used to analyze the samples for HCV antibodies. This is a rapid chromatographic immunoassay for the qualitative detection of antibody to HCV in serum (Acon laboratories, USA) .

Circulating plasma levels of IFN-γ, TNF-α**,** and IL-10 were measured by sandwich ELISA using commercial kits according to the manufacturers instructions. These Kits includes:

1. Human IFN-γ Bioassay ELISA Kit: (USA Biological)
2. Human Necrotic Factor Alpha TNF-α Bioassay ELISA Kit: (USA Biological)
3. Human IL-10 Bioassay ELISA Kit: (USA Biological)

Statistical Analysis:

Student t-test was used to measure the differences between two means. The results were expressed as means ± standard error (SE), using MiniTab program.

Results:

The mean serum levels of IFN-γ was observed in the investigated HCV patients with diabetes who showed higher means (43.87pg/ml) than Type 2 Diabetic patients (19.75pg/ml) and controls (8.08pg/ml) The statistical analysis revealed a significant difference between these three groups (P= 0.001, 0.01 and 0.000 respectively) table (1).

The estimated levels of TNF-α in the sera of the HCV patients with diabetes were higher than Type 2 Diabetic group (68.1 *vs.* 55.1 pg/ml respectively), but these values is not significant (P= 0.306), but when compared with the values of control group (31.4 pg/ml) they were significantly higher (P= 0.002) table (1).

Table (1) demonstrated also the mean serum levels of IL-10 in the studied groups. The mean value of serum IL-10 for Type 2 Diabetic patients group was significantly higher than HCV patients with diabetes (28.7 *vs.* 10.32 pg/ml respectively, P= 0.006). Type 2 Diabetic patients showed also significant elevation in IL-10 serum levels compared with controls (15.78 pg/ml) (P=0.047). A statistically difference of mean IL-10 concentration appears between HCV patients with diabetes and control groups (P= 0.024).

Table-1: Mean serum levels of hIL-10, hTNF-α, and hIFN-γ in Type 2 diabetic patients, HCV patients with diabetes and healthy control groups

	Type 2 Diabetic patients		HCV patients with Diabetes		Healthy Controls	
Parameters	Mean	SE	Mean	SE	Mean	SE
IFN-γ (pg/ml)	19.75	3.79	43.87	5.34	8.08	1.2
TNF-α (pg/ml)	55.10	6.1	68.1	10.8	31.4	3.1
IL-10 (pg/ml)	28.7	5.9	10.32	1.2	15.78	1.9

Discussion:

Although HCV is hepatotropic virus it has also been identified in extrahepatic tissues including kidney, lung, testis, peripheral blood mononuclear cells, and also in the pancreas (12). The present data demonstrated that the serum levels of TNF- α and IFN-γ were higher in HCV patients with diabetes. Several reports have shown an increase in serum levels of TNF- α in HCV patients with diabetes (13). Two main hypothesis are considered to explain the mechanism of this disorder, primary cytopathic effect of the virus and secondary induced autoimmunity (14). Many studies suggested that a possible immunological TH1 pattern could be the pathophysiological base of the association of HCV infection with T2D. Activation of TNF- α system in HCV- infected patients could be related to the immune response that is characteristically mediated by the TH1 cells, (15). These lymphocytes secrets IFN-γ as the predominant cytokines which is able to enhance the production of TNF- α and its two receptors (TNFR1 and TNFR2) by macrophages including those infiltrating the liver from systemic circulation and the Kupffer cells, (16). HCV infection of pancreatic beta cells may act by upregulating CXCL10 secretion in these cells that is responsible for TH1 lymphocyte recruitment. TH1 response leads to increase IFN-γ and TNF- α production that in turn stimulates CXCL10 secretion by the target cells, thus perpetuating the immune cascade, and may lead to the appearance of T2D in genetically predisposing subjects (2). In fact, in chronic hepatitis C patients, an increased intrahepatic TNF- α response, which results in insulin resistance (IR) and a high risk of developing T2D has been described, (13, 10). Hepatic fibrosis and inflammation appear to play key roles in the increase the insulin resistance in patients with chronic HCV infection (17).Interactions between the HCV core protein and intracellular lipid metabolism pathways as well as induction of insulin resistance are the suspected molecular mechanisms (18). Therefore, it seems that insulin resistance mediated by proinflammatory cytokines, but not a deficit in insulin secretion, is the main pathogenic mechanism involved in the pathogenesis of diabetes associated with HCV infection, (19, 20).

The results of this study indicated a high level of serum IL-10 in T2D patients as compared to the HCV patients with diabetes. On their study, Van Exel *et al.,* 2002 found that low IL-10 production capacity was reported to be associated with the development of metabolic syndrome and T2D (21). Interestingly, IL-10 is characterized as a potent B-cell activator and enhances MHC class II expression on B- cells which in turn accelerates the disease progress (22). The anti- inflammatory cytokine IL-10, is known to exert a protective role in hepatic damage caused by viruses, alcohol and autoimmunity. Its main biological function seems to be the limitation and termination of inflammatory responses and the regulation of differentiation and proliferation of several immune cells (21).

Conclusion:

It seems that the over production of proinflammatory (IFN-γ) and inflammatory (TNF- α) cytokines in addition to low level of IL-10 could play a crucial role in pathogenesis of HCV that leads to T2D. Knowledge of the pathogenic mechanisms involved in diabetes association with HCV infection will enable us not only to further identity those patients at high risk of developing diabetes but also to select the best therapeutic option.

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