Evaluation of Serum Level of Tumor Necrosis Factor Receptor II in Hepatitis C Virus (Genotype 4)-Infected Middle-Aged Men With and Without Diabetes and Its Complications in Egypt: A Pilot Study

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Background: Tumor necrosis factor α (TNF-α) is a type of cytokine produced by macrophages and other cell types in response to various stimuli. Many studies have shown that TNF-α is involved in the development of diabetes. It also has a pivotal role in the inflammatory process of chronic hepatitis C.

Objectives: This study aimed to examine the hypothesis that TNF is increased in patients infected with hepatitis C virus (HCV) and with diabetes rather than in patients infected with HCV or with diabetes alone.

Methods: Patients were divided into 5 groups: patients with diabetes without complications and without HCV infection (group 1), patients with diabetes and complications but without HCV infection (group 2), patients without diabetes but with HCV infection (group 3), patients with diabetes without complications but with HCV infection (group 4), and patients with diabetes and complications and with HCV infection (group 5).

Results: Results revealed an activation of the TNF axis in all tested patients when compared with the level of healthy Egyptians done in previous studies. However, although there was a gradual escalation in the activation of the TNF axis in these groups, the increase did not amount to a statistical difference between them ($P > 0.05$). However, the trend was toward the higher values in HCV infection with diabetes and its complications. The number of studied patients may be a limitation of this research. There was no correlation between the level of TNF receptor II and the levels of transaminases, albumin, and creatinine in the different groups or the degree of microalbuminuria in the groups of patients with diabetic complications. Also, there was no relation between the hepatic or splenic size and the level of TNF receptor II.

Conclusions: The presence of diabetes and its complications in patients with HCV infection could not be attributed only to the activation of the TNF system at least in Egyptian patients.

Key Words: TNF, diabetes mellitus, HCV, inflammation, hepatitis

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Tumor necrosis factor α (TNF-α) is a type of cytokine produced by macrophages and other cell types in response to various stimuli. Many studies have shown that TNF-α is involved in the development of diabetes because it inhibits insulin signaling activity,[1] inhibits insulin-stimulated glucose uptake,[2] decreases glucose uptake by the muscles, and decreases insulin-induced suppression of hepatic glucose production, and it may be also directly deleterious to pancreatic β cells.[3] Tumor necrosis factor α can induce proinflammatory cytokines and adhesion molecules and thereby increase monocyte-endothelial cell adhesion, which is now recognized as an early and rate-limiting step in the development of diabetic vascular complications.[4] Activation of the TNF-α system has a pivotal role in the inflammatory process of chronic hepatitis C. The number of TNF-α–producing cells and both types of soluble TNF receptor are increased in the liver in close correlation with markers of hepatocellular injury and the degree of histologic inflammation.[5] Data show that HCV infection is a significant risk factor for the development of type 2 diabetes. Tumor necrosis factor may be the link between HCV infection and diabetes.[6] Egypt has the highest HCV prevalence worldwide; 15% of the general population is infected with HCV.[7] This makes the Egyptian population prone to the combined effect and complications of both diseases. The aim of this work was to examine the following hypothesis: TNF is responsible for the development of diabetes in patients with hepatitis C or the development of diabetic complications. The level of soluble TNF receptor II (TNFRII) as a marker of TNF activation will be assessed in HCV-infected patients with and without diabetes and diabetic complications.

PATIENTS

The study was approved by the institutional ethical committee. A total of 88 patients were included in this study. They were recruited from the male outpatient clinic. Our patients' ages ranged from 40 to 60 years. We excluded patients with a body mass index greater than 25 kg/m^2, with hepatitis B virus infection, and with other medical diseases such as renal, cardiac, pulmonary diseases, advanced liver cell failure, hepatocellular carcinoma, or underlying infections that may alter TNF activity.

The patients were divided into 5 groups:

- Group 1: 18 patients with diabetes, without diabetic complications, and without HCV infection.
- Group 2: 16 patients with diabetes, with diabetic complications, but without HCV infection.
- Group 3: 18 patients without diabetes but with HCV infection.
- Group 4: 18 patients with diabetes, without diabetic complications, but with HCV infection.
- Group 5: 18 patients with diabetes, with diabetic complication, and with HCV infection.

All patients were subjected to a thorough medical examination including careful history and assessment of manifestations of liver cell failure; detailed ophthalmologic examination and neurologic assessment; abdominal ultrasound to detect ascites, hepatic, and splenic size to exclude the presence of malignancy;

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TABLE 1. Tested Variables in the 5 Different Groups

	Group 1	Group 2	Group 3	Group 4	Group 5
Age, y	51.5 ± 6.8	50.4 ± 8.2	49.5 ± 7.8	51.4 ± 8	49.4 ± 8.4
BMI, kg/m^2	23.5 ± 2.1	23.9 ± 1.1	21.7 ± 2.2	23.3 ± 1.5	23.2 ± 1.4
TNFRII, ng/mL	12.9 ± 1.6	14.4 ± 2.7	17.5 ± 2.1	18.8 ± 2.9	18.7 ± 2.9
AST, U/L	28.2 ± 1.8	34.8 ± 1.5	102.5 ± 8	80.3 ± 4.9	81.3 ± 6.2
ALT, U/L	31.4 ± 1.3	34.9 ± 1.1	88.3 ± 7.4	81.3 ± 6.2	80.3 ± 4.9
Albumin, g/dL	4.3 ± 0.1	3.9 ± 0.1	2.8 ± 0.2	3.2 ± 0.1	2.9 ± 0.1
Creatinine, mg/dL	1.1 ± 0.04	1.3 ± 0.1	1.1 ± 0.04	1.1 ± 0.1	1.2 ± 0.1
FBG, mg/dL	164.9 ± 8.8	154.7 ± 7.6	82.4 ± 2.6	106.9 ± 8.2	172.6 ± 8.8
PPBG, mg/dL	273.6 ± 16.8	287.4 ± 16.9	118.2 ± 3.3	229 ± 16.6	333.7 ± 16.6
Alb/CRE, mg/mmol	24.6 ± 0.9	*110.8 ± 11.8*	*23.8 ± 0.8*	*26.4 ± 0.5*	*130.8 ± 0.9*
PC, %	*91.9 ± 1.4*	*87.2 ± 1*	*66 ± 2.3*	*67.1 ± 2*	*61.9 ± 2*
Total bilirubin	*0.9 ± 1.4*	*0.9 ± 0.01*	*1.3 ± 0.1*	*1.2 ± 0.1*	*1.96 ± 0.1*
Splenomegaly, %	*0*	*0*	*100*	*94.4*	*100*
Hepatomegaly, %	*0*	*0*	*1*	*3*	*0*
Shrunken liver, %	*0*	*0*	*94*	*83*	*100*

Values are presented as mean ± SEM, unless indicated otherwise.

ALT indicates alanine transaminase; Alb/CRE, albumin/creatinine ratio; AST, aspartate transaminase; BMI, body mass index; FBG, fasting blood glucose; PC, prothrombin concentration; PPBS, postprandial blood glucose; TNFRII, TNF receptor II.

routine chest radiograph; and urine and stool analysis to exclude any infection in other systems.

METHODS

Six milliliters of venous blood was collected from each patient after an overnight fast. Three milliliters was evacuated into sterile plain tubes, and after complete clotting, serum was separated by centrifuging the tubes at 1500*g* for 20 minutes. Prothrombin time was examined using a coagulometer supplied by diagnostic StagoST2 (SC2; Stago ST2, Junior Instrument Stago Group, Gennevillers, Frances). Part of the serum was used for the measurement of transaminases (alanine transaminase, aspartate transaminase), serum albumin, and serum creatinine, in addition to the measurement of fasting glucose level using Auto analyzer (Synchron ALX) supplied by Beckman Coulter (Abbott Laboratories, Abbott Park, IL). Another part of the serum was used for in vitro quantitative determination of TNF receptor II. Serum samples were stored at 70-C till assayed using ELISA supplied by Invitrogen Biosource (KAC1771; Camarillo, CA). Soluble TNF receptor II was chosen rather than TNF-α itself because it is less liable to fluctuation, it has a longer half-life, and it is not affected by many other factors. In the meantime, it is a reliable indicator of TNF-α system activation.[1] Microalbuminuria was assessed by immunoassay.

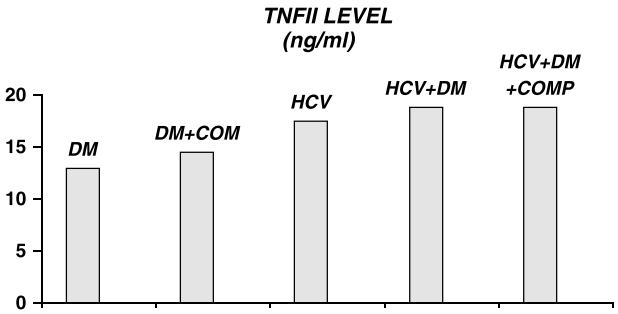

FIGURE 1. Level of TNFRII in the different groups.

STATISTICS

Statistical analysis was performed using the Statistical Package for the Social Sciences (version 12.0; SPSS, Inc, Chicago, IL). Results were expressed as mean and SEM. The Mann-Whitney test and the Kruskal-Wallis variance analysis were used to compare group results. Correlation analysis was performed using the Spearman test. Differences and correlation were considered significant at $P < 0.05$.

RESULTS

Table 1 shows the mean and SEM of different variables in different groups. Results showed activation of the TNF axis in the 5 groups in general (Fig. 1) when compared to the level in healthy Egyptians done in previous studies (1.27 ± 1.1 ng/mL).[8] However, although there was a gradual escalation in the activation

TABLE 2. Correlation Between TNFRII and Different Parameters in the Different Groups

TNFRII		AST	ALT	Albumin	Creatinine
Group 1	*R*	−0.009	0.070	−0.133	0.257
	P	0.971	0.781	0.656	0.303
Group 2	*R*	−0.382	0.066	0.006	−0.136
	P	0.144	0.808	0.983	0.617
Group 3	*R*	0.129	0.152	−0.268	0.005
	P	0.610	0.547	0.282	0.985
Group 4	*R*	0.237	0.067	−0.133	−0.310
	P	0.345	0.792	0.656	0.210
Group 5	*R*	−0.071	0.235	−0.118	−0.310
	P	0.780	0.349	0.641	0.211

R indicates the Spearman correlation value.

of the TNF axis in the groups, the increase did not amount to a statistical difference between them.

There was no correlation between the level of TNFRII and the levels of transaminases, albumin, creatinine in the different groups or the degree of microalbuminuria in the groups of patients with diabetic complications as shown in Table 2.

Also, there was no relation between the hepatic or splenic size and the level of TNFRII.

DISCUSSION

Hepatitis C virus is a common cause of hepatocellular injury that is associated with complex and vigorous immunologic mechanisms.[9] Inflammatory cytokines, including TNF, are an integral part of inflammation in chronic HCV infection. Serum level of TNF, both its soluble receptors, as well as TNF-producing cells and the expression of TNF and soluble TNF were increased in HCV-infected patients.[10,11] Tumor necrosis factor α has a pivotal role in both hepatitis C and diabetes. The higher incidence of insulin resistance and diabetes with HCV infection is becoming an increasing problem. This observation was confirmed in a large retrospective study, in a case-control study, and in a population-based study.[12,13] This constitutes an important issue because Egypt has the highest HCV prevalence worldwide; 15% of the general population is infected, and the HCV is the leading cause of HCC and chronic liver disease in the country.[7] This makes the Egyptian population prone to the combined effect and complications of both diseases. At the same time, TNF-α has been incriminated in the development of diabetic complications because it increases monocyte–endothelial cell adhesion, which is now recognized as an early and rate-limiting step in the development of diabetic vascular complications.[4] In 2005, Knobler and Schattner[6] reported that diabetic patients with HCV infection have significantly higher levels of sTNFRs compared to nondiabetic patients with HCV infection and controls. This was not in full agreement with our results, which failed to show such significant difference between the diabetic and nondiabetic patients with HCV infection. Their study was conducted on Jewish patients in Israel, where the most prevalent genotype type is 1b, not genotype 4 like ours. Therefore, the differences in race, ethnic group, and genotype may account for the differences noted. Nelson et al.[10] reported that patients with long-term HCV infection had higher levels of circulating TNF-α and its soluble receptors compared to the controls. However, in their study, these levels correlated to the degree of alanine transaminase elevation as a marker of hepatic injury, which does not agree with our results. In partial agreement with our results were those of El-Sammak et al.,[14] in 2005, who reported significantly higher levels of TNF in patients with HCV infection HCV and those with HCV infection and diabetes compared with patients with diabetes alone and control subjects, without any significant difference between patients with HCV infection and those with HCV infection and diabetes. El-Zayadi et al.[15] had demonstrated a significant difference between HCV-infected individuals and healthy blood donors within the Egyptian groups. They suggested that the inherited genetic variability might contribute, in part, to the difference between individuals in response to HCV infection. Our results showed a rise in the serum level of TNFRII in groups with diabetic complications when compared with the level of the healthy population, but no significant difference was detected between patients with diabetes with and without complications; it did not correlate to the degree of albuminuria. Concerning TNF axis activation in diabetic nephropathy, some studies showed that there is a significant correlation between TNF and degree of microalbuminuria.[16,17] The explanation to these different results could be attributed to the different ethnic groups studied, the genotypes, and the small number of examined population. In diabetic retinopathy, some studies showed a significant difference in TNF level between patients with diabetes with retinopathy and healthy controls.[18–21] This matches our results that show a significant difference in TNFRII level between the patients with diabetes and the healthy population. The small population studied may be a limitation of this study. It is important to conduct more prospective studies on larger groups of patients to find out if TNF plays a significant role in developing microvascular or macrovascular diabetic complications (any or all of them) and if blocking the TNF system may ameliorate or abolish these complications. The causal relationship between diabetes and HCV infection is a dilemma. It is important to try to find out answers for the conflicting and urgent questions we are facing. We believe that the small number of patients studied in each group and the dependence on the historic control group may be a limitation of our study.

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