Turk J Med Sci
33 (2003) 381-386

CLINICAL INVESTIGATION

The Serum Levels of IL-1β, IL-6, IL-8 and TNF-α in Nonalcoholic Fatty Liver

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Received: February 24, 2003

Abstract: Nonalcoholic fatty liver (NAFL) has an extensive clinical spectrum ranging from simple fatty liver to steatohepatitis and cirrhosis. Its pathogenesis is multifactorial. The aim of our study was to measure the serum levels of some cytokines (IL-1β, IL-6, IL-8 and TNF-α), which are thought to play a role in the pathogenesis of NAFL. Thirty subjects (18 males, 12 females) with clinical and laboratory features of hepatosteatosis were enrolled in the study as the patient group (group 1) and 30 healthy subjects (21 males, 9 females) served as controls (group 2). The levels of hepatic transaminases, gamma-glutamyltransferase, alkaline phosphatase, total cholesterol, IL-1β, IL-6, IL-8 and TNF-α were studied in both patients and controls. Serum IL-1β, IL-6, and TNF-α levels did not display significant differences between the patients and the controls ($P > 0.05$). On the other hand, the IL-8 level was significantly elevated in the patient group ($P < 0.05$). This increased level may play a more active role in the pathogenesis of fatty liver than IL-1β, IL-6 and TNF-α. Further studies are needed to elucidate to what extent the proinflammatory cytokines (especially IL-8 and TNF-α) are involved in the pathogenesis of NAFL.

Key Words: Nonalcoholic fatty liver, IL-1β, IL-6, IL-8, TNF-α

Introduction

Nonalcoholic fatty liver (NAFL) has an extensive clinical spectrum ranging from asymptomatic fatty liver to nonalcoholic steatohepatitis (NASH) and cirrhosis (1,2). In contrast to the benign course of NAFL, NASH may progress to cirrhosis and result in liver-related death in 10–25% of patients. The prevalence of NAFL is approximately 5% in the general population (3).

The histopathological changes in NAFL closely resemble those of alcoholic liver disease (1,4). NAFL commonly coexists with obesity, type II diabetes mellitus, hyperlipidemia, jejunoileal bypass surgery, drugs or toxins (1).

The pathogenesis of NAFL is multifactorial in origin. Oxidative stress, lipid peroxidation, amino acid imbalance, hyperglycemia, hyperinsulinemia, imbalance between ketogenic and anti-ketogenic hormones in portal blood, endotoxemia and cytokine upregulation due to endotoxemia are proposed factors in the pathogenesis of the disease (4,5).

Tumor necrosis factor-α (TNF-α) is an important cytokine in the development of various liver diseases. TNF-α recruits inflammatory cells and triggers the production of other cytokines, which initiate the healing process leading to fibrogenesis (6). The proinflammatory cytokines induced by TNF-α (IL-6, IL-8, etc.) are thought to be responsible for the pathogenesis of advanced liver diseases associated with alcohol (2,6).

The aim of this study was to investigate the serum level of some cytokines, namely IL-1β, IL-6, IL-8 and TNF-α, which are thought to play a significant role in the pathogenesis of NAFL.

Materials and Methods

Thirty patients with no history of alcohol intake (18 males, 12 females, mean age 50.2 ± 8) who demonstrated elevated serum aminotransferases lasting more than 3 months and with ultrasonographically documented hepatomegaly or steatosis or with histopathological evidence of hepatosetatosis in a liver biopsy were diagnosed as having NAFL (group 1). Other possible liver diseases were excluded with appropriate laboratory tests. Thirty healthy individuals (21 males, 9 females, mean age 46.8 ± 4) without any biochemical or ultrasonographical (US) abnormality comprised the control group (group 2). Individuals with a history of alcohol or drug use, small intestinal resection, total parenteral nutrition, diabetes mellitus or any other known liver disease or malignancies were excluded from the study.

Body mass index (BMI) was calculated for all patients and controls at admission. The normal BMI range was considered to be 18.5 ± 23.5 for males and 19.5 ± 24.5 for females, while overweightness was 23.5 ± 29.5 for males and 24.5 ± 29.5 for females, and obesity was over 29.5 both for males and females (7).

All patients and controls were investigated for the serum levels of aminotransferases, gamma-glutamyltranspeptidase (GGT), alkaline phosphatase (AP), total cholesterol and the cytokines IL-1β, IL-6, IL-8 and TNF-α. An abdominal US examination was performed in all patients and controls, and a liver biopsy was taken from 21 patients who gave informed consent for the procedure. Nine patients who refused to undergo a liver biopsy were diagnosed with NAFL on the basis of biochemical and ultrasonographical findings after excluding other liver diseases. The biopsy samples were evaluated by a single experienced pathologist. Serum cytokine levels were measured using commercial IL-1β, IL-6, IL-8 and TNF-α kits (Bio DPC, Los Angeles, USA) with a chemiluminescent immunometric method in Immulite 1000 hormone analyzer.

The results are expressed as mean ± standard error (mean ± SE). The Mann-Whitney U test was used to determine the differences between groups, and P values < 0.05 were considered significant.

Results

Mean ages and gender distributions were similar in the 2 groups. However, the mean BMI was higher in the patients than in the controls. In group 1, 17 cases presented with fatigue and 14 cases with right upper quadrant pain. Except for 1 patient, all subjects in group 1 were overweight; 10 were obese. Twelve individuals were overweight in group 2 (Table 1).

In the 21 patients in whom a liver biopsy was performed, the macrovesicular pattern of steatosis was observed in 7, mixed type in 11 and microvesicular in 3. Among those patients who were determined to have macrovesicular steatosis only 2 had intralobular inflammation.

Among the abnormalities revealed by liver function tests in the patient group, elevation of alanin aminotransferase (ALT) was observed in all cases (100%), aspartate aminotransferase (AST) in 22 (73%), GGT in 19 (63%), AP in 5 (16%) and high cholesterol in 13 (43%). The mean AST/ALT ratio was 0.5. The liver function tests were normal in all control subjects; however, 6 subjects displayed hypercholesterolemia (Table 1).

Serum cytokine levels showed a considerable variance in patients. IL-1β was elevated in 4 cases, IL-6 in 1, IL-8 in 9, and TNF-α in 12. Among the controls only 3 subjects displayed increased levels of TNF-α (Figure).

In the patient group, average serum levels of IL-1β, IL-6, IL-8 and TNF-α were 2.57, 4.29, 43.68 and 9.70 pg/ml, respectively. In the controls they were 1.29, 2.55, 9.51 and 6.59 pg/ml respectively. The levels of IL-1β, IL-6 and TNF-α did not show a significant difference between the groups ($P > 0.05$) whereas the IL-8 level was significantly increased in patients ($P < 0.05$) (Table 2). The cytokine levels in the 9 patients who did not undergo a liver biopsy did not differ from those of the patients who did undergo one ($P > 0.05$ for all).

Discussion

Hepatic steatosis is one of the most common forms of liver disease in industrialized countries. Hepatic steatosis itself is thought to be a relatively benign condition because it is seldom associated with liver-related morbidity or mortality (8). Many factors play a role in the etiology of NAFL. The disease occurs most commonly in

Table 1. Demographic, clinical and laboratory characteristics of subjects with NAFL.

Patient no.	Age (years)	Sex	BMI (kg/m^2)	ALT (U/l)	GGT (U/l)	ALP (U/l)	Cholesterol (mg/dl)	IL-1β (pg/ml)	IL-6 (pg/ml)	IL-8 (pg/ml)	TNF-α (pg/ml)
1	38	M	30.6	68	36	50	105	1.9	4	62.9	73.4
2	43	F	26.8	85	97	182	242	0.86	1.5	9.3	10.6
3	46	F	27.4	73	76	205	205	0.81	2.9	21.5	5.9
4	56	F	29	58	164	75	284	0.72	1.1	17.1	4.7
5	56	M	27.9	83	34	73	141	0.82	10.4	105	5.4
6	62	F	29.7	82	94	218	207	0.1	2.8	11.4	0.99
7	34	M	26.7	71	120	198	164	5	5	16.8	5.6
8	44	M	25.6	80	71	91	176	1	0.53	5.1	4.8
9	50	M	30.1	77	97	195	166	13.8	8.6	79	14.1
10	42	M	26.3	135	68	89	147	0.91	4	7.6	4.8
11	36	M	27.1	65	72	188	172	4	2.7	12.4	8.6
12	57	F	25.3	153	64	276	247	9.6	5	15.7	10.4
13	36	M	24.7	124	78	191	158	0.98	6.4	3	4.5
14	54	M	26.5	211	92	236	185	0.99	7.9	106	2.5
15	64	F	31.5	122	35	237	317	0.72	1.1	17.1	4.7
16	62	M	30.7	67	75	340	210	1.3	4	13.7	9.6
17	40	M	29.3	81	71	201	235	1.3	6.1	1	4
18	45	M	27.7	79	28	168	170	0.45	4	6	0.3
19	55	F	30	68	85	85	221	0.18	2.1	11.4	9.5
20	53	F	27.8	126	95	96	245	10.9	1.5	116	9.8
21	48	M	26.8	59	53	164	163	3.2	3.7	26.8	11.6
22	53	F	24.9	68	47	210	168	4.2	6.3	89.2	3.9
23	64	M	27.2	76	38	198	206	0.7	1.8	16.7	6.1
24	56	M	25.8	84	30	236	141	1.8	0.9	4.6	46.4
25	43	M	29.3	62	56	256	209	4.4	5.3	119.2	25.2
26	49	F	27.6	74	65	178	177	1.6	9.2	38.5	1.8
27	59	M	28	116	78	312	278	0.8	3.3	112	7.2
28	51	F	26.3	84	26	276	163	2.8	5.7	41.3	2.9
29	60	F	27.2	56	38	208	183	0.6	8.6	91.3	7.8
30	50	M	29.8	68	74	316	182	0.9	2.4	48.6	11.2

BMI: body-mass index, ALT: alanin aminotransferase (normal range); 0-41, GGT: gamma glutamyl transpeptidase (normal range); 0-49, AP: alkaline phosphatase (normal range); 0-270, cholesterol (normal range); 0-200. Cut-off value of cytokines: IL-1β; < 5 pg/ml, IL-6; < 9.7 pg/ml, IL-8; < 62 pg/ml, TNF-α; < 8.1 pg/ml

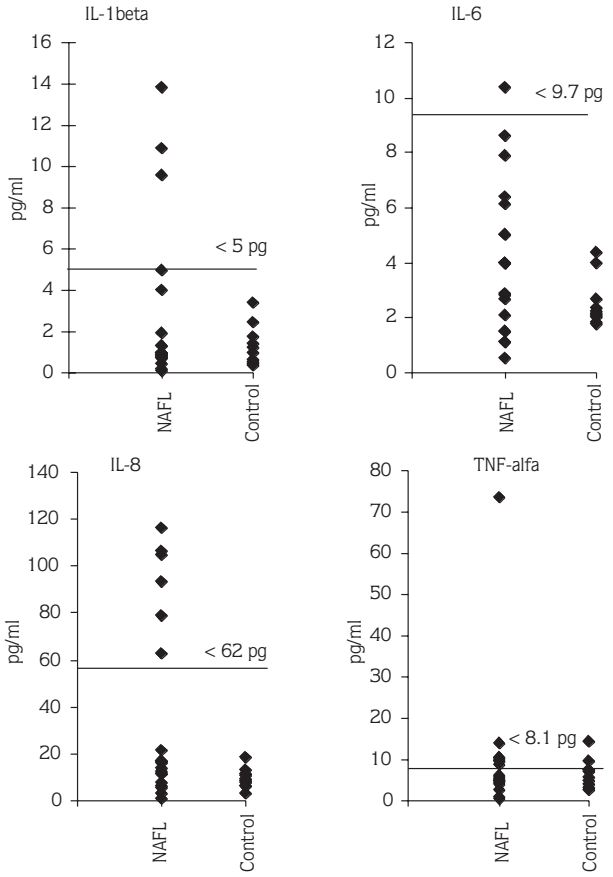

Figure. Serum cytokine values of NAFL and control groups.

obese middle aged women with diabetes. Obesity, rapid weight loss, total parenteral nutrition, jejunoileal bypass, Wilson's disease, drugs and toxic substances are among the most frequently encountered etiological factors (2,9,10).

The severity of steatosis is correlated with the severity of lipid peroxidation (5). Small intestinal bacterial overgrowth contributes to the development of NASH by increasing intestinal permeability and facilitating the absorption of endotoxins or other bacterial products from the intestinal lumen to the mesenteric circulation, thus increasing the formation of TNF-α and other proinflammatory cytokines (4,11,12). Extensive work by many groups has proven that LPS-liver injury is mediated by TNF-α in normal rats and mice (13).

Cytokines are pleotropical regulatory peptides synthesized by nucleated cells (6). Many hepatic cells, such as Kupffer cells, hepatocytes and stellate cells, can

Table 2. Serum cytokine values of NAFL and control groups.

	Group 1 (NAFL) (mean ± SE)	Group 2 (control) (mean ± SE)	P
IL-1β	2.57 ± 3.8	1.29 ± 0.1	0.552
IL-6	4.29 ± 2.6	2.55 ± 0.3	0.093
IL-8	43.68 ± 40.2	9.51 ± 1.2	*0.020
TNF-α	9.70 ± 15.34	6.59 ± 1.7	0.892

*P < 0.05

synthesize both proinflammatory (IL-1β, IL-1α, IL-12, IL-8, interferon-γ and TNF-α) and anti-inflammatory cytokines (IL-1 receptor antagonists, soluble TNF receptors p55 and 75, IL-4 and IL-10) (6,14). Cytokines cause necroinflammation in the liver by increasing the accumulation of fatty acids and inactivating the cytochrome P450 (15). In a healthy person, the proinflammatory cytokines and anti-inflammatory cytokines are in equilibrium. In progressive liver disease, this equilibrium is shifted towards the proinflammatory side (6).

TNF-α is a potent inhibitor of lipoprotein lipase (4,11). This cytokine conducts its effects through 2 specific receptors [TNFR1 (p55) and TNFR2 (p75)] on the cell surface (14). TNF-α kills cells by activating the caspases that cause apoptosis. However, healthy hepatocytes are normally not killed by TNF-α because when they are exposed to TNF-α they activate antiapoptotic transcription factors, such as nuclear factor-κB (NF-κB) (8). Increased levels of TNF-α also lead to an increase in the synthesis and accumulation of triglyceride in the liver by inhibiting lypolysis in the peripheral tissues. In addition, TNF-α accelerates the hepatic synthesis of other cytokines, enhances neutrophil chemotaxis and leads to a severe inflammatory response that results in hepatosteatosis and necrosis in the liver (4,6,14). Factors such as IL-12 and -18 and interferon (IFN) that enhance TNF-α activity generally exacerbate LPS liver injury, whereas those that inhibit TNF-α, such as IL-10, are hepatoprotective (13).

Interleukin 8 is a cytokine synthesized by hepatocytes, Kupffer cells and macrophages. TNF-α and IL-1 together with endotoxins increase IL-8 levels in the alcoholic liver. IL- 8 shows its effects by activating neutrophils. Serum IL-8 levels are markedly elevated in patients with alcoholic

hepatitis compared with those in patients with alcoholic cirrhosis, alcoholic fatty liver and nonalcoholic liver disease (16).

In our study, the mean TNF-α levels in NAFL cases without steatohepatitis were not significantly different from those of the control group. This finding may be due to the exclusion of cases in which certain risk factors such as diabetes and drug use were involved, or due to the absence of necroinflammatory activity (steatohepatitis or fibrosis) as seen in biopsy specimens. In contrast, previous studies in which elevated TNF-α levels were reported included patients with a disease at the steatohepatitis stage.

Several explanations concerning the correlation between NAFL and TNF-α were proposed in previous studies. TNF-α polymorphisms could represent a susceptibility genotype for insulin resistance, NAFL and steatohepatitis (17). TNF-α levels were found to be increased in patients with NAFL and accompanying bacterial overgrowth (18). In alcoholic steatohepatitis, serum concentrations of TNF-α and cytokines induced by TNF-α (IL-1β, IL-6 and IL-8) increase early in the course of the disease and then decrease during the convalescence period (6). Endotoxemia resulting from Gram-negative bacterial overgrowth in resting intestine or during total parenteral nutrition increases TNF-α production, and leads to hepatic damage and dysfunction (19). Yang et al. showed that in obese rats with severe steatosis both TNF-α and IFN-γ release increases hepatic damage (20).

There are some other studies with contradictory results. Jarvelainen et al. (21) showed that proinflammatory cytokines such as IL-1β and TNF-α did not increase in steatosis due to alcohol. Similarly Memon et al. (22) and Oliver et al. (5) showed that TNF-α was not a causative factor in hepatosteatosis in animal studies. Our results comply with those reported by Jarvelainen and Memon.

We did not encounter any previous study regarding the correlation between IL-1β, IL-6 and NAFL. However, an increased release of IL-6 in patients with alcoholic fatty liver was shown by Schafer et al. (23). We found that mean levels of serum IL-1β and IL-6 did not increase in NAFL cases.

In summary, in our NAFL cases, which were not at the steatohepatitis stage, serum mean IL-8 levels were higher than those in healthy individuals. IL-8 might play a more important role in the pathogenesis of liver steatosis than TNF-α, IL-1β and IL-6. Given the importance of proinflammatory cytokines in the pathogenesis of NAFL, therapeutic approaches directed to the inhibition of these factors may prove efficient.

Acknowledgment

This work was supported by the Yüzüncü Yıl University research foundation.

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