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Alpha fetoprotein levels and its relationship with histopathological findings in patients with non-alcoholic fatty liver disease

M. KARA[1], H. GENC[2], S. TAPAN[3], C. MERAL[4], C.N. ERCIN[2], M. ERDAL[5], T. DOGRU[2]

[1]Department of Gastroenterology, Etimesgut Military Hospital, Ankara, Turkey
[2]Department of Gastroenterology, Gulhane Medical School, Ankara, Turkey
[3]Department of Medical Biochemistry, Gulhane Medical School, Ankara, Turkey
[4]Department of Pediatrics, Haydarpasa Medical School, Istanbul, Turkey
[5]Department of Family Medicine, Etimesgut Military Hospital, Ankara, Turkey

Abstract. – BACKGROUND: Non alcoholic fatty liver disease (NAFLD) encompasses a wide spectrum of liver disorders ranging from simple steatosis (SS) to cirrhosis. In addition, increasing evidence indicates that hepatocellular carcinoma (HCC) may represent a late complication of NAFLD. Alpha-fetoprotein (AFP) serum levels can rise in adults with HCC.

AIM: In the present study, we aimed to investigate circulating AFP concentrations in subjects with histologically proven NAFLD. In addition, the relationship of AFP with liver histology was also searched.

PATIENTS AND METHODS: One hundred and three male NAFLD patients and 57 healthy male controls were enrolled in the study. In addition, patients with NAFLD grouped as nonalcoholic steatohepatitis (NASH) (n = 72) and SS (n = 31). AFP serum levels were measured in duplicate by the chemiluminescence's method.

RESULTS: Age and gender were similar in subjects with NAFLD and controls. AFP serum levels were not different between two groups. In subgroup analysis, AFP levels were also found to be similar in patients with NASH and SS. Moreover, no significant relationship was found between AFP and histopathological findings in patients with NAFLD.

CONCLUSIONS: The results of this preliminary study suggest that AFP is not involved in the pathogenesis of NAFLD.

Key Words:
Alpha-fetoprotein, Non-alcoholic fatty liver disease.

Introduction

Non alcoholic fatty liver disease (NAFLD) encompasses a wide spectrum of liver disorders characterized by histologic lesions typical of those in alcoholic liver disease, ranging from simple steatosis (SS) to cirrhosis[1]. The so-called nonalcoholic steatohepatitis (NASH) is a subtype of NAFLD in which steatosis is accompanied by hepatocytes ballooning and necrosis with or without Mallory hyaline and fibrosis[2]. Although NAFLD may remain asymptomatic and stationary for long periods, it also may insidiously progress to cirrhosis and end-stage liver disease[2,3]. Notably, there is evidence that a high percentage of patients who underwent liver transplantation for so-called cryptogenic cirrhosis probably had NAFLD[4]. In addition, increasing evidence indicates that hepatocellular carcinoma (HCC) may represent a late complication of NAFLD[5]. These data underlines the importance of screening for significant liver injury in patients with NAFLD and it will be an important medical challenge in the years to come because of the epidemics of obesity and diabetes mellitus. However, the inability of liver biopsy to meet this challenge makes the development of non-invasive, readily available, and easy to perform serum markers, a high priority.

Alpha-fetoprotein (AFP), a glycoprotein is normally excreted from the fetal liver and yolk sac. Circulating AFP level begins to fall before birth and is undetectable or low in healthy adults[6,7]. AFP serum levels can rise in adults with HCC or germ cell tumors. It is also found to be increased in acute or chronic viral hepatitis and various forms of chronic hepatic disorders[8]. Currently, AFP is widely used as a serum marker for diagnosing HCC, especially in patients with chronic liver disease[9].

Recently, AFP levels were reported to be elevated in patients with NAFLD who were diagnosed by ultrasonography[10]. On the other hand, to our best notice, AFP levels in biopsy proven NAFLD have not been studied in the relevant lit-

Corresponding Author: Muhammed Erdal, MD; e-mail: muhammederdal@yahoo.com

erature. So, in the present study, we aimed to investigate circulating AFP concentrations in subjects with histologically proven NAFLD. In addition, the relationship of AFP with liver histology was also searched.

Patients and Methods

Participants

One hundred and three male patients who were admitted to our Gastroenterology Clinic with the diagnosis of NAFLD were enrolled. Patients were included if they fulfilled all of the followings; elevated aminotransferase levels at least for 6 months, hyperechoic liver on ultrasound without any other liver or gall bladder pathology, presence of SS or NASH on liver biopsy. Exclusion criteria were ≥ 40 g/week alcohol intake, positive serum viral markers, autoimmune hepatitis, Celiac disease, abnormal copper and thyroid function tests, environmental hepatotoxins, and drug usage. Fifty-seven male volunteers who had been previously found to have normal liver function tests and ultrasonographic evaluation of the liver were taken as healthy controls.

All participants were informed about the study procedure and gave written consent to participate. The study protocol was approved by the Local Ethics Committee.

Biochemical Analysis

All blood samples were collected from an antecubital vein, between 08.00 and 09.00 a.m. after an overnight fasting. The samples were centrifuged for 15 minutes at 3000 rpm, aliquoted and immediately frozen at -80ºC for analyses until examination. All samples were run in the same assay.

Serum glucose, total cholesterol (TC), triglycerides (TG), high-density lipoprotein-cholesterol (HDL-C), lactate dehydrogenase (LDH), aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP) and gamma-glutamyl transpeptidase (GGT) levels were measured by the enzymatic colorimetric method with Olympus AU2700 auto analyzer using reagents from Olympus Diagnostics (Hamburg, Germany). Low density lipoprotein (LDL)-cholesterol level was calculated by Friedewald's formula[11]. Serum AFP levels and basal insulin levels were measured in duplicate by the chemiluminescence's method with Modular Analytics E170 auto analyzer using reagents from Roche Diagnostics (Mannheim, Germany).

Insulin resistance (IR) was calculated by homeostasis model assessment model (HOMA)[13] using the formula; HOMA-IR = Fasting insulin (□U/ml) × Fasting glucose (mg/dl)/405 and this index has been shown to be well correlated with the results of the euglycemic–hyperinsulinemic clamp method to determine insulin resistance[12]. Low HOMA-IR values indicate high insulin sensitivity, whereas high-HOMA-IR values indicate low insulin sensitivity.

Pathologic Examination

An experienced hepatopathologist blinded to subjects' details scored liver biopsy specimens using the classification of Kleiner et al[13]. Briefly, liver tissues were stained with hematoxylin–eosin, reticulin, and Gomori trichrome stains and scored. All cases showed macrovesicular steatosis affecting at least 5% of hepatocytes and these were classified as steatosis. In addition to steatosis, the minimum criteria for the diagnosis of steatohepatitis included the presence of lobular inflammation and either ballooning cells or perisinusoidal/pericellular fibrosis in zone 3 of the hepatic acinus.

Statistical Analysis

SPSS 15.0 (Chicago, IL, USA) was used for statistical analysis. Results are reported as the mean ± standard deviation (SD) and median (min-max). Kolmogorov-Smirnov test was used to determine the distribution characteristics of variables, and Levene's test was used to evaluate the equality of variance. Differences between groups were tested for significance by One Way ANOVA test, independent samples *t* test and Mann-Whitney U test, as appropriate. The relationship between variables was analyzed by Spearman's rho correlation. ROC (Receiver Operating Characteristic) curve was used for evaluating diagnostic value of AFP. Differences and correlations were considered significant at $p < 0.05$.

Results

Table I shows the characteristics and the laboratory data of the subjects with NAFLD and healthy controls. Age and gender were similar in two groups. Body mass index (BMI), glucose, TC, TG, ALT, AST, GGT and insulin levels, and HOMA-IR indexes were higher and HDL-C levels were lower in subjects with NAFLD than the control group. However, AFP serum levels were not different between two groups.

Table I. Comparison of some characteristics and laboratory findings between NAFLD and control group.

	NAFLD group (n = 103)	Control group (n = 57)	p
Age (year)	31.3 ± 5.8	30.2 ± 5.5	0.261†
BMI (kg/m^2)	28.3 ± 3.2	23.8 ± 2.5	< 0.001†
Glucose (mg/dl)	91 ±12	80 ± 9	< 0.001†
TC (mg/dl)	203 ± 43	178 ± 28	< 0.001†
TG (mg/dl)	201 ± 129	121 ± 54	< 0.001‡
HDL-C (mg/dl)	42 ± 7	45 ± 7	0.001†
LDL-C (mg/dl)	120 ± 37	108 ± 27	0.072†
ALT (IU/L)	101 ± 43	21 ± 9	< 0.001†
AST (IU/L)	48 ± 20	22 ± 5	<0.001†
GGT (IU/L)	71 ± 54	26 ± 17	< 0.001†
Insulin (mU/ml)	14.8 ± 9	7.5 ± 3.6	< 0.001‡
HOMA-IR	3.4 ± 2.3	1.5 ± 0.8	< 0.001†
AFP	1.99 ± 1.13	2.08 ± 1.16	0.536‡
Histology			
Fat score (1-3) (%)	55/28/17	–	–
Necroinflammation score (0-3) (%)	8/72/18/0	–	–
Fibrosis stage (0-4) (%)	35/57/6/2/0	–	–

Data is presented as the mean ± SD. NAFLD: non alcoholic fatty liver disease; BMI: body mass index; TC: total cholesterol; TG: triglyceride; HDL-C: high-density lipoprotein-cholesterol; LDL-C: low-density lipoprotein-cholesterol; ALT: alanine aminotransferase; AST: aspartate aminotransferase; GGT: gamma-glutamyl transpeptidase; HOMA-IR: homeostasis model assessment model-insulin resistance; AFP: Alpha-fetoprotein.†*t* test; ‡Mann Whitney U test.

Table II shows the characteristics and the laboratory data of the subjects with NASH, SS and controls. Age and gender were similar in three groups. BMI levels were higher in NASH and SS when compared to the controls. Glucose, TC, TG, ALT, AST, GGT and insulin levels, and HOMA-IR indexes were significantly higher in subjects with NASH and SS than in the control group. However, HDL-C levels in subjects with NASH and SS were significantly lower than in the control group. BMI, glucose, TC, TG, HDL-C, LDL-C, Insulin levels, and HOMA-IR indexes were not significantly different between NAFLD subgroups.

Table II. Comparison of some characteristics and laboratory findings between NAFLD subgroups and control.

	SS group (N = 31)	NASH group (N = 72)	Control group (N = 57)	p
Age (year)	31 ± 5.3	31.4 ± 5.9	30.2 ± 5.5	0.5
BMI (kg/m^2)	28.3 ± 3.5	28.3 ± 3.1	23.8 ± 2.5	< 0.05^{b,c}
Glucose (mg/dl)	88 ± 13	93 ± 12	80 ± 9	< 0.05^{b,c}
TC (mg/dl)	208± 45	201 ± 42	178 ± 28	< 0.05^{b,c}
TG (mg/dl)	136 ± 165	197 ± 126	121 ± 54	< 0.05^{b,c}
HDL-C (mg/dl)	42 ± 6	42 ± 7	46 ± 8	< 0.05^{b,c}
LDL-C (mg/dl)	124 ± 41	118 ± 36	109 ± 27	0.148
ALT (IU/L)	86 ± 36	109 ± 45	22 ± 10	< 0.05^{a,b,c}
AST (IU/L)	42 ± 12	52 ± 21	22 ± 5	< 0.05^{a,b,c}
GGT (IU/L)	95 ± 78	59 ± 33	26 ± 17	< 0.05^{a,b,c}
Insulin (mU/ml)	12.9 ± 7.6	15.9 ± 9.6	7.5 ± 3.6	< 0.05^{b,c}
HOMA-IR	2.8 ± 1.8	3.8 ± 2.6	1.5 ± 0.8	< 0.05^{b,c}
AFP	2.14 ± 1.35	1.93 ± 1.02	2.08 ± 1.16	0.618

Data is presented as the mean ± SD. SS: simple steatosis; NASH: nonalcoholic steatohepatitis; BMI: body mass index; TC: total cholesterol; TG: triglyceride; HDL-C: high-density lipoprotein-cholesterol; LDL-C: low-density lipoprotein-cholesterol; ALT: alanine aminotransferase; AST: aspartate aminotransferase; GGT: gamma-glutamyl transpeptidase; HOMA-IR: homeostasis model assessment model-insulin resistance; AFP: Alpha-fetoprotein. [a]There is significant difference between SS and NASH groups; [b]There is significant difference between Control and NASH groups; [c]There is significant difference between Control and SS groups.

ALT, AST, and GGT levels in NASH group were higher than in SS group. Serum AFP levels were found to be similar in all groups.

We also analyzed the association of AFP with histopathological findings in subjects with NAFLD. No significant relationship was found between these parameters. Moreover, AFP either did not discriminate NAFLD patients from controls, or NASH patients from SS patients. Hence, in the ROC analysis, area under the curve (AUROC) is just 0.530 and 0.537 respectively (Figures 1 and 2).

Discussion

Although the majority of subjects with NAFLD do not develop complications, one third of the patients may develop serious liver sequelae, including end-stage liver disease and HCC. Those at highest risk include patients with significant hepatic necro-inflammation and fibrosis[14,15]. Therefore, liver biopsy had been recommended for confirming diagnosis and for providing prognostic information in this condition[16]. However, there are several drawbacks in using liver biopsy for this purpose. This procedure is invasive, costly, and prone to complications, some minor, such as pain, others more severe with a reported risk of death of 0.01%[17]. More importantly, the number of subjects at risk for NAFLD in the general population is high enough that liver biopsy is not a practical and efficient tool for identifying those at risk of advanced fibrosis. Because liver biopsy is impossible to perform in such large cohorts of individuals, some investigators have tried to identify simple non-invasive markers of liver injury, in particular fibrosis, in subjects with NAFLD. Different studies have shown that an age of older than 45 years, obesity, type 2 diabetes, high levels of ALT and TG, insulin resistance, systemic hypertension, and high level of C-peptide are associated with advanced fibrosis in patients with NASH[18,19]. On the other hand, imaging techniques have moderate predictive values for advanced steatosis but not for bridging fibrosis[20].

Very recently, Babali et al., reported the association of AFP with NAFLD[10]. In this study, serum AFP levels were found to be higher in patients with NAFLD when compared to those of healthy subjects. They have also classified NAFLD patients according to their ultrasonography scores as described by Hamaguchi et al[20]; and further observed that subjects with grade 3 steatosis had higher AFP levels when compared with grade 1 and 2 steatosis. Since serum AFP testing is quite inexpensive and convenient, their results are clinically promising. In the present study, we investigated AFP serum levels in a large population of biopsy proven NAFLD patients and healthy controls. There was no difference between two groups regarding the AFP concentrations. In addition, AFP levels were not dif-

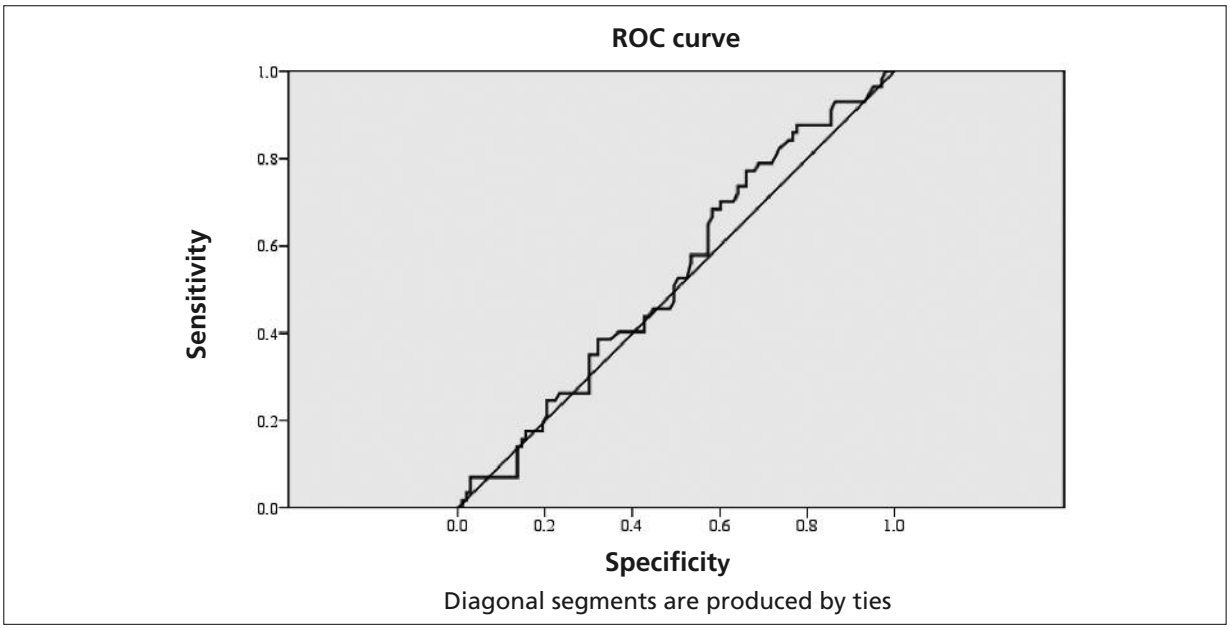

Figure 1. ROC curve of AFP in NAFLD and control goups.

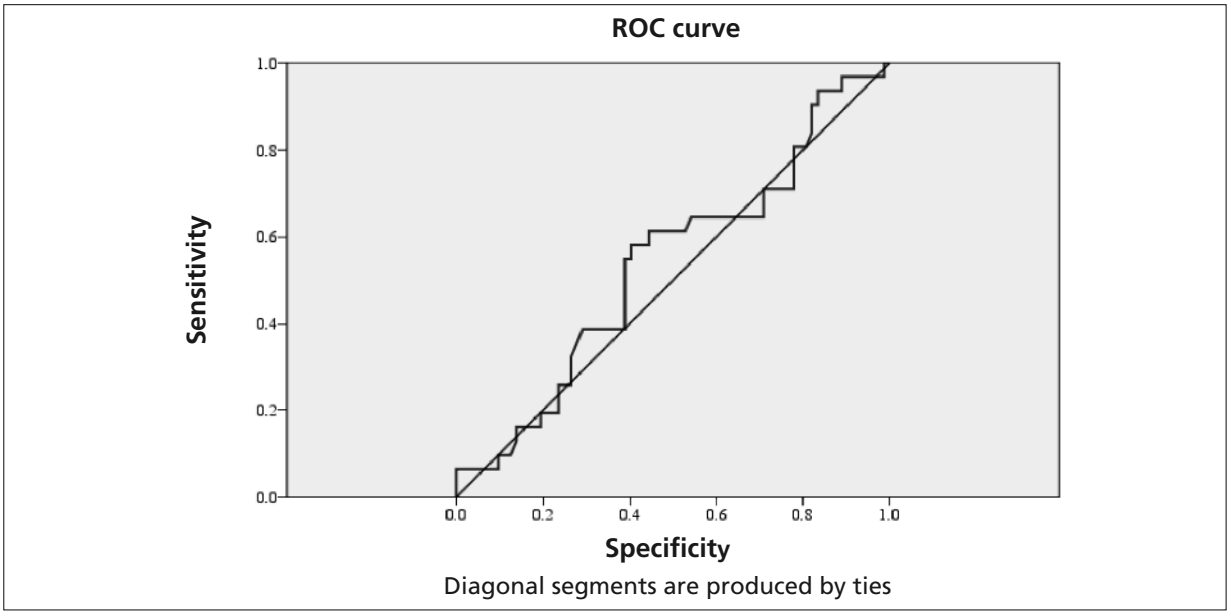

Figure 2. ROC curve of AFP in NASH and SS patients.

ferent between subjects with NASH and SS. Moreover, there was no significant relationship of AFP with the liver histology in NAFLD.

We suggest some possible explanations for the lack of association between AFP and NAFLD in our study. Firstly, as the Authors also mentioned, the main limitation of the study of Babali et al was the lack of histopathological confirmation of the patients' diagnoses. Herein, it is noteworthy that although the positive predictive value of ultrasonography in the diagnosis of NAFLD is around 80-100%[21], a sensitivity of 67% and a specificity of 77% have also been reported[22]. In addition, it should be kept in mind that the diagnostic value of ultrasound decreases in obese patients[23]. Furthermore, ultrasound can not be used to differentiate SS from NASH. Secondly, a correlation between elevated serum AFP and ALT levels has been reported in patients with acute exacerbation of chronic hepatitis B, especially in those showing bridging necrosis by liver histology[24-26]. In addition, AFP levels may be elevated in benign chronic liver disease, e.g. chronic viral hepatitis and liver cirrhosis without HCC[27-29]. These data indicate that AFP levels might be affected by the degree of liver pathology. Eventually, it can be hypothesized that the absence of significant fibrosis or cirrhosis on liver histology as in our patients may be another reason for the conflicting findings of two studies.

Though the sample size, the findings obtained may not be representative for all subjects with NAFLD. But, we think that the main strength of our study design is the involvement of subjects with histologically proven NAFLD and this was a requirement for the goals to achieve. Moreover, because of the relatively milder degree of liver pathology in our subjects, future studies consisting of patients with more advanced liver are injury required.

Conclusions

To the best of our knowledge, this is the first report that investigates circulating AFP concentrations in subjects with biopsy proven NAFLD. The findings of this study have shown that serum AFP levels are not different between male subjects with NAFLD and healthy controls. In addition, patients with SS and NASH were also found to be similar with regard to AFP levels. In addition, no significant association was found between AFP and histological findings. These data suggest that AFP is not involved in the pathogenesis of NAFLD.

Conflict of Interest

None to declare

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