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ORIGINAL ARTICLE / ÖZGÜN ARAŞTIRMA

Diagnostic performances of serum liver enzymes and cytokines in non-alcoholic fatty liver disease

Serum karaciğer enzimleri ve sitokinlerin alkolik olmayan yağlı karaciğer hastalığında tanısal performansları

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ABSTRACT

Objective: Non-alcoholic fatty liver disease (NAFLD) is affecting people worldwide with increasing prevalence. Non-invasive tests are required for both diagnosis and staging of the disease. We aimed to evaluate diagnostic accuracy of routine liver enzymes and cytokines in NAFLD.

Methods: A total of 88 cases, aged between 20 and 62 years, were included in the study. Serum ALT, AST, GGT, triglyceride, TNF-alpha, IL-6 and IL-8 were measured in 40 patients with NAFLD and in 48 healthy control patients with similar BMI and demographic characteristics. Diagnostic performances of serum biomarkers for diagnosis of NAFLD were evaluated with ROC analysis.

Results: ALT and AST showed good diagnostic performance in predicting patients with NAFLD in the overall group (AUC=0.817; 95% CI[0.721-0.913], AUC=0.815; 95% CI[0.718-0.911] respectively) but in obese subjects ALT and AST showed poor performance (AUC=0.659; 95% CI[0.478-0.841], AUC=0.680; 95% CI[0.498-0.861] respectively). Among cytokines TNF-alpha showed best performance in the diagnosis of NAFLD in both overall group and obese subjects (AUC=0.892; 95% CI[0.824-0.959], AUC=0.858; 95% CI[0.739-0.977] respectively). The optimal cut off value for TNF-alpha was 10.65pg/ml with a sensitivity of 75% and a specificity of 93% in the overall group. IL-6 and IL-8 showed poor performance.

Conclusion: TNF-alpha may be a good parameter for predicting patients with NAFLD. *J Clin Exp Invest 2015; 6 (1): 16-20*

Key words: TNF-alpha, IL-8, IL 6, non-alcoholic fatty liver disease

ÖZET

Amaç: Alkolik olmayan yağlı karaciğer hastalığı gittikçe artan prevalansla dünya çapında insanları etkilemektedir. Bu hastalığın tanısı ve evrelendirilmesi için invaziv olmayan testlere ihtiyaç vardır. Bu çalışmada rutin karaciğer enzimleri ve sitokinlerin alkolik olmayan yağlı karaciğer hastalığında tanısal performanslarını değerlendirmeyi amaçladık.

Yöntemler: 20-62 yaşları arasında 88 kişi çalışmaya dahil edildi. Serum ALT, AST, GGT, trigliserid, TNF-alfa, IL-6 ve IL-8 düzeyleri 40 yağlı karaciğer hastası ve benzer VKİ ve demografik özellikleri olan 48 sağlıklı bireyde ölçüldü. Serum biyobelirteçlerinin yağlı karaciğer hastalığındaki tanısal performansları ROC analizi ile değerlendirildi.

Bulgular: ALT ve AST tüm grupta yağlı karaciğer hastalığı olan bireyleri belirlemede iyi performans gösterdi (AUC=0,817; %95 CI[0,721-0,913], AUC=0,815; %95 CI[0,718-0,911] sırasıyla) ancak obez bireylerde ALT ve AST zayıf performans gösterdi (AUC=0,659; %95 CI[0,478-0,841], AUC=0,680; %95 CI[0,498-0,861] sırasıyla). Sitokinler arasında TNF-alfa yağlı karaciğer hastalığı tanısında tüm grupta ve obez hastalarda en iyi performansı gösterdi (AUC=0,892; %95 CI[0,824-0,959], AUC=0,858; %95 CI[0,739-0,977] sırasıyla). Tüm grupta TNF-alfa için en iyi cut-off değeri %75 sensitivite ve %93 spesifisite ile 10,65pg/mL bulundu. IL6 ve IL8 zayıf performans gösterdi.

Sonuç: TNF-alfa alkolik olmayan yağlı karaciğer hastalarını tespit etmede iyi bir parametre olabilir.

Anahtar kelimeler: TNF-alfa, IL-8, IL-6, alkolik olmayan yağlı karaciğer hastalığı

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INTRODUCTION

Non-alcoholic fatty liver disease (NAFLD) is a common disorder with growing incidence especially in developed countries. It becomes more common in several regions in the world and it has become a chronic liver disease [1]. NAFLD, which is considered as the hepatic indicator of metabolic syndrome, is the most common reason of liver test abnormalities in adults [2]. With the recent rise in body mass index in western countries, it has been revealed that more and more people suffer from NAFLD when evaluated in terms of abnormal liver tests [3]. The term NAFLD is used for the fatty infiltration of the liver as it includes different stages of the disease with or without inflammation. The exact cause of the disease is still unknown. There are strong evidences indicating that NAFLD is a part of metabolic syndrome or an indicator of it. Several risk factors such as insulin resistance, obesity, hypertension and hyperlipidemia have been related to NAFLD [4].

Non-alcoholic steatohepatitis (NASH) is a more aggressive form of NAFLD spectrum that carries advanced fibrosis risk. The clinical importance of NAFLD comes from the fact that it is seen frequently in general population and it has a potential to progress into cirrhosis [5]. Increase in free fatty acid synthesis, taking too much fatty acid to liver cells and macrovesicular fatty deposition play a role in the pathogenesis of the disease. Besides, as a result of fatty acid beta oxidation, oxidative stress, proinflammatory cytokines and endotoxemia, inflammation and fibrosis may develop. Proinflammatory cytokines that are effective in inflammatory, immune and metabolic phases have an important role in the development and advancing of NAFLD [2, 6]. The existence of NAFLD can be determined by ultrasound and liver biopsy with exclusion of too much alcohol consumption. Together with fatty liver inflammation it can lead to severe health problems such as cirrhosis and liver transplantation [7]. Due to serious results that this disease leads to, pathophysiological mechanism of this disease and means of treatment has been a subject of research. New biochemical indicators and new methods are still being researched [8]. ALT was used as a surrogate marker but it is not an ideal marker for diagnosis of NAFLD [9-10].

In the present study diagnostic accuracy of liver enzymes and cytokines to identify NAFLD were investigated.

METHODS

Population of the study

A total of 88 patients who admitted to Tepecik Teaching and Research Hospital gastroenterology outpatient clinic were included the study. The study comprised 40 patients with NAFLD and 48 control patients with similar demographic characteristics and BMI.

Patients with viral hepatitis, hemochromatosis, Wilson disease, biliary obstruction, sclerosing cholangitis, autoimmune hepatitis, ischemic cardiovascular disease, advanced cardiac or lung disease, acute infection, kidney function test abnormalities, thyroid disorders (hypothyroidism or hyperthyroidism) and malignant diseases were excluded from the study. In addition, patients with use of hormone replacement therapy, oral contraceptive, azotiopurin methotrexate, amiodarone, steroids, lipid-lowering medication, thyroid hormone therapy and antithyroid therapy or patients with alcohol consumption of more than 20 gr alcohols in a day were excluded from the study. Patients who underwent an abdominal ultrasonography screening and who did not have any of the exclusion criteria were invited to participate in the study. 40 subjects with ultrasonography proven NAFLD and 48 patients without NAFLD were prospectively enrolled for the measurement of biochemical parameters. Study protocol was approved by Hospital ethics committee and all participants provided written informed consent (Approval No.3/11 18-03-2009).

Data collection

Although liver biopsy is considered to be the gold standard for the diagnosis of NAFLD and NASH, due to the ethical reasons the diagnosis of NAFLD was based on ultrasound evaluation.

Ultrasound evaluation was performed by one experienced radiologist using a 3.5-MHz linear transducer with Siemens Acuson Sequoia 512 ultrasound system (Siemens, Germany). Ultrasound diagnosis for the fatty liver was based on the increased echogenicity of liver parenchyma.

Data on physical examination, anthropometric measurements and biochemical measurements of all patients were gathered. Body mass index was calculated (BMI=weight/(height)2). Blood pressure was measured at least 10 minutes after the patients rested with a sphygmomanometer that has appropriate collar. Smoking habits were asked. Insulin resistance for patients and control groups has been

calculated with HOMA-IR method. (HOMA-IR: fasting plasma insulin (μU/mL) x fasting plasma glucose (μU/mL)/22.5). HOMA-IR result <2.5 was considered as normal, HOMA-IR >2.5 was considered as insulin resistance [11]. All blood samples were collected within two weeks after ultrasound imaging. Blood samples were taken between the hours of 0800-0900 after fasting 12-hour for all the analyses. Samples were allowed 30 min to clot and then centrifuged for ten minutes at 1300 RCF. Routine biochemical measurements were performed in the same day with enzymatic method on the Olympus AU 2700 Autoanalyzer (Olympus, Tokio, Japan). Serum samples collected for fasting insulin, TNF-alpha, IL-6, IL-8 were stored at -20 degree until analyses. Fasting insulin, TNF-alpha, IL-6, IL-8 were measured with chemiluminescence immunometric method by using Immulite 2000 autoanalyzer (Siemens Medical Solutions Diagnostics, Llanberis, United Kingdom), and Immulite 1000 analyzer (Siemens Medical Solutions Diagnostics, Llanberis, United Kingdom).

Statistical analysis

For statistical analysis, SPSS (Statistical Package for Social Sciences) 17.0 program was used. Normality of the distribution for continuous variables was analyzed with Kolmogorov-Smirnov test. Mann-Whitney U test was used for comparison of continuous variables. For the comparison of categorical data, chi-square test was used. P<0.05 was significant. Diagnostic performances of variables were evaluated by ROC analysis. Area under curve (AUC) values was defined as follows: AUC of 1.0-0.90: excellent discrimination, AUC of 0.90-0.80 good discrimination, AUC of 0.80-0.70 fair discrimination, AUC of <0.70 poor discrimination. Best cut off point was the value that maximizes the sum of sensitivity and specificity.

RESULTS

The study was conducted between January 2010 and October 2010. Of the eligible 121 patients 33 refused participating in the study. A total of 88 cases (52female/36 male) were included in this study. Mean BMI was 29.6 ± 4.4 mean age was 42.1 ± 9.7 years. Thirty-six (40.9%) of the subjects were obese. Demographic and biochemical characteristics of the study population were presented at table1. There were no missing data for biochemical parameters and all results were included in analysis.

Table 1. Demographic and biochemical characteristics of the participants

	Overall Group			Obese Subjects		
	NAFLD (n=40)	Control (n=48)	p	NAFLD (n=19)	Control (n=17)	p
Sex (F/M)	20/20	32/16	0.113*	14/5	14/3	0.532*
Age	42.8 ± 10	41.5 ± 9.5	0.381	43.7±10	39.6±10	0.222
BMI (kg/m^2)	30.1 ± 3.8	29.1 ± 4.8	0.111	33.2±2.3	34.8±3	0.132
Glucose (mg/dL)	99 ± 12	101 ± 12	0.453	105±11	99±13	0.193
Insulin (mU/L)	14.5 ± 9.0	10.0 ± 6.4	0.010	16.8±9.7	13.3±7.4	0.247
HOMA-IR	3.7 ± 2.5	2.5 ± 1.6	0.017	4.5±2.8	3.3±1.9	0.141
TG (mg/dL)	178 ± 84	124 ± 69	<0.001	164±75	120±49	0.051
ALT (U/L)	57.5 ± 35.5	23.7 ± 12.1	<0.001	43.8 ± 28.9	27.3±14.9	0.102
AST (U/L)	40.9 ± 18.9	22.6 ± 6.8	<0.001	34.9±18.9	23.9±7.4	0.066
GGT (U/L)	41.3 ± 23.7	28.6 ± 22.7	<0.001	32.6±17.4	32±23.6	0.374
IL-6 (pg/mL)	3.4±1.8	2.9±1.0	0.815	3.7±2.4	3.2±1.1	0.974
IL-8 (pg/mL)	13.8±6.4	10.8±4.3	0.016	14±5.2	10.7±4.2	0.030
TNF-α (pg/mL)	14.0±5.4	7.6±2.2	<0.001	12.8±4.2	7.9±2.2	<0.001

Mann-Whitney U test were used unless otherwise indicated

* : Chi square P value NAFLD: Non-alcoholic fatty liver disease, BMI: Body mass index, AST: Aspartate aminotransferase, ALT: Alanine aminotransferase, TG: Triglyceride, TNF- α: Tumor necrosis factor alpha, GGT: gamma glutamyltransferase, IL-8: Interleukin 8, IL-6: Interleukin 6, HOMA-IR: Homeostasis model assessment of insulin resistance

In the overall group patients with NAFLD have significantly higher HOMA-IR, insulin, AST, ALT, triglyceride, GGT, TNF-alpha and IL8 levels compared to those without NAFLD (p<0.05 for all parameters). IL6 was not significantly different between patients with NAFLD and control patients (p=0.815). In the obese participants, patients with NAFLD have higher ALT, AST, GGT, HOMA-IR, insulin, triglyceride levels compared to control patients but the difference did not reach statistical significance(p=0.102, p=0.066, p=0.374, p=0.141, p=0.247, p=0.051 respectively). IL8 and TNF-alpha were significantly higher in obese subjects with NAFLD (p=0.030, p<0.001 respectively). IL6 was not significantly different between obese NAFLD patients and obese control patients (p= 0.974) (Table 1)

In ROC analysis TNF-alpha showed the best area under curve (AUC) for diagnosing NAFLD when calculated in the overall group and in obese subjects (AUC=0.892, AUC=0.858 respectively). The best cut off value for TNF-alpha was 10.65pg/ml with a sensitivity of %75 and a specificity of %93 in overall group. In the overall group ALT and AST showed good performance in predicting the presence of NAFLD (AUC=0.817, AUC=0.815 respectively) but in obese subjects ALT and AST showed poor performance (AUC=0.659, AUC=0.680 respectively). IL8 showed poor performance in the overall group (AUC=0.649) and fair performance in obese subjects (AUC=0.712). IL6 was of no use in predicting patients with NAFLD (AUC=0.514) (Table 2).

Table 2. Diagnostic performances of TNF-alpha, IL-8, IL-6, ALT, AST, GGT and TG in the diagnosis of NAFLD

	Overall Group (n=88)				Obese Subjects (n=36)			
	AUC (95%CI)	Sn (%)	Sp (%)	Cut-off	AUC (95%CI)	Sn (%)	Sp (%)	Cut-off
TNF-α (pg/mL)	0.892 (0.824-0.959)	75	93.7	10.65	0.858 (0.739-0.977)	63.2	94.1	11.5
IL-8 (pg/mL)	0.649 (0.535-0.764)	72.5	54.2	10.25	0.712 (0.536-0.888)	35.3	64.7	10.25
IL-6 (pg/mL)	0.514 (0.385-0.644)	35	81.2	3.75	0.497 (0.298-0.696)	26.3	94.1	4.6
ALT (U/L)	0.817 (0.721-0.913)	75	87.5	32.5	0.659 (0.478-0.841)	57.9	82.4	32.5
AST (U/L)	0.815 (0.718-0.911)	77.5	77.1	26.5	0.680 (0.498-0.861)	42.1	94.1	37.5
GGT (U/L)	0.733 (0.627-0.839)	72.5	68.7	26.5	0.587 (0.394-0.779)	73.7	47.1	21.5
TG (mg/dL)	0.722 (0.613-0.831)	70	70.8	135.5	0.690 (0.513-0.868)	63.2	76.5	145.5

Sn: Sensitivity, Sp: Specificity, AUC: Area Under Curve, AST: Aspartate aminotransferase, ALT: Alanine aminotransferase, TNF- α: Tumor necrosis factor alpha, TG: Triglyceride, GGT: gamma glutamyltransferase, IL-8: Interleukin 8, IL-6: Interleukin 6

DISCUSSION

Obesity is a major health issue in western countries and the association between obesity and NAFLD is well documented. The prevalence of NAFLD is estimated to be 20-30% in the general population in western countries [12]. Currently the diagnosis of NAFLD is based on ultrasonography and liver biopsy. There is a need for non-invasive tools that could be used in general population for both diagnosis and staging of NAFLD.

Elevated ALT and AST levels are the most common abnormalities detected in patients with NAFLD. In this study the diagnostic performances of AST and ALT were good in the overall group but in obese subjects ALT and AST showed poor performance. The limited diagnostic utility of ALT in obese individuals was previously reported [10]. NAFLD can be seen in individuals with normal ALT values [13, 14]. The results of this study make an emphasis that diagnostic utility of liver enzymes is substantially reduced in obesity which is a major risk factor for NAFLD. The reduced diagnostic utility of liver enzymes in obese individuals may be due to an elevation in liver enzymes because of obesity independent of NAFLD.

Elevated triglyceride and GGT were also reported to be associated with NAFLD [15, 16]. The

diagnostic performances of triglyceride and GGT were fair in both overall group and obese subjects.

TNF-alpha was the best parameter in predicting patients with NAFLD furthermore it showed good performance both in the overall group and obese subjects. Several studies investigated TNF-alpha in patients with NAFLD [2, 17, 18]. TNF-alpha was reported to be increased in patients with NAFLD but it is controversial whether it discriminates between NASH and simple steatosis. Lebensztejn et al reported that the ability of serum TNFα to differentiate obese children with liver steatosis from those without steatosis was significant with an AUC of 0.744 but the ability to differentiate children with advanced liver steatosis from those with mild steatosis was insignificant [19]. In a study by Manco et al it was reported that a value of TNF-alpha of 7.9 pg/mL or more has a sensitivity of 82% and a specificity of 96% to identify patients with a NAFLD activity score ≥5 in children with NAFLD [20]. The cut off for TNF-alpha was 10.65 pg/ml with a sensitivity of 75% and a specificity of 93.7% in the current study.

IL-6 and IL-8 were also reported to be higher in subjects with NAFLD (2, 17). Our results showed that IL-8 levels were higher in patients with NAFLD but IL-6 was not significantly different between two groups. The diagnostic performances of IL-8 and IL-6 were poor.

The most important limitation of the study is the fact that the diagnosis of NAFLD was done by noninvasive methods and the fact that there are no histopathologic data. For this reason, the relation between cytokine levels and the phase of the disease could not be evaluated. Fatty liver hepatitis couldn't be separated from simple steatosis which can lead to serious results and the role of cytokine levels in differentiating these two cases couldn't be evaluated.

In conclusion, our analysis showed that TNF-alpha may be a promising parameter in the diagnosis of NAFLD.

Conflict of Interest: Authors declare that they have no conflict of interest relevant to this article.

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