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The use of cytokeratin-18 and interleukin-6 as diagnostic markers for nonalcoholic fatty liver disease in Iraqi young adult

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Objective To study the prevalence of nonalcoholic fatty liver disease (NAFLD) among the study group in Karbala, Iraq and disease evaluation by inflammatory markers.
Methods A total of 816 patients referred for ultrasound checking, aged 18–40 years. They were studied to detect the prevalence of NAFLD. For comparison of level of CK18, IL-6 and other studied markers, we choose 45 patients with NAFLD. From the remaining standard individuals, we randomly selected 45 individuals as control group. Blood sample was collected to measure lipid profile, liver enzyme, and CK-18. Enzyme-linked immune sorbent assay (ELISA) was used for detecting and quantifying inflammatory markers.
Results The result shows that serum CK-18 was highly significant in patient with NAFLD when compared with control groups ($p = 0.001$). Also, serum IL-6 showed no significant increase in patient with NAFLD than in control group ($p < 0.05$).
Conclusion The prevalence of NAFLD in the study group (816 persons, age range 18–40 years) in Karbala, Iraq was 5,51%, and our results rely on finding from assays of inflammatory markers (CK-18 and IL-6) and that most patients in this study were found to have steatohepatitis.
Keywords Non-alcoholic fatty liver disease, keratin-18, interleukin-6.

Introduction

Nonalcoholic fatty liver disease (NAFLD) is a disease of increasing prevalence and has been approved as international health trouble. Influent around 1/3 of adult and 1/10 of children all over the world. The prevalence of NAFLD rise up with obesity, metabolic syndrome (MetS) and type 2 diabetes mellitus (T2DM).[1]

NAFLD has a lot of circumstances related to over accumulation of fat in the liver ranging from NAFLD to nonalcoholic steatohepatitis (NASH) and cirrhosis[2] NAFLD can be classified broadly into two subtypes:

(i) NAFLD that does not cause serious liver infection or liver-related death. (ii) NASH that causes serious liver infection or liver-related death. NAFLD has strong relation with metabolic disorders including obesity, hyperinsulinemia, glucose intolerance, and dyslipidemia.[3] Cytokeratin-18 (CK-18) is a main intermediate filament protein in liver cells and it is sustainable for hepatocyte integrity,[4,5] which is generated during apoptotic cell death of injured hepatocytes due to obesity-related damage, caspase-cleaved CK-18 fragments enter the bloodstream. The existence of CK-18 fragments in the blood will be distinct between steatohepatitis from simple steatosis (SS) because apoptotic does not appear in the SS. Previous study in adults reflected that each 50 U/L increase in the plasma level of CK-18 raise up the possibility of NASH by 30%.[6] CK-18 performs as a certain biomarker for evaluation of the existence of NASH, with sensitivity of 78% and specificity of 87%, actively differentiating NASH from nonalcoholic SS NAFLD.[7]

Interleukin-6 (IL-6) founded in 1986 as a B-cell stimulatory factor initiating IgG production after that; it was showed to be a multifunctional cytokine that regulates numerous biological processes including the organ development, acute-phase responses, inflammation, and immune responses.[8] IL-6 in liver diseases is very complicated, and its role in the development of NAFLD still not recognizable. IL-6 switch on number of cells, like immune cells, hematopoietic stem cells, hepatocytes and osteoclasts.[9] IL-6, in addition to being a pro-inflammatory cytokine, it also systemizes regenerative, metabolic, and neural processes.[10] A lot of researches indicate to increase the pro-inflammatory cytokines tumor necrosis factor (TNF)-α, and IL-6 in the people infected with NAFLD, while anti-inflammatory cytokines IL-4 level decreased and IL-10 level is still stable.[11,12,13] IL-6 is increased in plasma in NASH-infected people as compared with the infected people who suffer from fatty liver without inflammation.[3] IL-6 expressed in hepatocytes, and its ratio in blood, has a positive connection with the rate of liver inflammation, and fibrosis.[14]

Subjects and methods

Study design

A case–control study

Subjects selection

Individuals were selected from people who visited the Iraqi Medical Center in Kerbala City during the period from 11 January to 14 May 2017, aged 18-40 years for ultrasound examination. From the total of 1020 individuals who were referred by physician or surgeon for ultrasound checking, and after exclusion of 203 pregnant women and 1 alcoholic fatty liver patient, 204 individuals were excluded from this study; the remaining total of 816 individuals, the patients were diagnosed with fatty liver after examination by ultrasound, 45 individuals were diagnosed as nonalcoholic fatty liver patients, after receiving verbal consent (there was no refusal from the cases to take the necessary tests and to be included into this study). From the remaining standard individuals, we randomly selected 45 individuals as control group (the healthy individuals) for the purpose of comparison with the NAFLD

group after receiving verbal consent (a large number of healthy subject refused the tests and inclusion into this study); we collected the information from all individuals. This study was done in the Department of Medical Biochemistry, College of Medicine, University of Kerbala, from Nov. 2016 to Sep. 2017.

- Inclusion criteria: subjects who diagnosed by ultrasound as having NAFLD or healthy and were aged 18–40 years.
- Excluded criteria: should not suffer from liver cancer and hepatitis (by the history of the subjects and test results had negative virology), alcoholism, acute or chronic liver disease, and pregnant women.

Methods

Data included were body mass index (BMI) which was measured (weight (kg)/square height (m^2)), lipid profile (TG, TC, HDL), liver enzyme (aspartate aminotransferase, AST and alanine aminotransferase, ALT). They were measured by using Spectrophotometry. Gama Glutamyal Transferase (GGT) was measured by using Auto Analyzer Biochemistry (Cormay), IL-6 and CK-18 were measured by Enzyme Linked Immunosorbent Assay (ELISA).

Biometric analysis

The analysis of data was carried out using micro-software Excel and the available Statistical packages for social science, version 20.0 (SPSS-20.0). Data were presented in form of figures and tables of numbers and percentage. Chi-square test (χ^2-test) and *t*-test were used for testing the significance of association between variable under study.

Statistical significance was considered whenever the *p*-value was equal or less than 0.05.

Result

A total study sample was 816 patients (489 were male and 327 were female). As showing in Fig. 1. Forty-five patients out of 816 had disease and considered as cases. The prevalence of NAFLD in the study group (816 persons, age range 18–40 years) in Karbala, Iraq was 5.51%.

Results shows that the serum (TC, TG, HDL-C, VLDL-C, LDL-C, AST, ALT, GGT, BMI) significantly increases in patient with NAFLD when compared with the control group (*p*-value < 0.05, Table 1).

Results shows that the serum CK-18 significantly increases in patient with NAFLD when compared with the control group ($p = 0.001$). Also serum showed IL-6 no significant increase in patient with NAFLD than in control croup ($p = 0.2$), Table 2.

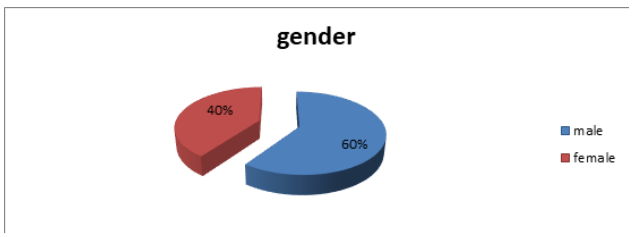

Fig. 1 **Gender distribution of study sample.**

Table 1. **The (mean ± SD) of Biochemical parameters to patients with NAFLD group and without NAFLD (control group)**

Variable	Status	N	Mean	Std. deviation	*p*-value
TG (mg/dl)	Case	45	263.77	162.55	0.0001**
	Control	45	100.62	36.22	
T C (mg/dl)	Case	45	257.67	56.49	0.003*
	Control	45	179.94	163.12	
HDL (mg/dl)	Case	45	42.05	5.86	0.0001**
	Control	45	55.16	5.82	
LDL (mg/dl)	Case	45	163.31	59.57	0.025*
	Control	45	105.08	160.09	
VLDL (mg/dl)	Case	45	52.30	32.57	0.0001**
	Control	45	20.12	7.24	
AST (U/L)	Case	45	29.78	12.85	0.0001**
	Control	45	9.41	1.87	
ALT (U/L)	Case	45	24.06	10.45	0.0001**
	Control	45	7.64	1.79	
GGT (U/L)	Case	45	45.22	36.78	0.001**
	Control	45	16.04	8.88	
BMI (Kg/m^2)	Case	45	34.97	4.80	0.001**
	Control	45	25.34	6.44	

No significant association $p<0.05$, *Significant association $p<0.05$, **Highly significant association $p<0.01$

Table 2. **The (mean ± SD) of the CK-18 and IL-6 to patients with NAFLD group and without NAFLD (control group)**

Variables	Status	N	Mean	Std. deviation	*p*-value
IL-6 (pg/mL)	Case	45	10.22	10.67	0.2
	Control	45	6.12	18.56	
CK-18 (mIU/mL)	Case	45	530.86	141.87	0.001**
	Control	45	397.92	196.01	

No significant association $p<0.05$, *Significant association $p<0.05$, **Highly significant association $p<0.01$

Discussion

In the current study, the prevalence of NAFLD in the study group was (5.51%). In a recent meta-analysis done by Younossi et al in Asia, it showed the prevalence of NAFLD in the general population was 25.24%.[15] Furthermore, in another study conducted by Michael et al in the USA, it showed the prevalence of NAFLD among 6000 subjects who were aged 18 years and older 30.0%.[16] Also, Elizabeth et al who showed the prevalence of NAFLD when checking by ultrasound in Asia, Africa and the Pacific Islands (Japan, China, Korea and India) in the General population and hospital health checkup was 9%–20% and Saudi Arabia and Egypt when checking by CT was 10%–18%.[17]

The difference in prevalence between our study and other studies is because of the sample size and specific age groups. In addition, age plays a significant role[18,19,20,21] where highest prevalence is reported in adults with range ages of 40–49, while the age of more than 60 years of age increases the prevalence of the disease twice compared to the age of 20 years.[19,21]

The data of the current study revealed no significant increase of serum IL-6 in patients with NAFLD when compared with the healthy group (Table 2). There are several studies showing strong association between IL-6 and NASH. Wieckowska et al, who showed gene expression of IL-6 increase in patients with NASH when compared with patients with SS or healthy liver. Hepatic IL6 expression was also in positive correlation with the degree of liver inflammation and fibrosis.[22] Naim et al, who showed levels of pro-inflammatory cytokines, such as TNFα and IL-6 increase in patient with the NASH compared with patient with SS NAFLD but the differences have not been significant enough to allow the use of these cytokines as noninvasive markers.[23] Also Abiru et al, who showed IL-6 levels are significantly increased in patients with NASH when compared with patients with the SS and healthy subjects, and that these increased levels may be implicated in the pathogenesis of NASH.[24] In another hand Bocsan et al study says plasma IL-6 showed highly significant increase in patients with NASH compared with the control group.[25] The no significant result can be explained because of sample size and that patients included in this study presented in the different degrees of steatosis and inflammation.

The results of this study showed significant increase in serum CK-18 in patients with NAFLD when compared with control group (p = 0.001), Table 2. This referred to presence of inflammation or apoptosis in the hepatocytes. CK-18 is a marker to predict steatohepatitis and differentiate steatohepatitis from SS according to guideline.[26] This is in agreement with Mohsen et al, who showed plasma CK-18 levels increase in the patients with NASH when compared with patients with NAFLD and the control group, with p-value <0.001. Also, there was no significant difference between the patients with NAFLD group when compared with healthy group.[2] And, similar results were found in a study done by Feldstein et al, on children, who found that CK-18 levels were significantly increased in patients with NASH compared with patients with NAFLD (p < 0.001). They also found that for every 10 U/L increase in CK-18 levels, the likelihood of having NASH increased by 70%.[27] These results are similar to results were found in a study done by Liang et al, who showed serum CK-18 M30 level was significantly increased in patients with NASH than in patients with non-NASH. CK-18 M30 showed the presence of NASH in liver cell, injury, and apoptosis in hepatocytes.[28] Furthermore, it has been shown that some liver enzymes such as alcohol dehydrogenase 2 and 3 are more active in nonalcoholic Iraqi persons.[29] The previous studies in this regard revealed that Plasma CK-18 is highly significant increased with the increased degree of steatosis, inflammation. and fibrosis (Cusi et al).[30]

Conflict of Interest

None

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