Original
ISSN 2521-8492

The role of biochemical markers in the assessment of non-alcoholic fatty liver disease in young Iraqi people

Shahad A. Qassam[1], Zaid K. Ahmed[2] , Shaymaa Z. Nada[1]

[1]Department of Biochemistry, College of Medicine, University of Karbala, Karbala, Iraq.
[2]Department of Anatomy, College of Medicine, University of Karbala, Karbala, Iraq.
Correspondence to Shahad A. Qassam (sahadsahad418@gmail.com)
(Submitted: 10 January 2019 – Revised version received: 17 February 2019 – Accepted: 03 March 2019 – Published online: 26 June 2019)

Objectives To study the clinical diagnostic markers and prognostic biochemical markers which helps to evaluate non-alcoholic fatty liver disease (NAFLD) in Iraqi Young adult.
Method A total of 816 patients aged 18–40 years were referred for ultrasound checking to detect the prevalence of NAFLD. For comparison of CK18 levels and other studied markers, we chose 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 immuno-sorbent assay used for detecting and quantifying inflammatory markers.
Results The results show that serum CK-18 was highly significant in patients with NAFLD when compared with control groups ($P = 0.001$). Also fatty liver index (FLI) values were significantly higher in patients with NALD than in control group ($P = 0.001$). They also showed markedly great diagnostic accuracy (AUROC curve: 0.968).
Conclusion Our result indicates from the results of inflammatory markers that most patients in this study are affected with steatohepatitis, FLI is the most sensitive diagnostic marker for diagnostic NAFLD.
Keywords Non-alcoholic fatty liver disease, non-alcoholic steatohepatitis, keratin-18, fatty liver index

Introduction

Non-alcoholic 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 rises up with obesity, metabolic syndrome (MetS) and type 2 diabetes mellitus.[1]

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

(1) NAFLD that does not cause serious liver infection or liver-related death. (2) 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 is the 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, and caspase-cleaved CK18 fragments enter the bloodstream. The existence of CK18 fragments in the blood will differ between steatohepatitis and simple steatosis because apoptotic does not appear in the simple steatosis. Previous study in adults reflected that each 50 U/L increase in the plasma level of CK18 raises up the possibility of NASH by 30%.[6] CK18 performs as a biomarker for evaluation of the existence of NASH, with sensitivity of 78% and specificity of 87%, active distinct NASH from non-alcoholic simple steatosis NAFLD.[7]

The fatty liver index (FLI), an algorithm based on gamma-glutamyl transferase (GGT) levels, triglyceride (TG) concentration, body mass index (BMI), and waist circumference (WC), was developed to diagnose fatty liver disease in the general population. The FLI has been validated by several studies and has been proven to have a strong association with T2DM, cardiovascular disease, hypertension, NAFLD, and MetS and are closely related[8] as follows:

$$\mathbf{FLI = Exp\,(0.953 \times In[TG] + 0.139 \times BMI + 0.718 \times In[GGT] + 0.053 \times WCF - 15.745)/(1 + Exp[0.953 \times In[TG] + 0.139 \times BMI + 0.718 \times In[GGT] + 0.053 \times WCF - 15.745]) \times 100\ ng.}\ [9]$$

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 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; the remaining total is 816 individuals. The patients were diagnosed for fatty liver after examination by ultrasound and 45 were diagnosed as non-alcoholic 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 November 2016 to September 2017.

- Inclusion criteria: Subjects who were diagnosed by ultrasound as having NAFLD or healthy, were aged 18–40 years.
- Exclusion 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 BMI (weight (kg)/square height (m^2)), lipid profile (TC, TG, HDL), liver enzymes (aspartate aminotransferase (AST) and alanine aminotransferase (ALT)). They were measured using Spectrophotometry. GGT were measured by using Auto Analyzer Biochemistry (Cormay), cytokeratin-18 (CK-18) was measured by enzyme linked immuno-sorbent 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 the 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 ≤0.05.

Results

The total study sample was 816 patients (489 were male and 327 were female). As shown in Fig. 1, 45 patients out of 816 had disease and were 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 show that the serum TC, TG, HDL-C, VLDL-C, LDL-C, AST, ALT, GGT, and BMI is significantly increased in patients with NAFLD when compared with the control group (*P* value <0.05; Table 1).

Results show that the serum CK-18 is significantly increased in patients with NAFLD when compared with the control group ($P = 0.001$). Also FLI values are significantly higher in patients with NAFLD than in control croup ($P = 0.001$, Table 2).

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

	Status	N	Mean	Std. deviation	*P* value
FLI	CASE	45	99.59	0.80	0.001**
	CONTROL	45	90.55	9.27	
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 results of 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, the prevalence of NAFLD in the general population was 25.24%.[10] Furthermore, in another study conducted by Michael et al in the United States, the prevalence of NAFLD among 6000 subjects aged 18 years and older was 30.0%.[11] Also, Elizabeth et al showed the prevalence of NAFLD checking by ultrasound in Asia, Africa and the Pacific Islands (Japan, China, Korea and India) among the general population and hospital health checkup was 9%–20% and in Saudi Arabia and Egypt checking by CT was 10%–18%.[12]

The difference in prevalence between our study and other studies was because of sample size and specific age groups. Also age plays important role.[13,14,15,16] The highest prevalence in adults was with range between the ages of 40 and 49, while

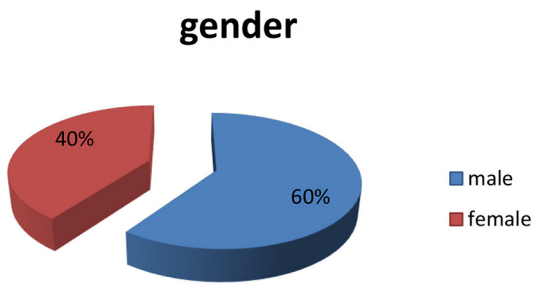

Fig. 1 **Gender distribution of study sample.**

Table 3. **Sensitivity and specificity of diagnostic values of CK-18 and FLI for detection of NAFLD**

Area under the curve								
Test result variable(s)	Area	Std. error	Sig.	Asymptotic 95% confidence interval		Sensitivity %	Specificity %	Cut-off value
				Lower bound	Upper bound			
FLI	0.968	0.017	.000	0.934	1.00	95.6	80.0	98.01
CK-18	0.702	0.056	.001	0.593	0.811	64.4	62.2	535.55

Results show that diagnostic performance of non-invasive markers for the presence of NAFLD from the area under the curve (AUC) was 0.968 (95% CI: 0.934–1.00). The cut-off value was 98.0123, with specificity (80%) and sensitivity (95.6%) in the diagnosis of NAFLD; $P < 0.0001$ for FLI, and 0.702 (95% CI: 0.593–0.811). The cut-off value was 535.55, with specificity (62.2%) and sensitivity (64.4%); $P < 0.0001$ for CK-18, the FLI showed most sensitive diagnostic marker for diagnostic NAFLD (AUC curve: 0.968) compared with the CK-18 (AUC curve: 0.702).

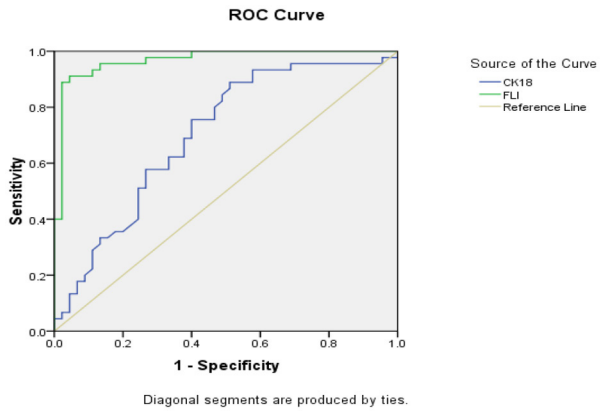

Fig. 2 **Receiving operating characteristic curve of CK18 and FLI.**

the age of more than 60 years of age increases the prevalence of the disease twice compared to the age of 20 years.[14,16]

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 refers to the presence of inflammation or apoptosis in the hepatocytes. CK-18 is a marker to predict steatohepatitis and differentiates steatohepatitis from simple steatosis according to guidelines.[17] This is in agreement with Mohsen et al, who showed plasma CK18 levels, increase in 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.[18] And similar results were found in a study done by Feldstein et al. on children, who found that CK18 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 CK18 levels, the likelihood of having NASH increased by 70%.[19] These results are similar to results 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.[20] The previous studies in this regard revealed that plasma CK-18 is significant increased with the increased degree of steatosis, inflammation and fibrosis.[21] Furthermore, it has been shown that some liver enzymes such as alcohol dehydrogenase 2 and 3 are more active in non-alcoholic Iraqi persons.[22]

The results of the current study showed higher significance of FLI values in the patients group when compared with the control group (Table 2). This agrees with Sviklāne et al.,[23], Cuthbertson et al., who showed FLI values significantly increased in patients with steatosis when compared with subjects without steatosis.[24]

In the current study, diagnostic performance of non-invasive markers for the presence of NAFLD from AUC was 0.968 (95% CI: 0.934–1.00). A cut-off value was 98.0123, with specificity (80%) and sensitivity (95.6%) in the diagnosis of NAFLD; $P < 0.0001$ for FLI, and 0.702 (95% CI: 0.593–0.811). The cut-off value was 535.55, with specificity (62.2%) and sensitivity (64.4%); $P < 0.0001$ for CK-18, the FLI showed most sensitive diagnostic marker for diagnostic NAFLD (AUC curve: 0.968) compared with the CK-18 (AUC curve: 0.702, Table 3). This in agreement with Evangeline et al.[25] Also, Kantartzis et al. showed that FLI was a better marker to predict NAFLD.[26] Huang et al. demonstrated that the FLI could detect NAFLD accurately with a good AUC of 0.834 and this comes in agreement with our studies.[27]. Motamed et al. proved that FLI has a high sensitivity in the diagnosis of NAFLD as well.[28]

References

1. Chalasani N, Younossi Z, Lavine JE, Diehl AM, Brunt EM, Cusi K, et al. The diagnosis and management of non-alcoholic fatty liver disease: practice guideline by the American Association for the Study of Liver Diseases, American College of Gastroenterology, and the American Gastroenterological Association. Hepatology 2012; 55(6):2005–2023.
2. Bellentani S, Scaglioni F, Marino M, Bedogni G. Epidemiology of non-alcoholic fatty liver disease. Dig Dis. 2010; 28:155–161.
3. Sanal MG. Biomarkers in nonalcoholic fatty liver disease the empero has no clothes? World J Gastroenterol. 2015;21(11):3223–3231 .
4. Xinhuan Wei, MM, Hongshan Wei, MD, Wei Lin, MM, Zhongjie Hu, MD, and Jing Zhang, MD. Cell death biomarker M65 is a useful indicator of liver inflammation and fibrosis in chronic hepatitis B. Medicine (Baltimore). 2017;96(20):e6807.
5. Gian Paolo Caviglia1, Rinaldo Pellicano. Serum cytokeratin-18 levels for liver fibrosis prediction. Hepat Mon. 2015;15(8):e30853.
6. Huang, J.F., Yeh, M.L., Huang, C.F., Huang, C.I., Tsai, P.C., Tai, C.M., Yang, H.L., Dai, C.Y., Hsieh, M.H., Chen, S.C. and Yu, M.L. Cytokeratin-18 and uric acid predicts disease severity in Taiwanese nonalcoholic steatohepatitis patients. PLoS One.2017; 12(5):.e0174394.
7. John Vizuete, Alfredo Camero, Mazyar Malakouti, Karthik Garapati and Julio Gutierrez. Perspectives on nonalcoholic fatty liver disease: An overview of present and future therapies. J Clin Transl Hepatol. 2017;5(1):67–75.
8. Yuan-Lung Cheng, Yuan-Jen Wang, Keng-Hsin Lan, Teh-la Huo, Yi-Hsiang Huang, Chien-Wei Su, Wei-Yao Hsieh, Ming-Chih Hou, Han-Chieh Lin, Fa-Yauh Lee, Jaw-ChingWu, and Shou-Dong Lee. Fatty liver index and lipid accumulation product can predict metabolic syndrome in subjects without fatty liver disease. Gastroenterol Res Pract. 2017. Article ID 9279836:12.
9. Agnieszka Zwolak, Agnieszka Szuster-Ciesielska, Jadwiga Daniluk, Justyna Semeniuk, Martyna Kandefer-Szerszen, Chemerin, retinol binding protein-4, cytokeratin-18 and transgelin-2 presence in sera of patients with

non-alcoholic liver fatty disease. Ann Hepatol Off J Mex Assoc Hepatol 2016;15(6):862–869.
10. Younossi, Z.M., Koenig, A.B., Abdelatif, D., Fazel, Y., Henry, L. and Wymer, M., Global epidemiology of nonalcoholic fatty liver disease—Meta-analytic assessment of prevalence, incidence, and outcomes. Hepatology 2016; 64(1):73–84.
11. Michael H. Le, Pardha Devaki, Nghiem B. Ha, Dae Won Jun, Helen S. Te, Ramsey C. Cheung, Mindie H. Nguyen. Prevalence of non-alcoholic fatty liver disease and risk factors for advanced fibrosis and mortality in the United States. PLoS One 2017; 12(3):e0173499.
12. Elizabeth M. Brun, Vincent W.-S. Wong, Valerio Nobili, Christopher P. Day, Silvia Sookoian, Jacquelyn J. Maher, Elisabetta Bugianesi, Claude B. Sirlin, Brent A. Neuschwander-Tetri and Mary E. Rinella, Nonalcoholic fatty liver disease. Article number: 15080 17 December 2015; 1.
13. Lonardo A, Ballestri S, Marchesini G, Angulo P, Loria P. Nonalcoholic fatty liver disease: a precursor of the metabolic syndrome. Digest Liver Dis. 2017; 47(3):181–190.
14. Than NN, Newsome PN. A concise review of non-alcoholic fatty liver disease. Atherosclerosis 2015; 239(1):192–202.
15. Pappachan, J.M., Antonio, F.A., Edavalath, M. and Mukherjee, A. Non-alcoholic fatty liver disease: a diabetologist's perspective. Endocrine.2014;45(3):344–353.
16. Hashimoto, E., Taniai, M. and Tokushige, K. Characteristics and diagnosis of NAFLD/NASH. J Gastroenterol Hepatol 2013; 28(S4):64–70.
17. European Association for the Study of the Liver, European Association for the Study of Diabetes, European Association for the Study of Obesity. EASL-EASD-EASO Clinical Practice Guidelines for the management of non-alcoholic fatty liver disease. J Hepatol 2016; 64:1388–402.
18. Mohsen M. Maher, Wesam A. Ibrahim, Shereen A. Saleh, Lobna Shash, Hoda Abou Gabal, Mohammed Tarif, Mohammed Gamal. Cytokeratin 18 as a non-invasive marker in diagnosis of NASH and its usefulness in correlation with disease severity in Egyptian patients. Egypt J Med Human Genet, 2015;16(1):41–46.
19. Feldstein, AE, Alkhouri, N, De Vito, R, Alisi, A, Lopez, R and Nobili, V. Serum cytokeratin-18 fragment levels are useful biomarkers for nonalcoholic steatohepatitis in children. Am J Gastroenterol 2013;108(9): 1526–1531.
20. Liang, J., Liu, F., Wang, F., Han, T., Jing, L., Ma, Z. and Gao, Y. A noninvasive score model for prediction of NASH in patients with chronic hepatitis B and nonalcoholic fatty liver disease. BioMed Res Int. 2017; 2017.
21. Cusi, K., Chang, Z., Harrison, S., Lomonaco, R., Bril, F., Orsak, B., Ortiz-Lopez, C., Hecht, J., Feldstein, A.E., Webb, A. and Louden, C. Limited value of plasma cytokeratin-18 as a biomarker for NASH and fibrosis in patients with non-alcoholic fatty liver disease. J Hepatol, 2014; 60(1):167–174.
22. Fadhil Jawad Al-tuma, Hedef Dafer El-yassin and Kammela Hadi Shammam, Alcohol dehydrogenase genotype (ADH2 and ADH3) in alcoholism of Iraqi people, J Cont Med Sci 2015, 1(2):9–13.
23. Sviklāne L, Olmane E, Dzērve Z , Kupčs K, Pīrāgs V V, Sokolovska J. Fatty liver index and hepatic steatosis index predict non-alcoholic fatty liver disease in type 1 diabetes. J Gastroenterol Hepatol. 2017; doi: 10.1111/jgh.13814. PMID: 28464337.
24. Cuthbertson, D.J., Weickert, M.O., Lythgoe, D., Sprung, V.S., Dobson, R., Shoajee-Moradie, F., Umpleby, M., Pfeiffer, A.F., Thomas, E.L., Bell, J.D. and Jones, H. External validation of the fatty liver index and lipid accumulation product indices, using 1H-magnetic resonance spectroscopy, to identify hepatic steatosis in healthy controls and obese, insulin-resistant individuals. Eur J Endocrinol. 2014;171(5):561–569.
25. Evangeline Vassilatou, Sophia Lafoyianni, Dimitra-Argyro Vassiliadi, Dimitrios Ioannidis, Stavroula Paschou, Maria Mizamtzidi and Andromachi Vryonidou. Diagnostic performance of non-invasive markers for the presence of nonalcoholic fatty liver disease in premenopausal women with and without polycystic ovary syndrome. 2017;49 EP1139.
26. Kantartzis, K., Rettig, I., Staiger, H., Machann, J., Schick, F., Scheja, L., Gastaldelli, A., Bugianesi, E., Peter, A., Schulze, M.B. and Fritsche. An extended fatty liver index to predict non-alcoholic fatty liver disease. Diab Metab. 2017;43(3):229–239.
27. Huang, X., Xu, M., Chen, Y., Peng, K., Huang, Y., Wang, P., Ding, L., Lin, L., Xu, Y., Chen, Y. and Lu, J., 2015. Validation of the fatty liver index for nonalcoholic fatty liver disease in middle-aged and elderly Chinese. Medicine, 94(40).
28. Motamed, N., Sohrabi, M., Ajdarkosh, H., Hemmasi, G., Maadi, M., Sayeedian, F.S., Pirzad, R., Abedi, K., Aghapour, S., Fallahnezhad, M. and Zamani, F., 2016. Fatty liver index vs waist circumference for predicting non-alcoholic fatty liver disease. World J Gastroenterol, 22(10):3023.