The Egyptian Journal of Medical Human Genetics (2015) **16**, 41–46

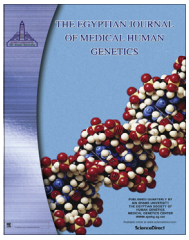
HOSTED BY

ELSEVIER

Ain Shams University

The Egyptian Journal of Medical Human Genetics

www.ejmhg.eg.net
www.sciencedirect.com



ORIGINAL ARTICLE

Cytokeratin 18 as a non invasive marker in diagnosis of NASH and its usefulness in correlation with disease severity in Egyptian patients

Mohsen M. Maher [a], Wesam A. Ibrahim [a], Shereen A. Saleh [a,*], Lobna Shash [b], Hoda Abou Gabal [b], Mohammed Tarif [c], Mohammed Gamal [a]

[a] *Department of General Internal Medicine, Faculty of Medicine, Ain Shams University, Cairo, Egypt*
[b] *Department of Surgical Pathology, Faculty of Medicine, Ain Shams University, Cairo, Egypt*
[c] *Department of Clinical Pathology, Faculty of Medicine, Ain Shams University, Cairo, Egypt*

Received 14 October 2014; accepted 10 November 2014
Available online 19 December 2014

KEYWORDS

Cytokeratin 18;
NASH;
NAFLD;
NAFLD activity score;

Abstract *Background:* A simple noninvasive test that accurately distinguishes NASH from NAFL as well as determines the disease severity is urgently needed. Recently, it was found that determination of cytokeratin-18 (CK-18) fragments in the blood, predicts and correlates with histological NASH in which there is development of lobular inflammation, cell ballooning and fibrosis, supporting its usefulness in clinical practice.

Abbreviations: ALT, alanine transaminase; ANA, antinuclear antibody; Anti LKM Ab,anti-liver kidney microsomal antibodies; ASMA, anti smooth muscle antibody; AST, aspartate transaminase; BMI, body mass index; CBC, complete blood count; CK-18, cytokeratin-18; CK18-Asp396, caspase-cleaved cytokeratin 18; DM, diabetes mellitus; ELISA, enzyme-linked immunosorbent assay; FBS, fasting blood sugar; HA1C, glycated hemoglobin; HBcAb, hepatitis B core antibody; HBsAg, hepatitis B surface antigen; HCC, hepatocellular carcinoma; HCV Ab, hepatitis c antibody; HS, highly significant; IBM, *International Business Machines*; IR, insulin resistance; LDL, low density lipoprotein; mRNA, messenger ribonucleic acid; NAFL, non alcoholic fatty liver; NAFLD, non alcoholic fatty liver disease; NAS, NAFLD activity score; NASH, non alcoholic steatohepatitis; NS, insignificant; PT, prothrombin time; ROC, receiver operating characteristic; S, significant; SD, standard deviation; SPSS, statistical package for special science; T2DM, type 2 diabetes mellitus; TPS ELISA, tryptase ELISA.

* Corresponding author at: 5th Settlement, 90th Road, Compound Lake View, 51/1, Cairo, Egypt. Tel.: +20 26131342 (home), mobile: +20 01227834104.
E-mail address: shereen_saleh2014@hotmail.com (S.A. Saleh).

Peer review under responsibility of Ain Shams University.

http://dx.doi.org/10.1016/j.ejmhg.2014.11.003

Liver biopsy;
Keratins

Aims: To evaluate the role of CK-18 as a non invasive marker in diagnosis of NASH and its usefulness in correlation with disease severity in Egyptian patients.

Patients and methods: 90 subjects were divided into 3 groups: group I: including 30 patients with NASH, group II: including 30 patients with NAFL, and group III: including 30 healthy subjects as control. Diagnosis of NASH and its discrimination from NAFL was done by liver biopsy. CK-18 level in plasma was measured for all subjects using ELISA.

Results: CK-18 was significantly elevated in patients of group I in comparison to group II and III patients, with mean ± SD: 460 ± 279, 167 ± 56 and 149 ± 57, respectively, and *P* value: 0.001. The (ROC) curve diagnostic performance of CK18 in diagnosis of NASH shows: cutoff value of >240 U/L, with sensitivity 76.7%, specificity 95.0%. Ck-18 was found to correlate with disease severity assessed by NAS scoring system with *P* value: 0.001.

Conclusion: Measurement of CK18 in NASH is a useful screening, diagnostic and staging biomarker.

1. Introduction

Nonalcoholic fatty liver disease (NAFLD) encompasses a wide spectrum of conditions associated with over accumulation of fat in the liver ranging from nonalcoholic fatty liver disease NAFLD to nonalcoholic steatohepatitis (NASH) and cirrhosis [1].

Although NAFL typically follows a benign non progressive clinical course, NASH is a potentially serious condition; as many as 25% of patients may progress to cirrhosis and experience complications of portal hypertension, liver failure, and hepato-cellular carcinoma [2].

It was suggested that progression from NAFL to NASH and to advanced fibrosis results from two distinct events; first, insulin resistance (IR) leading to the accumulation of fat within hepatocytes, and second, mitochondrial reactive oxygen species causing lipid peroxidation and cytokine induction [3].

Cytokeratins are proteins of keratin-containing intermediate filaments found in the intracytoplasmic cytoskeleton of epithelial tissue. In 2006 a new systematic nomenclature for keratins was created and now the proteins previously called "cytokeratins" are simply called keratins [4].

When hepatocytes are chronically exposed to oxidative stress and toxic substances, they become ballooned, accumulate fat, show a disruption in the keratin intermediate filament network, and form Mallory bodies [5]. A Mallory body is composed of abnormally phosphorylated and cross-linked keratins, such as cytokeratin (CK) 8 and 18 and stress-induced proteins [6].

Since hepatocytes containing Mallory bodies are susceptible to apoptosis, so those levels of Mallory body-associated proteins released from hepatocytes into peripheral blood may be increased in NASH patients and change in accordance with disease activity [7].

At present, liver biopsy remains the only reliable and the "gold standard" way of diagnosing NASH, grading the severity of liver damage and for assessing fibrosis and architectural alterations [8]. Liver biopsy is an invasive procedure that carries a risk of complications. It is an imperfect tool; due to sampling errors, biopsy size (5–30 mm) and intra- and inter-observer variability, it is now agreed that biopsy is an "imperfect Gold Standard diagnostic tool" [9]. Hence new simple and non-invasive test that both accurately distinguishes NASH from NAFLD and determines the stage and grade of the disease is urgently needed [10].

In the current study we investigated the role of serum cytokeratin 18 as a noninvasive maker in differentiating NASH from NAFL, and its correlation with the disease severity.

2. Patients and methods

This study was conducted on 90 Egyptian individuals selected from Internal Medicine and Hepatology outpatient clinics and inpatient wards of Ain Shams University Hospitals (from Dec 2012 to Jul 2013).

They were classified into 3 groups:

- *Group I:* included 30 patients with NASH diagnosed by abdominal ultrasonography and liver biopsy, with no history of consumption of alcohol or hepatotoxic drugs.
- *Group II:* included 30 patients with NAFL diagnosed by abdominal ultrasonography and liver biopsy with no consumption of alcohol or hepatotoxic drugs.
- *Group III:* included 30 age-matched healthy controls attending Blood Bank as blood donors or coming for pre-employment check up with normal abdominal ultrasonography, normal aminotransferases, and no history of chronic liver disease.

Inclusion criteria: patients with NASH and NAFLD on top of Diabetes Mellitus (DM), obesity diagnosed by laboratory tests, ultrasonography and liver biopsy.

Exclusion criteria: liver diseases other than fatty liver as: chronic viral hepatitis, autoimmune hepatitis, liver cirrhosis, hepatocellular carcinoma (HCC) patients and significant alcoholic consumption (more than 21 drinks/week in males and 14 drinks/week in females).

- A consent was taken from all individuals in this study. The study was approved by the ethical committee of Ain Shams University School of medicine. The work has been carried out in accordance with the code of Ethics of the Wold Medical Association (Declaration of Helsinki) for experiments in humans.