Diabetes & Metabolic Syndrome: Clinical Research & Reviews 13 (2019) 2226–2229

Contents lists available at ScienceDirect

Diabetes & Metabolic Syndrome: Clinical Research & Reviews

journal homepage: www.elsevier.com/locate/dsx

Original Article

Serum Ferritin level, microalbuminuria and non-alcoholic fatty liver disease in type 2 diabetic patients

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ARTICLE INFO

Article history:
Received 3 May 2019
Accepted 24 May 2019

Keywords:
Type 2 diabetes mellitus
Non-alcoholic fatty liver disease
Microalbuminuria
Serum ferritin

ABSTRACT

Background: Non-alcoholic fatty liver disease (NAFLD) was considered one of the most common causes of chronic liver disease and is considered the hepatic manifestation of type 2 diabetes mellitus (T2DM). The factors that lead to marked fibrosis and liver cell injury in NAFLD are still remaining undiscovered.
Patients and methods: This study included (40) type 2 diabetic patients with NAFLD and (40) diabetic patients without NAFLD beside 15 healthy persons as a control group. All of them were subjected to full history taking, thorough clinical examination with especial stress on body weight (BW), height, body mass index (BMI), waist-hip ratio, blood pressure. Laboratory tests included serum total cholesterol (TC), triglycerides (TG), low density lipoprotein (LDL) and high-density lipoprotein (HDL), fasting blood glucose (FBG) and 2-h postprandial blood glucose (PBG), serum Ferritin and urine microalbuminuria (MAU).
Results: Duration of diabetes, BW, BMI and blood pressure were significantly higher in NAFLD group ($P = 0.001$). FBG, PBG, TC, TG, LDL, serum Ferritin and MAU were significantly increased in NAFLD group with significant difference between two studied groups as regard HDL. There was a highly significant correlation between serum Ferritin with BW, BMI, duration of diabetes, TC, TG, LDL and MAU. There was a significant correlation between serum Ferritin with age, waist hip ratio, duration of diabetes, SBP, FBG, PBG and HDL. There was a significant correlation between MAU and age, weight, BMI, waist hip ratio, duration of diabetes, DBP, FBG TC, TG, LDL and HDL.
Conclusion: NAFLD is a common liver disorder in diabetic patients. NAFLD is significantly associated with microalbuminuria and elevated serum Ferritin.

1. Introduction

Increase obesity in the world is the most common cause in increasing the incidence of T2DM which is a fertile condition for the development of NAFLD and increase of its complications [1]. Adipose tissue dysfunction is associated with central obesity with production of adipokines that promote the development of insulin resistance in T2DM. Insulin resistance facilitates lipolysis of triglycerides by hormone sensitive lipase. This leads to increase free fatty acid flux to the liver and development of NAFLD which is considered the hepatic manifestation of T2DM [2]. NAFLD is considered to be one of the most common causes of chronic liver disease [3]. It is associated with increase lipid deposition in the hepatocytes. It is ranged from mild steatosis (simple fatty liver), to nonalcoholic steatohepatitis (NASH), [fatty changes with inflammation and hepatocellular injury), to advanced sever fibrosis and cirrhosis [4].

Studies reported that although the simple fatty liver is a benign condition, NASH can lead to end-stage liver disease and even may lead to liver transplantation [5]. The disease is mostly silent and discovered through incidentally elevated liver enzyme levels [6]. The factors that lead to fibrosis and liver cell injury in NAFLD are still remaining undiscovered and further researches are needed to recognize them in order to develop an effective treatment [7]. Some studies suggested that an increase in the iron level may be a reason for more liver injury in NAFLD but the mechanism by which this occurs still remain undiscovered [8]. The presence of MAU in T2DM patients with NAFLD may increase the incidence of cardiovascular

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https://doi.org/10.1016/j.dsx.2019.05.030
1871-4021/

risk and chronic kidney disease [9].

The pathogenic association between NAFLD in T2DM patients and MAU is still unclear however, some researchers suggested to be caused by pro-inflammatory cytokines secreted by the liver. It is unknown whether MAU is associated with NAFLD among patients with T2DM [10]. However, up to our knowledge, there are no available reports demonstrate the relationship between serum Ferritin level and MAU in diabetic patients with NAFLD.

1.1. Patients and Methods

This is a case control, cross-sectional study, which conducted in Internal Medicine Department, Tanta Faculty of Medicine and Internal Medicine Department, El-Sinbellawin General Hospital. It included (40) type 2 diabetic patients diagnosed to have NAFLD (group I) and (40) diabetic patients without NAFLD (Group II) besides, 15 apparently healthy persons of matched age and sex as a control group (group III).

Exclusion criteria:

Patient was excluded from this study if he has any of the following:

1. Conditions which affect serum Ferritin level, e.g.: rheumatoid arthritis, hyperthyroidism, leukemia, iron poisoning, or frequent blood transfusion.
2. Benign or malignant tumors.
3. Patients aged less than 18 years.

All patients will be subjected to:

- Full History taking.
- Thorough clinical examination with especial stress on measuring body weight, height, body mass index (BMI), waist-hip ratio and blood pressure.
- **Laboratory tests: b**lood sample were taken from all subjects to evaluate:
  - Lipid profile: serum TC, TG, LDL and HDL. TC as well as TG are measured by cholesterol oxidase, glycerol-3-phosphateoxidase (GPO), phenol and amino phenazone (PAP) methods (Roche Life Science, US). LDL and HDL were measured by direct immune suppression method (DESAY diagnostics system Co. Ltd., Shanghai, China).
  - FBG and 2-h PBG were determined by the hexokinase method.
  - Serum Ferritin level measured by commercially available kits (Human Ferritin Elisa Kits). Ferritin ELISA is used for the quantitative determination of Ferritin in the serum. This measurement is used to diagnose diseases which is affected by iron level. Ferritin Quantitative Test principle is a solid phase enzyme-linked immunosorbent assay [11]. This assay utilizes rabbit anti-Ferritin for solid phase (microtiter wells) immobilization and mice monoclonal anti-Ferritin in the antibody-enzyme (horseradish peroxidase) conjugate solution. The sample react with antibodies, result in the Ferritin molecules to be trapped between the solid phase and enzyme-linked antibodies. After 45 min incubation at room temperature, the tubes were washed by water to erase unbound labeled antibodies. We add solution of 3, 3′, 5, 5′-Tetramethylbenzidine (TMB) incubated for 20 min which results in development of blue color. The development of the color stopped with addition of 1 N HCl, and the produced yellow color was measured spectrophotometrically at 450 nm. Concentration of Ferritin is directly proportional to the intensity of the color produced. Normal range in male (20–250) and female (10–120).
  - Urine samples were taken to evaluate MAU. Albumin was measured by immune turbidimetry utilized by antibody against human albumin in an automated immune analysis system. (Including Tina-Quant Albumin reagents kit used to determine albumin in urine Roche Diagnostics, Indianapolis 2007). The preferred specimen is a 24-h collection, also 10-h through night collection (10 p.m.–8 a.m.) also randomized collection is accepted [12] with a reference values, 24-Hour excretion: <30 mg/24 h
- Abdominal ultrasound was done to diagnose fatty liver disease. All subjects did a pelvi-abdominal ultrasonography and the presence of fatty liver was graded by a professional radiologist who doesn't know laboratory results of the subjects. Fatty Liver was graded on a scale of 0–3: 0, no fatty liver; 1, mild; 2, moderate; 3, severe. On the principle of intense level echoes comes from the hepatic parenchyma, echo is penetrated into deep portion of the liver and show liver blood vessel structure. Steatosis was graded by Saverymuttu et al., 1986 [13].

1.2. Statistical methods

Data entry and statistical analyses are done by SPSS (statistical package of social sciences) version 21 (SPSS Inc., Chicago, IL, USA) [14]. Normally distributed data are expressed in mean and standard deviation. Categorized data are expressed in number and percentage. Continuous none normally distributed data were expressed in median and range. The quantitative data were examined by Kolmogorov Smirnov test for normality of data. Chi square test was used to compare categorical data. Independent sample *t*-test (student *t*-test) was used for continuous normally distributed data. Analysis of variance (ANOVA) test was used for multivariate continuous normally distributed data. Statistical significance was considered when probability (P) value was less than or equal to 0.05.

2. Results

Body weight, BMI, systolic blood pressure (SBP) and diastolic blood pressure (DBP) are significantly higher in DM with NAFLD group compared to other groups ($P = 0.0001$, 0.0001, 0.001 and 0.011respectively). Diabetes duration was significantly higher in DM with NAFLD group compared to non NAFLD group. There is no significant difference between the studied groups as regard height and waist-hip ratio ($P > 0.05$). (Table 1).

Laboratory investigations of the studied groups revealed a significant increase of FBG, PBG, TC, TG, LDL, HDL, serum Ferritin and MAU in DM with NAFLD compared to other two groups. (Table 2). Table 3 revealed a significant positive correlation between serum Ferritin and age, diabetes duration, weight, BMI, waist hip ratio, systolic blood pressure, FBG, PBG, TC, TG, LDL and MAU in NAFLD group. There was a significant correlation between serum Ferritin and diabetes duration, SBP, DBP FBG and PBG in non-NAFLD group. It is obvious that correlation between serum Ferritin and clinical and laboratory parameters has higher significant value in the NAFLD group compared to the non-NAFLD group.

3. Discussion

This study aims to know the relationship between serum Ferritin levels, MAU in T2DM patients with NAFLD and compare results with diabetic patients without NAFLD in Egyptian population. Body weight, BMI, blood pressure is significantly higher in DM with NAFLD group. Duration of diabetes in DM with NAFLD is significant higher than in DM without NAFLD ($p = 0.001$) while, height and waist hip ratio did not show any significant difference ($p > 0.05$). Visceral obesity and increased BMI are obvious risk

Table 1
Revealed clinical data of the studied groups.

Items	DM with NAFLD *(N = 40)*	DM without NAFLD *(N = 40)*	Control *(N = 15)*	P
	M±SD	M±SD	M±SD	
Weight	89 ± 11.47	79.3 ± 7.1	79.6 ± 10.01	0.0001**
Height	168.9 ± 7.4	167 ± 5.2	171.2 ± 9.2	0.285
BMI	31.2 ± 4.3	28.1 ± 2.2	27.1 ± 2.2	0.0001**
WHR	0.929 ± 0.07	0.905 ± 0.07	0.88 ± 0.08	0.122
SBP	126.2 ± 8.9	120.8 ± 10.6	118.6 ± 7.4	0.001*
DBP	83.3 ± 5.4	78.5 ± 7.6	77.6 ± 5.6	0.011*
Diabetes duration	81.5 ± 56.4	41.5 ± 42.5	–	0.001*

*p < 0.05 is significant.
**p < 0.0001 is highly significant.

Table 2
Revealed laboratory data of the studied groups.

Items	DM with NAFLD *(N = 40)*	DM without NAFLD *(N = 40)*	Control *(N = 15)*	P
	M±SD	M±SD	M±SD	
Weight	89 ± 11.47	79.3 ± 7.1	79.6 ± 10.01	0.0001**
Height	168.9 ± 7.4	167 ± 5.2	171.2 ± 9.2	0.285
BMI	31.2 ± 4.3	28.1 ± 2.2	27.1 ± 2.2	0.0001**
WHR	0.929 ± 0.07	0.905 ± 0.07	0.88 ± 0.08	0.122
SBP	126.2 ± 8.9	120.8 ± 10.6	118.6 ± 7.4	0.001*
DBP	83.3 ± 5.4	78.5 ± 7.6	77.6 ± 5.6	0.011*
Diabetes duration	81.5 ± 56.4	41.5 ± 42.5	–	0.001*

*p < 0.05 is significant.
**p < 0.0001 is highly significant.

Table 3
Correlation between serum Ferritin and clinical and laboratory parameters in studied groups.

	NSFLD group		Non-NAFLD group	
	R_1	P_1	R_2	P_2
Age	0.51	0.001*	0.25	0.113
Diabetes duration	0.719	0.0001**	0.45	0.003*
Weight	0.67	0.0001**	0.08	0.624
Height	−0.023	0.077	−0.12	0.45
BMI	0.82	0.0001**	0.207	0.201
Waist hip ratio	0.48	0.001*	0.12	0.454
SBP	0.51	0.001*	0.32	0.041*
DBP	0.24	0.12	0.39	0.013*
FBG	0.42	0.006*	0.37	0.018*
PBG	0.89	0.001**	0.31	0.047*
TC	0.76	0.0001**	0.07	0.664
TG	0.79	0.0001**	0.31	0.05
LDL	0.76	0.0001**	0.17	0.284
HDL	0.23	−0.042*	−0.303	0.057
MAU	0.73	0.0001**	0.86	0.0001**

*p < 0.05 is significant.
**p < 0.0001 is highly significant.

factors for NAFLD. The incidence of NAFLD exceed 90% and about 5% of patients may have suffer from cirrhosis in patients with marked obesity and has undergone bariatric surgery [15,16]. Studies suggested that reduction of body weight has a therapeutic effect in NAFLD with ALT elevation in obese patients. The ALT normalization rate has a positive correlation with the body weight reduction percentage in 45 patients who has achieved 48 weeks' of body weight reduction program. Score of fatty liver which assess the severity of fatty liver estimated by the ultrasonography was remarkably decreased after body weight reduction [17]. In our study there was a high significant difference between NAFLD group and non-NAFLD one regarding FBG, P2HBG, TC, TG, LDL, HDL, serum Ferritin and MAU. Previous studies revealed a significant positive association between serum TG and NASH, also level of TG and degree of periportal fibrosis [18]. Our study revealed that serum Ferritin level was higher in patients with NAFLD than in without NAFLD ts with high significant association between serum Ferritin and degree of fatty liver in NAFLD group. Some studies predict liver injury by increasing serum Ferritin concentration. Liver injury is attracted by hepatic iron itself, although the location of iron in the cells of the liver vary in genetically distinct populations. There is an increasing link between adipose tissue iron and adipose tissue dysfunction with the dysregulation of adipokines which enhance adipose tissue lipolysis and inflammation.

Many theories try to explain the mechanisms that link adipose tissue iron to liver injury. It was found that serum Ferritin is associated with liver injury among patients with fatty liver [19]. In our study none of our subjects has any sign included with hemochromatosis and the level of Ferritin was high. There was a theory that observe that not all steatosis progresses to steatohepatitis which is called the two −hit theory [20,21]. Some studies suggest minimizing foods which are high in iron, as red meat to prevent NAFLD [22]. Healthy obese patients suffering from fatty liver should also limit alcohol, vitamin C, and nutrition supplements containing iron due to their effect in enhancing the absorption of iron [23].

In our study there was a high significant correlation between serum Ferritin with body weight, BMI, duration of diabetes, TC, TG, LDL and MAU in NAFLD patients. There was a significant correlation in serum Ferritin with age, waist hip ratio, and systolic blood pressure, FBG, PBG and HDL. These correlations are more significant than in DM without NAFLD patients. In our study there was a high significant association between MAU and degree of fatty liver. There was a high significant correlation between MAU with age, weight, BMI, waist hip ratio, duration of diabetes, TC, TG and LDL. These correlations are more significant in NAFLD patients. Some studies found that diabetes mellitus patients with NAFLD have a poorer long term glycemic control. Also, lipid profile deterioration in which there is s higher TG and TC levels and low HDL is associated with presence of NAFLD compared with controls.

We have found that, incidence of MAU is remarkably higher in diabetic patients with NAFLD. Microalbuminuria prevalence in

2489 non-diabetic, non-hypertensive patients was investigated and reported that the NAFLD group had a significantly higher prevalence of microalbuminuria compared with the non-NAFLD group. Glomerular endothelial dysfunction may be caused by iron-mediated cell injury, which leads to MAU [24]. This finding agrees with the studies reported that presence of fatty liver in diabetic patients increases the microvascular complications, as retinopathy and nephropathy [25]. Some studies suggest that NAFLD enhance inflammation, may be by releasing the injured hepatocytes proinflammatory cytokines, which is caused by reactive oxygen species derived from steatosis stimulated fatty liver oxidation. This circle is thus promoted increasing consequently hepatic injury and subclinical systemic inflammation [26].

Increased oxidative stress, subclinical inflammation, lipid abnormalities, endothelial dysfunction and an abnormal adipocytokine profile are the mainly accused factors linking hepatic steatosis with vascular diseases [27,28]. Other study assess the relationships between liver histology and MAU in 87 adults who was proven NAFLD by a biopsy. They defined that 14 patients had MAU and those cases had a significantly higher insulin resistance values as well as the mean fibrosis scores compared with the cases without microalbuminuria [29].

4. Recommendation

We suggest that MAU and serum Ferritin should be evaluated among NAFLD cases especially with advanced stages and if present patients should be followed more strictly. As MAU and increased serum Ferritin were linked with NAFLD, more studies which will focus on the mechanism, causal effect relationship with Ferritin and MAU and new plan in treatment of NAFLD is needed.

Financial support

No financial support.

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