

## Study of serum ferritin, serum uric acid and plasma malondialdehyde (MDA) levels in non-alcoholic fatty liver disease

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### Abstract

**Background:** Non-alcoholic fatty liver disease (NAFLD) is one of most important cause of fatty liver that may lead to end-stage liver disease. Various studies have reported that serum ferritin, serum uric acid and plasma malondialdehyde (MDA) levels are related to the development of NAFLD. Diabetes and obesity are the major risk factors which are associated with NAFLD. The aim of this study was estimation of serum ferritin, serum uric acid and plasma MDA levels in NAFLD patients with diabetes and with obesity.

**Materials and Methods:** In the present study, total (n = 230) subjects were recruited for the study and divided in three groups. Group I; (n = 79) diagnosed cases of non-alcoholic fatty liver disease with diabetes, group II; (n = 71) diagnosed cases of non-alcoholic fatty liver disease with obesity patients and group III included (n = 80) healthy control subjects. Serum ferritin was measured by ELISA method. Estimation of serum uric acid was done by uricase peroxidase colorimetric method. Plasma MDA was estimated by spectrophotometric method.

**Results:** The present study shown that serum ferritin, serum uric acid and plasma MDA levels were significantly increased (p<0.001) in NAFLD with diabetes mellitus patients (333.42±82.93, 14.29±1.87, 7.75±3.35, respectively) as compared with healthy controls (126.30±72.12, 5.19±1.72, 2.79±0.52, respectively), and also significantly increased (p < 0.001) in NAFLD with obesity patients (300.87±85.80, 12.08±2.81, 7.43±3.05, respectively) when they compared with healthy controls (126.30±72.12, 5.19±1.72, 2.79±0.52, respectively).

**Conclusion:** Serum ferritin, serum uric acid and plasma MDA are associated with the increased risk for non-alcoholic fatty liver disease.

**Keywords:** Malondialdehyde (MDA), Non-alcoholic fatty liver disease (NAFLD), Reactive oxygen species (ROS)

### 1. Introduction

Non-alcoholic fatty liver disease (NAFLD) is characterized the major cause of chronic liver diseases that may lead to end-stage liver disease [1]. NAFLD enhance the progression of fibrosis, liver cirrhosis, and liver failure [2]. Fat accumulation in the liver cell is characteristic feature of development of NAFLD [3]. The pathogenesis of NAFLD has not been fully understood, but it is observed that obesity, diabetes and hyperlipidemia are the major risk factors which are associated with development of non-alcoholic fatty liver disease [4, 5, 6]. NAFLD is most prevalent disease in developed [7] as well as in developing countries. In India the prevalence rate of NAFLD is 9-32% in among population [8]. In addition to its high prevalence rate, the most important challenge is early diagnosis and risk stratification of NAFLD.

Serum ferritin is an acute-phase protein and its concentration fluctuated in inflammation of liver, hepatic necrosis and alcoholism [9]. Increased levels of serum ferritin have been reported in patients with type 2 diabetes mellitus [10, 11] and different metabolic abnormalities [12] includes high fasting plasma glucose and high serum total cholesterol. Increased ferritin may be intermediated by undiagnosed non-alcoholic fatty liver disease [13] However, the relationship of serum ferritin with NAFLD is not clear.

Uric acid is the end product of purine nucleotides metabolism and excreted through the urine [14]. Previous studies have been shown that increased serum uric acid has also been implicated in the development of metabolic syndrome, hypertension, kidney disease, and cardiovascular disease [15]. The mechanism includes oxidative stress, systemic inflammation, and endothelial dysfunction which

are induced by increased serum uric acid [16, 17]. However, the NAFLD was considered a hepatic manifestation of the metabolic syndrome [18]. Evidence suggests that increased serum uric acid levels associated with increased serum liver enzymes, liver cirrhosis and the development or progression of NAFLD [19, 20, 21]. Therefore, there might be a relationship between uric acid levels and NAFLD.

Malondialdehyde (MDA) is a product of lipid peroxidation and marker for oxidative stress [22]. Oxidative stress is an important player in the pathogenesis of fatty liver to non-alcoholic fatty liver disease [23]. The increased production of reactive oxygen species (ROS) is most common cause of lipid peroxidation, followed by an inflammatory response, and activation of hepatic cells leading to liver fibrosis and liver damage [24, 25, 26].

There are several laboratory investigations have been used for diagnosis of NAFLD. However, a very little information available that the serum ferritin, uric acid and MDA are related to the NAFLD. Therefore, the present study was undertaken to estimate the levels of serum ferritin, serum uric acid and plasma MDA in non-alcoholic fatty liver disease patients.

## 2. Material and Methods

The present study was carried out in the Department of Biochemistry, Sri Aurobindo Institute of Medical sciences (SAIMS), Indore, M.P., India between July 2014 to September 2015. Patients were enrolled from Department of Medicine, Gastroenterology clinic, SAIMS College and Hospital, Indore. Total (n = 230) subjects were selected for the study and divided in three groups. Group I; included (n = 79) diagnosed cases of non-alcoholic fatty liver disease with diabetes, group II; included (n = 71) diagnosed cases of non-alcoholic fatty liver disease with obesity. The age range was between 25 to 75 years for both males and females. Group III included (n = 80) healthy control subjects with age and sex matched for cases. Cardiovascular associated liver disease, malignancy, kidney disease, asthma, gout and other infectious diseases were excluded from the study. The study was approved by the Institutional Ethical Committee and patients were recruited for the study after taking their written informed consent. A detailed physical examination was done which included measuring of height, weight and blood pressure.

### 2.1. Sample collection and analysis of biochemical parameters

The blood samples from patients necessary for biochemical parameters were obtained after 12 hours fasting. Blood samples transferred in EDTA tubes were centrifuged at 3000 rpm for 15 min and their plasma fractions were used to measure MDA levels and plasma glucose, and blood samples collected in plain tubes were used for the analysis of serum ferritin, serum uric acid, serum liver function tests and serum lipid profile. Determination of serum ferritin was done by

commercial ELISA kit [27]. Estimation of serum uric acid was carried out by uricase peroxidase colorimeter method at 505 nm [28]. Plasma MDA was measured by spectrophotometric method at 531nm [29].

### 2.2 Statistical analysis

The statistical analysis was carried out by the SPSS statistics version 20.0. Values are presented as means  $\pm$  standard deviation (means  $\pm$ SD).  $p < 0.05$  was considered as significant level.

## 3. Results

### 3.1. Age distribution in study subjects

The age distribution in non-alcoholic fatty liver disease and healthy control subjects are listed in [Table 1]. Out of total (n = 150) subjects, seventy nine NAFLD patients were having diabetes mellitus and seventy one were having obesity. According to age distribution, 41 to 60 years age group subjects were in higher numbers as compare to other age groups.

### 3.2 Gender distribution in study subjects

Gender distributions in non-alcoholic fatty liver disease are shown in [Table 2]. Out of the 79 NAFLD with diabetes cases, 41 patients were males and 38 were females. Of the 71 NAFLD with obesity, 28 patients were males and 43 were females. In healthy controls, 53 were males and 27 were females. In age group 41-60 years, the prevalence of diabetes and obesity with NAFLD is more in females as compare to males.

### 3.3 Demographic and biochemical characteristic profile of NAFLD with diabetes patients

The basal metabolic rate (BMI), plasma glucose, serum SGPT, SGOT, triglyceride, and total cholesterol levels in NAFLD with diabetes mellitus patients and healthy control are shown in [Table 3]. The plasma glucose, serum SGPT, SGOT, triglyceride, and total cholesterol levels were found significantly increased as compared to healthy controls. However, no significant change was found in BMI when NAFLD with diabetes patients compared to healthy controls.

### 3.4 Demographic and biochemical characteristic profile of NAFLD with obesity patients

BMI, plasma glucose, serum SGPT, SGOT, triglyceride, and total cholesterol levels in NAFLD with obesity patients and healthy control are shown in [Table 4]. The BMI, plasma glucose, serum SGPT, SGOT, triglyceride, and total cholesterol levels were found significantly increased in NAFLD with obesity patients as compared to healthy controls.

### 3.5 Serum ferritin, serum uric acid and plasma MDA levels in NAFLD with diabetes mellitus patients

Serum ferritin level, serum uric acid and plasma MDA levels in NAFLD with diabetes mellitus patients and healthy control are shown in [Table 5]. The serum ferritin, serum uric acid, and plasma MDA levels were found

significantly increased in NAFLD with diabetes mellitus patients as compared to healthy controls.

### 3.6 Serum ferritin, serum uric acid and plasma MDA levels in NAFLD with obesity patients and healthy controls

Serum ferritin, serum uric acid and plasma MDA levels in NAFLD with obesity patients and healthy controls were listed in [Table 6]. The serum ferritin, serum uric acid, and plasma MDA levels significantly increased in NAFLD with obesity patients as compared to healthy controls.

**Table 1: Age distribution of all non-alcoholic fatty liver disease patients and healthy controls**

Age (year)	NAFLD with diabetes n = 79	NAFLD with obesity n = 71	Healthy controls n = 80
25 - 30	9	5	13
31 - 40	17	11	21
41 - 60	37	43	28
61-75	16	12	18
Total	79	71	80

**Table 2: Gender distribution of all non-alcoholic fatty liver disease patients and healthy controls**

Age (year)	NAFLD with diabetes n = 79		NAFLD with obesity n = 71		Healthy controls n = 80	
	Male	Female	Male	Female	Male	Female
25 - 30	4	5	2	3	9	4
31 - 40	11	6	4	7	13	8
41 - 60	17	20	16	26	17	11
61 -75	9	7	6	7	10	8
Total	41	38	28	43	49	31

**Table 3: Demographic and biochemical characteristics of NAFLD with diabetes mellitus patients and healthy controls**

Parameters	NAFLD with diabetes (n =79)	Healthy controls (n = 80)	p value
BMI (kg/m <sup>2</sup> )	18.6 ± 3.1	18.8 ± 1.9	NS
Plasma glucose (mg/dL)	176.11 ± 20.17	74.14 ± 8.66	< 0.001
SGPT (IU/L)	53.34 ± 11.61	26.00 ± 9.01	< 0.001
SGOT (IU/L)	48.59 ± 10.95	32.18 ± 8.12	< 0.001
Triglyceride (mg/dL)	203.82 ± 24.99	120.95 ± 13.61	< 0.001
Total cholesterol (mg/dL)	254.2 ± 84.22	165.38 ± 24.9	< 0.001

Data are presented as mean ± SD, p < 0.05 was considered as significant level

**Table 4: Demographic and biochemical characteristics of NAFLD with obesity patients and healthy controls**

Parameters	NAFLD with obesity (n =71)	Healthy controls (n = 80)	p value
BMI (kg/m <sup>2</sup> )	32.14 ± 7.23	18.8±1.9	< 0.001
plasma glucose (mg/dL)	157.21 ± 17.26	74.14±8.66	< 0.001
SGPT ( IU/L)	56.40 ± 12.32	26.00 ± 9.01	< 0.001
SGOT (IU/L)	49.77 ± 10.69	32.18 ± 8.12	< 0.001
Triglyceride (mg/dL)	199.49 ± 29.60	120.95 ± 13.61	< 0.001
Total cholesterol (mg/dL)	267.11 ± 87.39	165.38 ± 24.9	< 0.001

Data are presented as mean ± SD, p < 0.05 was considered as significant level

**Table 5: Serum ferritin, serum uric acid and plasma MDA levels in NAFLD with diabetes mellitus patients and healthy controls**

Parameters	NAFLD with diabetes (n = 79)	Healthy controls (n = 80)	p value
Ferritin (ng/dL)	333.42 ± 82.93	126.30 ± 72.12	< 0.001
Uric acid (mg/dL )	14.29 ± 1.87	5.19 ± 1.72	< 0.001
MDA (µmol/mL)	7.75 ± 3.35	2.79 ± 0.52	< 0.001

Data are presented as mean ± SD, p < 0.05 was considered as significant level.

**Table 6: Serum ferritin, serum uric acid and plasma MDA levels in NAFLD with obesity patients and healthy control**

Parameters	NAFLD with obesity (n =71)	Healthy controls (n = 80)	p value
Ferritin (ng/dL)	300.87 ± 85.80	126.30 ± 72.12	< 0.001
Uric acid (mg/dL)	12.08 ± 2.81	5.19 ± 1.72	< 0.001
MDA (µmol/mL)	7.43 ± 3.05	2.79 ± 0.52	< 0.001

Data are presented as mean ± SD, p < 0.05 was considered as significant level.

#### 4. Discussion

In the present study, we observed that obesity and diabetes mellitus were more prevalent in patients between 41-60 years age group. In this study, BMI was found statistically significant in NAFLD with obesity patients as compared with controls. A statistically significant difference was found for plasma glucose levels in NAFLD with diabetes and with obesity patients when compared both the groups with healthy controls. We observed mild statistically significant increase in SGPT and SGOT in NAFLD with diabetes and with obesity patients. We also observed statistically significant increased levels of triglyceride and total cholesterol in NAFLD with diabetes and with obesity in the present study. Hence, the results support that BMI is associated with the degree of fat infiltration in the liver [30]. In the Indian population, obesity is major cause for increased risk of steatosis [31]. According to Ludwig J *et al*, NAFLD in obese female case is related to the status of fibrosis [32]. The increased prevalence of diabetes and obesity are well thought out to be most important causes for non-alcoholic liver disease.

In the present study, we observed significantly increased serum ferritin levels ( $< 0.001$ ) in non-alcoholic fatty liver disease with diabetes mellitus and with obesity as compared with control group. In support to our results, anbakan *et al*, have found serum ferritin levels were higher in patients with NASH than in those with simple steatosis [33]. Puljiz *et al*, have also reported a statistically significant correlation between serum ferritin and NAFLD stages [34]. Some studies have shown that concentration of ferritin associated with an increased risk of obesity and diabetes and increased levels of serum ferritin is marker of non-alcoholic fatty liver disease patients [35, 36]. In contrast to our results, Angulo *et al*, have reported that serum ferritin, when controlled for age, obesity, diabetes and the SGOT/SGPT ratio, did not correlate with the severity of NAFLD [37]. The mechanism of increased levels of serum ferritin in patients with NAFLD might be due to systemic inflammation, increased iron stores, or both. Increased serum ferritin in non-alcoholic fatty liver disease with diabetic and with obesity patients is a reflector of body iron stores. Accumulation of iron in the liver cells may cause insulin resistance and repress hepatic glucose production [38, 39]. Increased formation of free radicals also responsible for the oxidative stress which can lead to hyperglycemia via altered glucose metabolism [40].

In the present study, we observed statistically significant increased serum uric acid levels ( $< 0.001$ ) in non-alcoholic fatty liver disease with obesity and with diabetes mellitus patients. The significant association between uric acid and non-alcoholic fatty liver disease suggest that high uric acid levels may play an important role in the development of NALFD. Previous studies have shown that the increase serum uric acid levels were independently and

positively associated with the risk for incident non-alcoholic fatty liver disease with obesity and with diabetes [41, 42, 43]. The injured liver cells released uric acid and induced inflammation [44, 45] In the normal condition the liver can be exported and transformed of large crystals of uric acid. Non-alcoholic steatohepatitis, is one of the important stage of liver damage which induce cell death results accumulation of uric acid and prevent the release of uric acid in the extracellular environment [46].

The level of plasma MDA, which is the end product of lipid peroxidation, was significantly increased ( $< 0.001$ ), in NAFLD with diabetes and with obesity patients as compared to control group. Previous studies have shown that the plasma MDA levels significantly increased in non-alcoholic fatty liver disease with obesity and with diabetes mellitus patients than healthy control subjects [47, 48]. It is reported that liver MDA, which is an indicator of lipid peroxidation, explain the presence of an enough antioxidant pool in the early stages of the disease before development of fibrosis [49]. Therefore, estimation of MDA would be an ideal approach in the pathogenesis of non-alcoholic fatty liver disease.

#### 5. Conclusion

The present study shows that the serum ferritin, serum uric acid and plasma MDA levels are associated with development of non-alcoholic fatty liver disease which may lead to liver cirrhosis. Diabetes mellitus and obesity are risk factors for increasing non-alcoholic fatty liver disease.

#### Conflict of interest statement

The authors declare that there is no conflict of interest.

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