ORIGINAL RESEARCH PAPER

Clinical Biochemistry

STUDY OF ASSOCIATION OF SERUM URIC ACID AND SERUM MAGNESIUM LEVELS IN NON-ALCOHOLIC FATTY LIVER DISEASE

KEY WORDS: NAFLD, serum magnesium, serum uric acid

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ABSTRACT

INTRODUCTION: Non alcoholic fatty liver disease (NAFLD) describes a spectrum of liver abnormalities from benign steatosis to non alcoholic steatohepatitis (NASH). Magnesium is a vital cation in our body. Hypomagnesaemia is associated with diabetes mellitus, hypertension and NAFLD. Serum uric acid (SUA) levels increased in metabolic syndrome. The aim of present study was to study the association of Serum Uric Acid and Serum Magnesium levels in NAFLD. **METHODOLOGY-**This cross sectional study was conducted in department of biochemistry and gastroenterology in SMS Medical College and hospital Jaipur. 30 NAFLD patients and 30 healthy controls were included. SUA and Mg levels calculated and compaired between NAFLD patients and healthy controls. **RESULT-** Significantly high ($p<0.01$) levels of uric acid was seen in patients (7.37±1.03mg/dl) with NAFLD in comparison to normal healthy controls (4.88±1.10 mg/dl). Significantly low ($p<0.01$) levels of Magnesium was seen in patients (1.29±0.27mg/dl) with NAFLD in comparison to normal healthy controls (2.15±0.29 mg/dl). **CONCLUSION-** Regular estimation of Serum uric acid and serum magnesium levels could be considered as a simple and non-invasive investigation for estimation of NAFLD.

INTRODUCTION

Non alcoholic fatty liver disease (NAFLD) describes a spectrum of liver abnormalities from benign steatosis to non alcoholic steatohepatitis (NASH). NASH is characterized by chronic and progressive liver pathology and can cause advanced fibrosis, cirrhosis, hepatocellular carcinoma, end-stage liver disease. NAFLD including both NAFL and NASH, is the most common cause of abnormal liver enzymes in the developed countries. The prevalence of NAFLD and NASH in adults in the United States is 30% to 40% and 3% to 12% respectively. It can develop at any age, and its prevalence increases with aging.[1,2]

NAFLD is defined as the ectopic accumulation of fat in the liver (hepatic steatosis) when no other causes of secondary liver fat accumulation are present. The diagnosis of NAFLD should not be made in a patient who has a history of significant alcohol consumption. The acceptable level of daily alcohol consumption is considered to be < 20 g/day in men and <10 g/day in women. NASH can progress to liver fibrosis, cirrhosis, and hepatocellular carcinoma with about 30% to 40% of patients developing fibrosis. NASH can be associated with other liver conditions such as chronic hepatitis C. NASH is classified into two types, primary; which is related to obesity and diabetes in the absence of excessive alcohol intake, and secondary; which is toxin or drug induced.[3-6]

Magnesium (Mg) is an abundant cation in the human body that is known to be involved in multiple physiological pathways like cellular energy metabolism, DNA transcription, protein synthesis and electrolyte balance.[8] Magnesium is also a vital cation in neuromuscular function and bone formation. Hypomagnesemia is therefore associated with osteoporosis, seizure, depression and several neuromuscular abnormalities.[7] Hypomagnesemia is also associated with diabetes mellitus, hypertension and dyslipidemia.

Uric acid is the major end product of purine metabolism, and the level of serum uric acid (SUA) is maintained by the balance between uric acid production and excretion.[11] Over the past few years, an association between SUA level and metabolic syndrome has been repeatedly demonstrated.[6,7] SUA levels are often increased in subjects with metabolic syndrome.[7,8] NAFLD is a hepatic manifestation of metabolic syndrome and an association between SUA level and metabolic syndrome has been well documented, the association between NAFLD and SUA level is controversial in the literature. Some studies have reported that NAFLD patients had higher SUA levels than controls. Further clarifying the relationship between SUA level and NAFLD may have significant clinical implications for the prevention and treatment of NAFLD by modulating the SUA level.

Therefore in lieu of conflicting data and paucity of data in our region, we conducted this study with the aim to study the association of Serum Uric Acid and Serum Magnesium levels in NAFLD.

AIM AND OBJECTIVES : To Study the levels and association of Serum Uric Acid and Serum Magnesium levels in NAFLD and compare among normal age and sex matched healthy control.

REVIEW OF LITERATURE

EPIDEMIOLOGY: The prevalence of NAFLD among the general population in India ranges from 9% to 53%.[8]. More recently, a population-based study from coastal south India reported an overall NAFLD prevalence rate of 49.8%; urban domicile was found to be associated with a higher risk for NAFLD after adjusting for sex, body mass index (BMI), DM, and metabolic syndrome. As a part of the ongoing community-based Prospective Urban Rural Epidemiology (PURE) cohort study in north India, prevalence of NAFLD was found to be higher in urban communities (53.7%) in comparison with rural communities (30.2%) ($P < 0.001$).[10] Among the high-risk groups, prevalence has been reported to be higher among those with type 2 DM, prediabetes, obesity, and metabolic syndrome. Further worrisome are the recent data showing a high prevalence of NAFLD in obese Indian children.

RISK FACTORS AND ETIOLOGY

Gender and age: Higher prevalence in males. Prevalence increases with age (20% in people younger than age 20) to greater than 40% in those who are older than 60 years of age.

Diet, smoking and lifestyle are independent risk factor for the development of NAFLD.

Polycystic ovarian syndrome(PCOS): PCOS is a common endocrine disorder in reproductive aged women and is typically characterized by obesity and insulin resistance. women with PCOS are at a heightened risk of developing T2DM and NAFLD.

GENETICS: In 2008, the first genome wide association study was published; hepatic triacylglycerol (HTAG) accumulation was found and identified association with increased HTAG and the PNPLA3 gene. This single nucleotide polymorphism is a nonsynonymous cytosine to guanine nucleotide transversion mutation that results in an isoleucine to methionine amino acid change. Subsequent work has confirmed this variant (PNPLA3 rs738409) in Japanese, Indian, and Chinese NAFLD patients.

Laboratory findings

serum markers like aminotransferases (AST, ALT), are mild to moderately elevated but can be nonspecific in NAFLD[11,12]. ALT elevations are more common than elevations of AST. The ALT levels tend to be higher in NASH than in simple steatosis. serum ferritin levels are commonly elevated and increased transferrin saturation is found in 6–11% of patients. ALP can be abnormal and even be elevated 2-3 times the upper limit of its normal value. Both albumin and bilirubin levels may be high in patients who have developed chronic progressive disease.

Imaging in NAFLD

Ultrasound reveals a hyperechoic texture or a bright liver because of diffuse fatty infltration. The sensitivity and the specifcity of US are respectively 89% and 93% in detecting increased fibrosis and steatosis. It is the cheapest and most commonly used .

Vibration-controlled transient Elastography (VCTE) is a non-invasive method for excluding advanced fibrosis in measuring liver stiffness with VCTE.

CT, MRI, and magnetic resonance spectroscopy (MRS): Both imaging modalities are able to detect steatosis, but lack sensitivity to detect inflammatory or fibrotic process of the liver. In general, the sensitivity of CT, MRI and MRS to detect steatosis of the liver was 33, 50, and 88%, respectively. Specificity of all three for detection of hepatic steatosis was 100, 83, and 63% respectively.

Histological findings: Liver biopsy is considered to be the gold standard in diagnosing NAFLD. It can be very helpful in assessing the amount of hepatic damage in general, but also in patients with unclear diagnosis after non-invasive assessments. The NAFLD Activity Score (NAS) is a validated score that is used to grade disease activity. The NASH has several components and each of them has a minimum and maximum score; steatosis (0 to 3), lobular inflammation (0 to 3), hepatocellular ballooning (0 to 2). Fibrosis is not included in the NASH.

NAFLD and Uric acid: The role of uric acid in causing NAFLD was recently explained via uric acid mediated generation of ROS and pro-inflammatory cytokines, which lead to increased expression of thioredoxin (TXN)-interacting protein (TXNIP), and ROS-dependent dissociation of TXN from TXNIP, which then interacts with NLRP3, activating the inflammosome in parenchymal and non- parenchymal liver cells, and resulting in release of IL-1b and IL-18. The ROS-TXNIP pathway inflammatory signaling induces deregulation of lipid metabolism related gene expression and lipid accumulation, through overexpression of the lipogenic enzyme, acetyl-coenzyme A (COA) carboxylase 1, fatty acid synthase and stearoyl-COA desaturase 1. Another mechanism for uric acid-mediated fat accumulation in liver proposed that uric acid induces oxidative stress in hepatocytes endoplasmic reticulum followed by cleavage into active form and nuclear translocation of the transcription factor, sterol regulatory element-binding protein (SREBP), which regulates the expression and activity of lipogenic enzymes[12].

Magnesium:. Normal serum magnesium concentration is 0.5-2 mEq/l and about one-third of this is bound to protein. Usually about a third of the magnesium intake is excreted in the urine, though under conditions of deprivation renal excretion is reduced to less than 0.5 mmol (mEq)/day. Ahad Eshraghian et alin 2018 showed that lower serum magnesium concentration was independently associated with biopsy-proven hepatic steatosis and steatohepatitis. While comparing those with only steatosis and those with steatohepatitis in liver biopsies, serum magnesium was significantly lower in participants with steatohepatitis.

A cross-sectional study in Brazil conducted by Lima Mde L et al in 2009 showed that serum and intracellular magnesium levels were lower in patients with components of metabolic syndrome like obesity, insulin resistance and patients with moderate to severe hepatic steatosis on ultrasound.[13]

MATERIALS AND METHODS :

Place of study: Department of Biochemistry and Central Lab, in association with Department of Gastroenterology, SMS Medical College and Hospital, Jaipur.

Study Type , Design: Hospital based comparative observational study, Cross sectional study.

Study Period: - March 2021 to Oct 2022.

Sample size:- The sample size was 30 subjects in each group. First group included patients with NAFLD or case group and second group included healthy controls

Inclusion Criteria : Cases:

Patients with NAFLD diagnosed on clinical, laboratory and radiological evaluation in OPD or admitted in our hospital with written consent and age between 20-60 years.

Control:-Demographically matched healthy individuals who were willing to participate in the study and had given written consent.

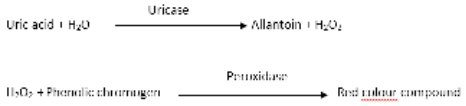
Exclusion criteria:- Age above 60 years and below 20 years, Patients with chronic liver disease (Except NAFLD), Dyslipidemia, Cardiovascular Diseases, Hypertension, Malignancy, Pregnant females, any other chronic illness.

Sample collection and Storage: - The blood samples of the patients and controls were taken in plain vials from outdoor & indoor in morning. The plain vial samples were left standing for 30 minutes, Serum was separated at 2500 rpm centrifugation and analyzed on fully automated analyzer Beckman Coulter AU-680.

Principle of Assay:-

URIC ACID: Liquid Uric acid is a reagent set for determination of Uric Acid based on enzymatic method using Uricase and Peroxidase.

PRINCIPLE: Uricase converts uric acid into allantoin and hydrogen peroxide. In presence of peroxidase, hydrogen peroxide oxidatively couples with phenolic chromogens to form a red coloured compound, which has maximum absorbance at 510 nm.(500- 530 nm.).The concentration of the red coloured compound is proportional to the amount of uric acid in specimen.



Calculation:

$$\text{Conc. (mg\%)} = \frac{\text{Withstandard Absorbance of Sample}}{\text{Absorbance of Standard}} \times 6$$

With factor for wavelength range : 500 - 510 nm. Conc. (mg%) = 28 x Absorbance of sample

Normal Values of SUA :- 3.7 - 6-5 mg/dl
Magnesium - Calmagite - EGTA. Colorimetric Quantitative determination

REAGENTS: R1- Buffer Amino-methyl-propanol 1 mmol/L, EGTA 0.21 mmol/L

R 2 - Chromogen Calmagite 0.30 mmol/L
MAGNESIUM STD Magnesium aqueous primary standard 2mg/dl PREPARATION Of Working reagent (WR): Mix equal volumes of R 1 Buffer and R 2 Chromogen. The working reagent is stable for 4 days at refrigerator (2-8°C) or 24 h at room temperature (15-25°C).

STORAGE AND STABILITY: All the components of the kit are stable until the expiration date on the label when stored tightly closed at 2-8°C protected from light and contaminations prevented during their use. Do not use reagents over the expiration date.

MAGNESIUM STD: Store at 2-8°C.The Standard is stable until the expiry date stated on the label. Signs of reagent deterioration: Presence of particles and turbidity. Blank absorbance (A) at 520 > 1.4.

PROCEDURE: Assay conditions: Wavelength:520 nm (500-550) , Cuvette 1 cm light path . Temperature 37°C / 15-25°C, Adjust the instrument to zero with distilled water. Pipette into a cuvette:

	Blank	Standard	Test
Reagent	1ml	1ml	1ml
Standard	-	10µl	-
Sample	-	-	10µl

1. Mix and incubate for 5 min at room temperature or 3min at 37°C.
2. Read the absorbance (A) of the samples and calibrator, against the Blank.The colour is stable for at least 30 minutes.

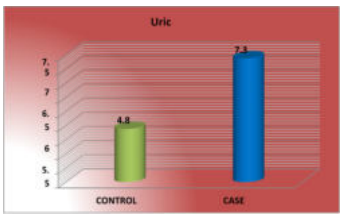
CALCULATIONS : (A)Sample x 2(Stan.con) = mg/dL magnesium (A)Standard Conversion factors: mg/dL x 0.412 = mmol/L or 0.5 mmol/L = 1.0 mEq/L = 1.22 mg/dL = 12.2 mg/L
REFERENCE VALUES: Serum magnesium: 1.6 – 2.5 mg/dL = 0.66 –0.03 mmol/L

OBSERVATIONS AND RESULTS

Table 1: Mean Uric Acid Levels between NAFLD and controls

Test/ Parameters	Control (n=30)	CASES (n=30)	P-value
Serum Uric Acid	4.88 ± 1.10	7.37 ± 1.03	< 0.01 (S)

Mg/dlDifference between mean Uric acid of both groups was statistically significant(p<0.01). P-value as obtained on applying students' t-test

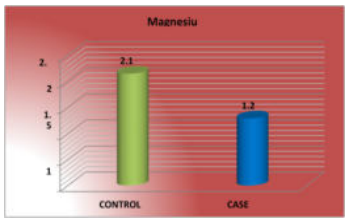


Graph 1: Comparison of Mean SUA between NAFLD Patients & controls

Table 2:Mean S. Magnesium Levels in NAFLD and controls

Test/ Parameters	Controls(n=30)	CASES (n=30)	P-value
Sr. Magnesium (mg/dl)	2.15 ± 0.29	1.29 ± 0.27	< 0.01 (S)

Difference between mean S. magnesium of both groups was statistically significant (p<0.01). *P-value as obtained on applying students' t-test.

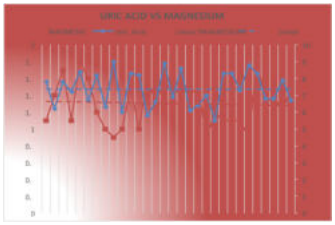


Graph 2: Comparison of Mean S. Mg in NAFLD & controls

Table 3: Correlation between SUA and Sr Magnesium in NAFLD Patients

Parameter	P value	R Score	R2	Significance
Uric acid vs Magnesium	0.002022	-0.5405	0.2921	S

Above table shows negative correlation(R= -0.5405) between Uric acid and magnesium levels in patients with NAFLD. This correlation was statistically significant(p<0.01). *Data analysis using Pearson correlation analysis



Graph 3: Correlation between SUA and Serum Magnesium in NAFLD Patients

DISCUSSION

The development of NAFLD is closely associated with obesity, type 2 diabetes mellitus, dyslipidemia and hypertension, which form a cluster of metabolic disorders that is now identified as metabolic syndrome. For this reason, NAFLD has been regarded as a hepatic manifestation of metabolic syndrome.[14]

In this study, 30 cases of NAFLD were included Uric acid, Magnesium and other routine parameters were compared with normal healthy controls and 0our cases and controls were age matched.

20(67%) males and 10(33%) females were included in the cases and controls both groups. It shows that male female ratio in our study was 2:1. It shows male preponderance of our study. According to our study, prevalence of NAFLD is more in males as compare to females.

A similar study conducted by Lonardo A et al in 2015 epidemiological review showed that NAFLD is more common in men and has been shown to increase in those who are younger to middle aged with a decline noted after the age of 50-60 years. In contrast, NAFLD has been shown to spare those women who are pre-menopausal and then a rise in incidence occurs after the age of 50 with a peak at 60-69 years, and the preponderance of evidence does seem to suggest that NASH is histologically more severe in women when compared to men.

SERUM URIC ACID : the present study shows that serum uric acid level was higher in patients with NAFLD in comparison to controls.This difference was statistically significant ($p<0.01$).

Result of the present study matches with the study conducted by Fengjiang Wei et al17 in 2020 who performed the Cox regression analysis, and their results showed that the HRs of NAFLD (95% CI) were 1.431 (95% CI, 1.123–1.823),1.610 (95% CI, 1.262–2.054), and 1.666 (95% CI, 1.287–2.157) across the second to the fourth quartile of SUA vs. the first quartile after adjusting for other confounders. The sex-specific association analysis between SUA and NAFLD has a similar tendency in males and females. Their findings suggest that elevated SUA levels promote the development of NAFLD, which is consistent with the previous hypothesis that high SUA might be an important contributor to the development of NAFLD.

Magnesium: The present study shows decreased levels of magnesium in NAFLD patients in comparison to controls and this difference was statistically significant ($p<0.01$).

Results of the present study are in concordance with study conducted by Ahad Eshraghian et al in 201815 which showed that lower serum magnesium concentration was independently associated with biopsy-proven hepatic steatosis and steatohepatitis. While comparing those with only steatosis and those with steatohepatitis in liver biopsies, serum magnesium was significantly lower in participants with steatohepatitis.

Insulin resistance may probably be the main underlying mechanism in pathogenesis of NAFLD. Magnesium plays a central role in insulin action and insulin regulates cellular magnesium concentration. A decreased magnesium level has been suggested to cause decreased tyrosine kinase activity and subsequent decreased ability of insulin to stimulate glucose uptake in adipose tissue and skeletal muscle. It seems that magnesium is involved in glucose transport in the insulin signaling pathway.

In the present study negative (R= -0.5405) and statistically significant ($p<0.01$) correlation was seen between Uric acid and magnesium levels in patients with NAFLD. It shows that in patients with NAFLD magnesium levels decreases with increase in level of uric acid significantly

CONCLUSION: The present study suggested that decreased serum magnesium is associated with NAFLD. Hypomagnesemia is not only a laboratory symptom of fatty liver but due to its connection with increased oxidative stress it might be a risk factor in the progression of fatty liver to NAFLD. Present study also demonstrated a significant correlation between high SUA level and NAFLD. So the present study suggests that regular estimation of Serum uric acid and serum magnesium levels could be considered as a simple and non-invasive investigations for estimation of NAFLD.

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