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RESEARCH ARTICLE

EVALUATION OF RENAL MICROVASCULAR DAMAGE BY PROTEINURIA IN NON ALCOHOLIC FATTY LIVER DISEASE (NAFLD)

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Abstract

Non alcoholic fatty liver disease (NAFLD) is a clinicopathological syndrome that is closely associated with obesity, dyslipidaemia, insulin resistance and diabetes mellitus, thus suggesting that NAFLD represents another component of metabolic syndrome. It is postulated that NAFLD, like metabolic syndrome, is associated with increased risk of atherosclerosis. In the present study 100 healthy subjects with incidental NAFLD were subjected to detailed history, physical examination including body-mass index (BMI) and biochemical investigations including renal function tests, lipid profile and liver function tests. Proteinuria was measured in these subjects to evaluate microvascular renal damage. Subjects with alcoholic liver disease, chronic viral hepatits, renal disease, etc were excluded. The results obtained were entered in Microsoft excel and was analysed using Epi info software version 7. The statistical analysis was done by using the ANNOVA test. Results showed there was highly significant ($p<0.001$) correlation between proteinuria and NAFLD. This correlation was more positive and progressive with increased grades of NAFLD. This signifies increased renal microvascular damage and progression to chronic kidney disease (CKD) in patients with NAFLD. The prevalence of NAFLD is increasing mainly due to increasing obesity and metabolic syndrome. The potential clinical implications of these finding for patient care are the detection of NAFLD by routine ultrasonography (USG) especially in obese and urine analysis for proteinuria in these persons. This will alert the clinician about cardiovascular risks and risk of CKD in these apparently healthy persons.

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INTRODUCTION

Non Alcoholic Fatty Liver Disease represents a spectrum of medical conditions in which there is increased infiltration of fat, predominantly triglycerides, inside the hepatocytes[1]. In 1980, Ludwig and colleagues from the Mayo Clinic coined the term "non-alcoholic steatohepatitis" (NASH) to describe a form of liver disease observed in middle aged patients with abnormal liver biochemical test results and histological evidence of alcoholic hepatitis but no history of alcohol abuse[2]. It is now clear that non-alcoholic steatohepatitis is a part of spectrum of Non Alcoholic Fatty Liver Disease (NAFLD) which encompasses simple fatty liver, NASH and NAFLD associated cirrhosis[3].

NAFLD is a clinicopathological syndrome that is closely associated with obesity, dyslipidaemia, insulin resistance and type 2 diabetes mellitus (T2DM), thus suggesting that NAFLD represents another component of metabolic syndrome[4].

NAFLD is a rapidly growing health problem in India along with other diseases like diabetes, hypertension, dyslipidemia and obesity[1,5]. The association of NAFLD with the features of the metabolic syndrome has raised an interest in its role in the development and progression of chronic kidney disease (CKD) and coronary artery disease

(CAD). Many studies suggested that NAFLD, CAD and CKD share common risk factors and pathogenic mechanisms and that NAFLD, in particular, is associated with an increased prevalence and incidence of these diseases[6]. This association appears to be independent of other potentially confounding factors, and they found it in diabetic as well as non diabetic patients. The mechanism responsible for this association has yet to be clearly described. Very few studies were carried out in India associating NAFLD with diabetic and prediabetic patients[7]. Microalbuminuria is a known surrogate indicator of subclinical cardiovascular and renal disease as well as vascular endothelial dysfunction.

Ultrasonographic grading of NAFLD varies depending on the amount of fat deposited and whether deposits are diffuse or focal[8].

Diffuse steatosis may appear as follows:

- Grade I -- Minimal diffuse increase in hepatic echogenicity with normal visualization of diaphragm and intrahepatic borders.
- Grade II -- Moderate diffuse increase in hepatic echogenicity with slightly impaired visualization of intrahepatic vessels and diaphragm.
- Grade III -- Marked increase in echogenicity with poor penetration of posterior segment of right lobe of liver and poor or no visualization of hepatic vessels and diaphragm[8].

Other non-invasive tests are available that are able to assess liver fat only within the spectrum of nonalcoholic fatty liver disease. Ultrasonography remains the recommended first-line imaging modality for diagnosing hepatic lipid accumulation (steatosis) in clinical practice, which is noted when a hyperechogenic or bright appearance is seen on imaging. Ultrasonography provides a subjective and qualitative assessment of hepatic fat content and generally is believed to be of only limited sensitivity (60%-90%) if <30% of hepatocytes are steatotic[9,10]. A recent meta-analysis has shown that the overall sensitivity and specificity of ultrasonography for the detection of moderate to severe fatty liver compared to histology were 84.8% and 93.6%, respectively[11].

Several lines of evidence link NAFLD to CKD. Large surveys showed an association between decreases in glomerular filtration rate (GFR) and/or proteinuria/albuminuria and liver biochemistry[12-14]. Several cross-sectional epidemiological studies in type 1 and type 2 diabetes[15], as well as in glucose intolerant patients[16], showed an association between CKD and hepatic steatosis assessed by ultrasonography. Finally, longitudinal studies, either in diabetic[17] or healthy subjects showed that patients with NAFLD more frequently developed CKD. That temporal evolution might suggest a cause-effect relation.

Microalbuminuria is an established marker for monitoring progression of chronic kidney disease and elevated urinary levels may be indicative of proximal tubular damage[18]. Measurement of albuminuria is also helpful for monitoring nephron injury and the response to therapy in many forms of CKD, especially chronic glomerular diseases, while an accurate 24 hours urine collection is the criterion standard for measurement of albuminuria, the measurement of protein to creatinine ratio in a spot first morning urine sample is often more practical to obtain and correlates well but not perfectly, with 24 hour urine collections. Microalbuminuria refers to the excretion amounts of albumin too small to detect by urinary dipstick or conventional measures of urine protein. It is a good screen test for early detection of renal disease and may be a marker for the presence of microvascular disease in general[19].

The high morbidity, mortality, and health care costs associated with CKD have led investigators to seek novel modifiable risk factors. Non-alcoholic fatty liver disease (NAFLD), the hepatic manifestation of the metabolic syndrome, affects 30% of the general adult population and up to 60%–70% of diabetic and obese patients[9].

The possible link between nonalcoholic fatty liver disease and CKD recently has attracted considerable scientific interest. Accumulating clinical evidence indicates that the presence and severity of NAFLD is associated significantly with CKD (defined as decreased estimated glomerular filtration rate and/or proteinuria) and that nonalcoholic fatty liver disease predicts the development and progression of CKD, independently of traditional cardiorenal risk factors. Experimental evidence also suggests that NAFLD itself may exacerbate systemic and hepatic insulin resistance, cause atherogenic dyslipidemia, and release a variety of proinflammatory, procoagulant, pro-oxidant, and profibrogenic mediators that play important roles in the development and progression of CKD. However, despite the growing evidence linking nonalcoholic fatty liver disease with CKD, it has not been definitively established whether a causal association exists. The clinical implication for these findings is that patients with nonalcoholic fatty liver disease may benefit from more intensive surveillance or early treatment interventions to decrease the risk of CKD[20].

The present study was undertaken in apparently healthy subjects with incidental finding of non alcoholic fatty liver disease on ultrasound abdomen for evaluation of Renal micro-vascular damage by measuring 24 hours urinary protein excretion. The aim of our study was to determine whether there was an association between NAFLD and Renal microvascular damage.

Material and methods

In this randomized open study, 100 apparently healthy subjects in age group 21-50 years with incidental finding of NAFLD on ultrasound abdomen, attending Sri Guru Ram Das Institute of Medical Sciences and Research Amritsar were included. Patients were divided into three different groups having 35 patients with fatty liver grade I and grade II each and 30 patients with fatty liver grade III. Proteinuria was measured by 24 hours urinary protein excretion. Informed consent was taken from every patient. Routine biochemical investigations were done. BMI (Body Mass Index) was measured by weight (Kg)/Height (m^2)

Patients of alcoholic liver disease, chronic viral hepatitis, renal disease, diabetes, hypertension, malignancy, autoimmune or connective tissue disease, taking medications which are renal or hepatic toxicity were excluded.

Proteinuria was measured by collecting 24 hours urine and proteins were measured in it. 24 hour urine was collected by discarding first early morning urine and then urine was collected for next 24 hours including next day early morning urine. An albumin excretion rate less than 30mg/day was considered within the reference range where as albumin excretion rate from 30 to 300mg/day was considered to indicate microalbuminuria and albumin excretion of more than 300 mg/day was considered as overt albuminuria or macroalbuminuria.

The results obtained were entered in Microsoft excel and was analysed using Epi info software version 7. The statistical analysis was done by using the ANNOVA test.

Results

In the present study, mean body mass index (BMI) in patients with grade I NAFLD was 28.54 ± 5.12 kg/m^2 and with fatty liver grade II was 31.89 ± 4.68 Kg/m^2 and that of study subjects with fatty liver grade III was 35.42 ± 4.71 Kg/m^2. This difference in mean BMI in study subjects with different fatty liver grades was found to be statistically highly significant ($p<0.001$). The results of the present study are in concordance with the study by Mahmut Acikel et al, which showed BMI in grade I was 26.5±4.5 , grade II was 29.3±4.0 and grade III was 31.7±3.3 and was statistically highly significant ($p<0.0001$)[21]. Similar significant correlation was seen in study by Ghobad Abangah and others and observations were statistically significant ($p<0.001$)[22]. The study by Afshin Mohammadi et al showed BMI of 28.79±2.99 in grade I NAFLD, 29.71±4.10 in grade II and 31.77±4.18 in grade III NAFLD with p value of <0.001 which was also statistically highly significant[23].

Considering correlation of serum triglyceride with grades of NAFLD, the present study showed that mean serum triglyceride of study subjects with fatty liver grade I was 176.43 ± 111.37 mg/dl, with fatty liver grade II was 223.60 ± 73.48 mg/dl and that of study subjects with fatty liver grade III was 351.27 ± 175.58 mg/dl. This difference in mean serum triglyceride in study subjects with different fatty liver grades was found to be statistically highly significant ($p<0.001$). Similar results were obtained in study by Abhijit Sen et al which showed that triglyceride level was also higher among the fatty liver Grade III (431.61±48.76 mg/dl) patients as compared to Grade I (203.81±33.55 mg/dl) and Grade II (278.34±70.41 mg/dl) and the differences were statistically significant ($p<0.0001$)[24]. This study is also in concordance with study by Mahmut Acikel et al and is statistically significant ($p<0.0001$)[21].

While studying the effect of NAFLD grades on proteinuria, statistically highly significant correlation ($p<0.001$) between grades of fatty liver and proteinuria was found. The study showed that mean 24 hour urinary protein of study subjects with fatty liver grade I was 191.21 ± 185.13 mg, with fatty liver grade II was 315.54 ± 225.91 mg and that of study subjects with fatty liver grade III was 549.60 ± 261.10 mg. This study is in concordance with Yilmaz et al. which showed a positive correlation between microalbuminuria and histological fibrosis stages in nondiabetic patients with NAFLD[25]. The study by Ivana Mikolasevica et al found that the severity of liver steatosis, defined by CAP values, was positively correlated with serum creatinine levels and negatively correlated with eGFR ($p<0.01$)[26]. Similar significant and independent trends were also found for albuminuria ($p<0.001$) in patients with histological defined fibrosis stages of nonalcoholic steatohepatitis than matched nonsteatotic controls and that the severity of nonalcoholic steatohepatitis by Giovanni Targher et al[27].

While correlating proteinuria with BMI, it showed that the difference in distribution of BMI of study subjects according to 24 hour urinary protein was found to be statistically highly significant($p<0.01$). The present study showed that patients with high BMI had higher range of proteinuria. Similar results were obtained in study by David N et al, where albumin excretion rate was found to be high in patient with high BMI ($p<0.001$)[28].

Discussion

This randomized open study was conducted at Sri Guru Ram Das Institute of Medical Sciences and Research, Amritsar to find the association between Non alcoholic fatty liver disease (NAFLD) and Renal microvascular damage.

1. There was strong association between different grades of NAFLD with proteinuria. As the Grade of NAFLD increased, there was proportional increase in proteinuria ($p<0.001$).
2. The difference in mean BMI in study subjects with different fatty liver grades was found to be statistically highly significant ($p<0.001$). As the BMI increased in participants, there was increase in incidence of NAFLD with increased Grades.
3. Serum total cholesterol and serum LDL levels had highly significant ($p<0.001$) correlation with increasing Grades of NAFLD. With the rise in serum triglycerides and LDL levels, there was proportional increase in the Grade of NAFLD.
4. Proteinuria had significant correlation ($p<0.01$) with BMI of participants. As the BMI of study subjects increased there was proportional increase in proteinuria.

In conclusion, there was highly significant ($p<0.001$) correlation between NAFLD and proteinuria. This correlation was more positive and progressive with increasing grades of NAFLD. This signifies increased renal microvascular damage and progression to CKD in patients with NAFLD. The prevalence of NAFLD is increasing mainly due to increasing obesity and metabolic syndrome.

The potential clinical implications of these finding for patient care are the detection of NAFLD by routine USG especially in obese and urine analysis for proteinuria in these persons. This will alert the clinician about cardiovascular risks and risk of chronic kidney disease in these apparently healthy persons.

Table 1

	Fatty Liver Grade I	Fatty Liver Grade II	Fatty Liver Grade III	p value
BMI (Kg/m^2)	28.54±5.12	31.89±4.68	35.42±4.71	<0.001
T. Cholesterol (mg/dl)	172.03±54.37	203.20±57.85	257.43±62.14	<0.001
S. Triglyceride (mg/dl)	176.43±111.37	223.60±73.48	351.27±175.58	<0.001
S. HDL (mg/dl)	36.34±14.65	37.40±12.75	36.83±12.16	>0.05
S. LDL (mg/dl)	108.17±43.41	128.63±42.69	154.47±42.39	<0.001
24 hour Urinary Protein (mg)	191.21±185.13	315.54±225.91	549.60±261.10	<0.001

Figure 1

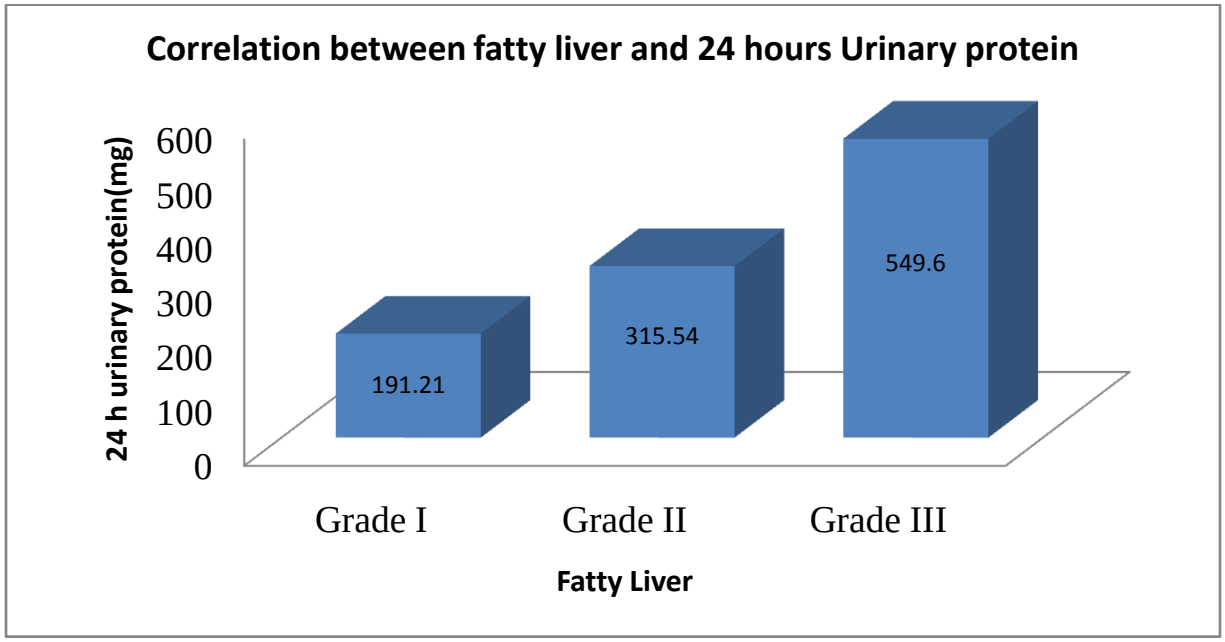
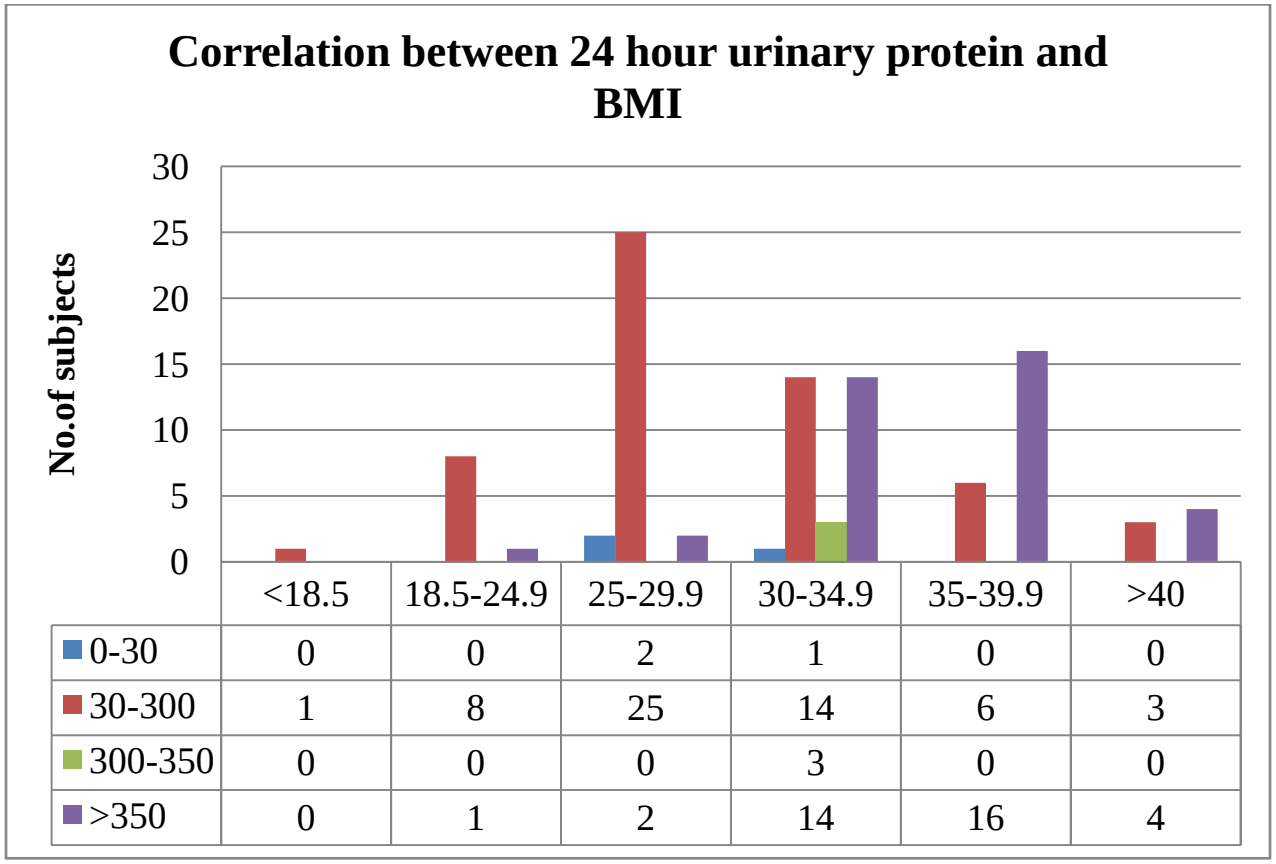


Figure 2



	<18.5	18.5-24.9	25-29.9	30-34.9	35-39.9	>40
0-30	0	0	2	1	0	0
30-300	1	8	25	14	6	3
300-350	0	0	0	3	0	0
>350	0	1	2	14	16	4

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