Evaluation of Renal Microvascular Damage by Measuring Microalbuminuria in Non-Alcoholic Fatty Liver Disease – A Study from Punjab, India

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ABSTRACT

BACKGROUND

Non-alcoholic fatty liver disease (NAFLD) is a clinicopathological syndrome characterized by obesity, dyslipidaemia, insulin resistance and diabetes mellitus (DM). NAFLD represents another component of metabolic syndrome. NAFLD and metabolic syndrome are associated with increased risk of atherosclerosis.

METHODS

In the present comparative cross-sectional study, 100 cases with incidental NAFLD and 100 healthy controls were subjected to detailed history, physical examination including body-mass index (BMI) and biochemical investigations including renal function tests (RFT), lipid profile and liver function tests (LFT). Microalbuminuria was measured in these subjects to evaluate microvascular renal damage. Subjects with alcoholic liver disease, chronic viral hepatitis, renal disease, etc were excluded.

RESULTS

The mean urine albumin creatinine ratio (UACR) in cases and controls was 35.81 ± 27.81 and 7.88 ± 0.78 respectively. The mean UACR in grade 1 fatty liver was 16.31 ± 5.45, in grade 2 fatty liver was 28.64 ± 12.91, and 72.8 ± 27.10 in grade 3 fatty liver and they were compared with each other and were found to be statistically significant with P value = 0.001.

CONCLUSIONS

The present study showed highly significant (P < 0.001) correlation between microalbuminuria and NAFLD. There was a positive correlation which progresses with increased grades of NAFLD. This correlation points towards a positive relationship between increased renal microvascular damage and progression to chronic kidney disease (CKD) in patients with NAFLD. The occurrence of NAFLD is increasing mainly due to increasing obesity and metabolic syndrome. The clinical implications of these findings for patient care are the detection of NAFLD by routine ultrasonography (USG) especially in obese people and urine analysis for microalbuminuria in these persons. This will help the clinician in determining cardiovascular risks and risk of CKD in these apparently healthy persons.

KEYWORDS

NAFLD, CKD, Albuminuria

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BACKGROUND

Non-alcoholic fatty liver disease represents a clinicopathological spectrum of medical conditions characterized by 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, dyslipidaemia and obesity.[5] Many studies suggested that NAFLD, coronary artery disease (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] 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

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 non-alcoholic 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.[12,13,14] 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] For monitoring nephron injury and the response to therapy in many forms of CKD, especially chronic glomerular diseases, measurement of albuminuria is helpful. 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 than 24-hour urine collection. Microalbuminuria refers to the amount 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, 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]

There is an experimental evidence which suggests that NAFLD itself may exacerbate systemic and hepatic insulin resistance, causing atherogenic dyslipidaemia, and release a variety of proinflammatory, pro-oxidant, procoagulant and profibrogenic mediators that play important roles in the development and progression of CKD.[20]

Objectives

1. To determine and compare the occurrence of micro albuminuria in patients with and without NAFLD.
2. To compare the occurrence of microalbuminuria among those with different grades of fatty liver

METHODS

The present study was undertaken in 100 cases of either sex with incidental finding of non-alcoholic fatty liver disease on ultrasound abdomen and 100 healthy controls with normal ultrasound abdomen attending Sri Guru Ramdas Institute of Medical Sciences and Research, Amritsar. Informed consent was taken from every patient. It was comparative cross-sectional study done from January 2019 to December 2020.

All patients were interviewed and detailed history was undertaken along with thorough general and systemic physical examination.

Inclusion Criteria

Patients of age more than 18 years having non-alcoholic fatty liver disease diagnosed on ultrasound abdomen.

Exclusion Criteria

1. Patients of alcoholic liver disease (i.e. male patient taking alcohol > 30 gm/day and female patient taking alcohol > 20 gm/day)
2. HBsAg, HCV and HIV positive patients
3. Patients taking drugs known to induce fatty liver like oestrogens, valproate, methotrexate, tamoxifen, corticosteroids, amiodarone, diltiazem.
4. Patients with history of diabetes mellitus and hypertension.
5. Patients with renal parenchymal disease.
6. Patients taking nephrotoxic drugs.
7. Patients with history of nephrotic or nephritic syndrome.
8. Patients with polycystic kidney disease.
9. Patients with malignant disorders.
10. Patients with autoimmune disorders.
11. Patients taking angiotensin converting enzyme (ACE) inhibitors or angiotensin II receptor blockers (ARBs).
12. Obese patients with BMI > 30.
13. Patients with history of smoking.

Physical examination included height and weight measurement for calculating body mass index. BMI = weight\height2 in kg\m2. Waist and hip measurements were taken in standing position and waist hip ratio (WHP) was calculated. Waist measurement was the smallest horizontal girth between the costal margin and iliac crests and hip measurement means greatest circumference at the level of greater trochanter. Routine biochemical investigations were done which included haemoglobin (Hb), total and differential leukocyte count, fasting plasma glucose levels, blood urea, serum creatinine, lipid profile (after 8 - 12 hours. fasting), liver function test [serum bilirubin total, direct bilirubin, alkaline phosphatase, serum glutamic oxaloacetic transaminase (SGOT), serum glutamic pyruvic transaminase (SGPT), gamma glutamyl transferase (GGT)], total serum proteins, serum albumin, serum globulin anti-HBs Ag, anti HCV, HIV rapid test.

Fasting plasma insulin levels and fasting plasma glucose levels were done and the index of homeostasis model assessment of insulin resistance (HOMA-IR) was calculated. Index of HOMA-IR = fasting plasma insulin levels (uIU/ml) x fasting plasma glucose / 22.5. Grading of non-alcoholic fatty liver was done on ultrasound abdomen as grade 1, grade 2 and grade 3 and was compared with level of albuminuria. Albuminuria was measured by urine albumin creatinine ratio (UACR). The first morning urine spot samples were collected to access UACR.

The level of albuminuria in non-alcoholic fatty liver disease diagnosed on ultrasound abdomen was determined and compared to the level of albuminuria in patients having normal ultrasound abdomen. eGFR was calculated by MDRD (Modification of Diet in Renal Disease) formula i.e., eGFR= 175 x (serum creatinine x $0.011^{)-1.234}$ x $(age)^{-0.179}$ x (0.79 if female). The association between NAFLD and CKD was established when eGFR comes < 60 ml/min/1.73m^2 All tests were conducted in the Department of Biochemistry, Sri Guru Ram Das Institute of Medical Sciences and Research, Amritsar who were not aware of the clinical status of the study patients. Data generated from the study was analysed according to standard statistical methods.

RESULTS

	Cases	Controls	P Value
Waist circumference	89.96 ± 3.92	86.55 ± 2.9	0.001*
BMI	24.12 ± 1.34	21.93 ± 1.07	0.001*
Cholesterol	142.4946.21	111.27 ± 12.58	0.001*
Triglyceride	175.92 ± 64	122.02 ± 11.60	0.001*
LDL	109.55 ± 29.10	94.82 ± 12.27	< 0.001*
UACR	35.81 ± 27.81	7.88 ± 6.78	< 0.001*
Creatinine clearance	81.73 ± 24.88	94.89 ± 22.46	< 0.001*

Table 1. Comparison of Different Parameters in Cases and Controls

*indicates statistically significant difference

	Fatty Liver Grade 1	Fatty Liver Grade 2	Fatty Liver Grade 3	P Value
UACR	16.31 ± 5.45	28.64 ± 12.91	72.8 ± 27.10	< 0.05*
Creatinine clearance	100.34 ± 19.82	78.2 ± 22.57	62 ± 15.29	< 0.05*

Table 2. Comparison of Different Parameters in Different Grades of Fatty Liver

*indicates statistically significant difference

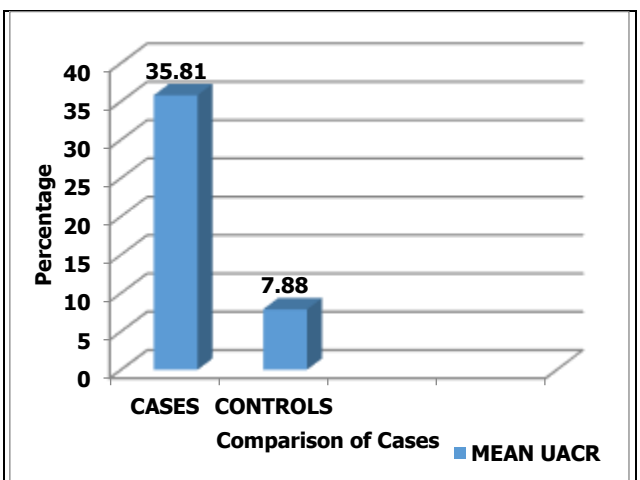

Figure 1. Comparison of UACR among Study Cases and Controls

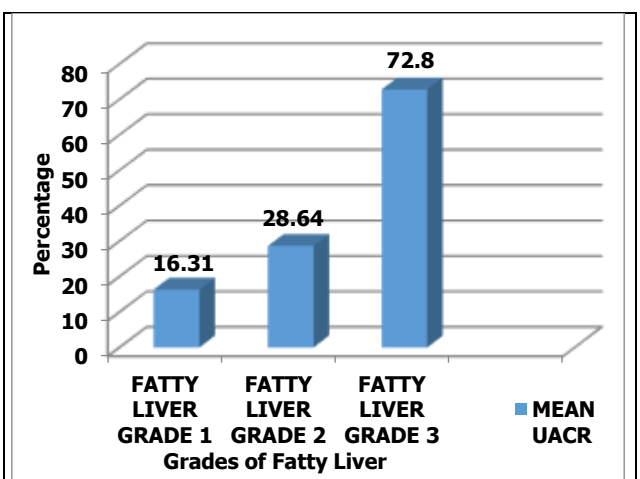

Figure 2. Comparison of UACR among Different Grades of Fatty Liver

The mean UACR in cases and controls was 35.81 ± 27.81 and 7.88 ± 0.78 respectively and comparison was statistically significant (P value = 0.001).

DISCUSSION

The present study was conducted for evaluation of renal microvascular damage by measuring microalbuminuria in non-alcoholic fatty liver disease. The study included 2 groups in which 100 individuals had fatty liver (categorized as 'cases') and 100 individuals did not have fatty liver (categorized as controls) on ultrasound abdomen. The group containing fatty liver individuals was further divided into subgroups based on different grades of fatty liver.

In the present study, the mean waist circumference in study cases was 89.96 ± 3.93 and in controls the mean waist circumference was 86.55 ± 2.9 with P value of 0.001 which was statistically significant. It was comparable to study conducted by Seok Hui Kang et al. in which mean waist circumference was in cases 94.7 ± 6.7 and 81.7 ± 7.8 in controls (P value < 0.001).[21]

In our study, the mean BMI in cases and controls was 24.12 ± 1.34 and 21.93 ± 1.07 respectively with P value = 0.001 which was statistically significant. It was comparable to study conducted by Takemi Akahane et al. in which mean BMI in cases and controls group was 22.0 ± 2.7 and 25.8 ± 3.3 respectively (P value < 0.001).[22]

Considering correlation of serum triglyceride with grades of fatty liver, in the present study mean triglyceride levels in cases and controls were 175.92 ± 64 and 122.02 ± 11.60 respectively. This difference was statistically significant with P value = 0.001. similar results were obtained in study by EL Azeem et al. in which mean triglyceride levels in cases and controls were 175.68 ± 51.48 and 151.49 ± 44.24 respectively (P value < 0.05).[23]

In the present study, mean total cholesterol in cases and controls were 142.49 ± 46.21 and 111.27 ± 12.58 respectively. They were compared and found to be statistically significant with P value = 0.001 and were in accordance to study by Melania Manco et al. which showed mean total cholesterol levels in cases and controls were 169.1 ± 35.2 and 132.2 ± 8.9. (P value < 0.05).[24]

In the present study, the mean low-density lipoprotein (LDL) levels in cases and controls were 109.55 ± 29.10 and 94.82 ± 12.27 respectively. They were compared with each other and were found to be statistically significant with P value < 0.001 and was according to study by Takemi Akahane et al. in which LDL levels in cases and controls were 135 ± 30 and 124 ± 29 respectively.[22]

The mean UACR in cases and controls was 35.81 ± 27.81 and 7.88 ± 0.78 respectively. The comparison was statistically significant with P value < 0.001 and was comparable to study conducted by H R Jang et al. in which mean UACR in cases and controls was 188 ± 30 and 212 ± 23 respectively.[25] The mean UACR in grade 1 fatty liver was 16.31 ± 5.45, in grade 2 fatty liver was 28.64 ± 12.91, and 72.8 ± 27.10 in grade 3 fatty liver and they were compared with each other and were found to be statistically significant with P value = 0.001. it was comparable with the study conducted by B. Kasapoglu et al. in which mean UACR in grade 1 fatty liver was 9.2 ± 7.9, 11.9 ± 9.2 in grade 2 fatty liver, 19.6 ± 11.5 in grade 3 fatty liver.[26]

The mean GFR in cases was 81.73 ± 24.88 and 94.89 ± 22.46 in controls. When compared, it was found to be statistically significant with P value < 0.001 and was comparable to study done by H R jang et al. in which mean GFR in cases and controls was 61.8 ± 17.6 and 57.3 ± 16.8 respectively.[25] The mean GFR in grade 1 fatty liver was 100.34 ± 19.82, 78.20 ± 22.57 in grade 2 fatty liver and 62 ± 15.29 in grade 3 fatty liver. It was comparable to study done by Mona Adb-Elmonem hegazy et al. in which mean GFR was 72.07 ± 8.06, 59.74 ± 9.77 and 58.03 ± 9.01 in grade 1, grade 2 and grade 3 fatty liver respectively.[27]

CONCLUSIONS

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 NAFLD and waist circumference among study cases. The waist circumference was more in cases than controls and it was statistically significant (P value < 0.05).
2. There was strong association between NAFLD and BMI among study cases and controls. The BMI was more in cases than controls and it was statistically significant (P value < 0.05).
3. There was strong association between NAFLD and the serum total cholesterol, serum triglycerides and serum LDL levels among study cases and controls. The serum total cholesterol, serum triglycerides and serum LDL levels were more in cases than controls and it was statistically significant (P value < 0.05).
4. There was strong association between NAFLD and microalbuminuria among study cases and controls and UACR was more in cases than controls it was statistically significant (P value < 0.05). microalbuminuria had significant correlation (P < 0.05) with different grades of fatty liver of participants. As the grade of fatty liver of study subjects increased there was proportional increase in microalbuminuria.
5. There was strong association between NAFLD and creatinine clearance (eGFR) among study cases and controls and it was statistically significant (P value < 0.05). The creatinine clearance (eGFR) had significant correlation (P < 0.05) with different grades of fatty liver of participants. As the grade of fatty liver of study subjects increased there was proportional decrease in creatinine clearance.

The conclusion was that, in our study there was highly significant (P < 0.001) correlation between NAFLD and microalbuminuria. This correlation was more positive and it progresses as the grades of NAFLD increases. This shows 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.

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