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Frequency of non-alcoholic fatty liver disease in non-obese type 2 diabetic patients and its association with sex and age

Muhammad Umair[1a], Muhammad Omar Malik*[2a], Syed Hamid Habib[2], Mohsin Shah[2], Atifullah[3]

[1]*Department of Physiology, Gomal Medical College D. I. Khan, Khyber Pakhtunkhwa*
[2]*Department of Physiology, Institute of Basic Medical Sciences, Khyber Medical University, Peshawar, Khyber Pakhtunkhwa*
[3]*Department of Physiology, Nowshera Medical College, Nowshera, Khyber Pakhtunkhwa*
[a]*Contributed equally*

ABSTRACT

Objective: This study aims to explore the frequency of non-alcoholic fatty liver disease (NAFLD) in non-obese Type 2 Diabetes Mellitus (T2DM) patients and its association with diabetic control in different age and sex groups.

Methodology: This study involved 100 already diagnosed T2DM patients with BMI ranging from 18.5 to 29.9. 55% were male and 45% were female. Age limit was 30-60 years. Mean age of the patients was 46.78 ± 8.39 years. Diagnosis of NAFLD was performed by ultrasonography.

Study Design: Cross sectional observational study.

Place and Duration of Study: The study was conducted at outpatient department of District Headquarter Hospital Dera Ismail Khan, Pakistan from a period of March 2016 to August 2016.

Results: The results showed fifty eight (58%) of these patients had fatty liver. NAFLD had no significant association with different age (< 45 years and ≥ 45 years, p=0.847) and sex groups (p = 0.392), although males were found to have higher prevalence than females (61.8 % vs. 53.3 %).

Conclusion: NAFLD is a highly prevalent disorder in non-obese T2DM patients and was found to have no significant association with different age and sex groups.

Keywords: Non-alcoholic fatty liver disease (NAFLD), Type 2 diabetes mellitus (T2DM), Age, Gender, Frequency, hyperglycemia

***For Correspondence**

Muhammad Omar Malik
Assistant Professor,
Department of Physiology,
Institute of Basic Medical Sciences, Khyber Medical University, Peshawar,
Khyber Pakhtunkhwa

Email:
omarmalik786@gmail.com

INTRODUCTION

The presence of diffusely accumulated fat in liver hepatocytes is a characteristic of fatty liver or hepatic steatosis.[1] Non-alcoholic fatty liver disease (NAFLD) is the term used for fatty liver in individuals who have not ingested alcohol in significant amount previously.[2,3] NAFLD may progress from pure steatosis and non-alcoholic steatohepatitis (NASH)[4] to more serious problems like cirrhosis[5] and hepatocellular carcinoma.[1,3] Obesity, type-2 diabetes mellitus and hyperlipidaemia are found to have strong association with NAFLD.[6,7] Moreover, metabolic syndrome manifestations like hyperinsulinemia (due to peripheral insulin resistance), hypertension and hypertriglyceridemia are all related to its hepatic component.[3,6,8] The highest prevalence of NAFLD (35-75%) is reported among the diabetic and obese people.[9,10,11] The prevalence of NAFLD is associated with body mass index (BMI); ranging from 10% in normal BMI subjects compared to 80% in obese.[12] Furthermore, NAFLD independently can be correlated with impaired glucose metabolism.[13] In type-2 diabetes mellitus, hepatic insulin resistance severity is influenced by fatty liver.[14] The hepatic insulin resistance is also associated with impaired fasting glucose levels (IFG).[15]

NAFLD is known as "silent liver disease" as it is mostly asymptomatic or found accidentally by mildly elevated levels of serum aminotransferase[16] or hepatomegaly.[17,18] The histological finding of NAFLD is simple steatosis.[19] Liver biopsy and histological examination is confirmatory diagnosis of NAFLD. However, biopsy is not done routinely as the process is not only invasive and expensive but may lead to complications as well. Alternatively, fatty liver can easily be detected by means of non-invasive radiologic imaging of the liver with sonography, computed

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tomography (CT) or magnetic resonance imaging (MRI). Ultrasonography is 80% sensitive and 99% specific test in diagnosing NALFD and is mostly used due to its cost effectiveness and easy accessibility.[10,20] Furthermore, it gives almost similar results as CT, MRI or MRS which are considered more sophisticated gold standard imaging techniques.

NAFLD is getting attention due to its increasing prevalence (around 30%) in the developed world[3,21] and is on top of the list of common liver diseases in United States.[22] However, in Pakistan, hepatic disorders are mainly due to viral hepatitis B and C23 and differences in hepatic disorders due to obesity or viral hepatitis have not been clearly demonstrated. NAFLD is characterized by features like bright hepatic echo texture, deep and vascular blurring on ultrasonography.[24] In Western countries NAFLD has been explored vastly but as it remains asymptomatic, it is rarely diagnosed in Asian people and limited work has been done in our clinical set-up in this regard.[25,26] Furthermore, available studies show the association of NAFLD with development of type 2 diabetes and obesity; however, limited data is available showing its frequency in non-obese type 2 diabetic patients. Therefore, in this study we explored the frequency of NAFLD in non-obese T2DM patients. Moreover, we explored the distribution of NAFLD in different age and sex groups along with the diabetic control.

METHODOLOGY

Study population: This was a cross sectional observational study. The study population included already diagnosed T2DM patients both sexes between the age of 30-60 years and having BMI range of 18.5-29.9. The study was conducted at outpatient department of District Headquarter Hospital Dera Ismail Khan, Pakistan. The sampling was done during 6 months duration; March 2016, to August 2016. The protocol was approved by the Institutional Review Committee, and a prior written informed consent was obtained from each participant before entry into the study.

Study inclusion criteria: Included participants were already diagnosed Type 2 diabetic patients attending OPD within the BMI range of 18.5–29.9. Subjects from both genders in the age range of 30-60 years with no co-morbid conditions were included.

Study exclusion criteria: Subjects with history of usage of alcohol (daily alcohol consumption > 20g in females and > 30g in males) and/or drugs causing fatty liver were excluded. Furthermore, subjects with anemia, pregnancy, chronic hepatitis (HBsAg or Anti HCV positive), renal and cardiac diseases were not recruited in the study. Informed consent was obtained from all the participants before inclusion into the study. Patients with hepatitis B and C were excluded using rapid test kit consisting of test cassette. BMI less than 18.5 and more than equal to 30 were excluded

Sample size: Sample size was calculated by using G*Power 3.1.9 by keeping the power of 0.80 and α value of 0.05 and using mean and standard deviation of HbA1c of published literature. A total sample of 100 diagnosed T2DM patients of both sexes were included in the study as previously done by Ijaz et. al.[27]

Assessment program: Informed consent was obtained from all the participant before inclusion into the study. All patients underwent a detailed clinical history (including anti-diabetic drugs), examination (to look for hepatomegaly) and anthropometric measurements [height, weight, waist circumference, hip circumference, fat mass and BMI (weight in kg/m^2)].

Venous Blood Sample Collection: Venous blood samples were collected and processed immediately by centrifugation at 3000 rpm for 10 min. The clear supernatant collected was used for analysis and then frozen stored at −20 °C until assayed for triglycerides, cholesterol, HDL, urea, creatinine, ALT, and ALP levels using Cobas C501. For HbA1c and complete blood picture, blood was collected in the EDTA tube with anticoagulant and analysed by Cobas C501. Plasma fasting glucose (mg/dl) and 2 hours post prandial glucose after taking breakfast was performed using Accu-Chek (Roche) glucometer.

Sonographic measurements: Assessment of liver fat content was performed by ultrasonography (Sono ACE R3 Samsung Medison). Evaluation of hepatic fat content was performed by using convex transducer (2-5 MHz frequency). The fatty infiltration and severity of liver pathology was graded according to scoring system for fatty liver as described by Kumar et al. Subjects were positioned in supine position during ultrasound assessment and measurements were recorded during expiration. Fatty liver was seen as bright liver, more echogenic as compared to right kidney. The grading system used for steatosis[28] is as follows:

Mild steatosis (I): increased echogenicity of liver, normally seen diaphragm and intrahepatic vessels; moderate steatosis (II): moderate increase echogenicity,

mildly obscured visualization of diaphragm and intrahepatic vessels; severe steatosis (III): marked increase in echogenicity, obscured penetration, poor or non-visualization of diaphragm and intrahepatic vessels.28

Statistical analysis: Questionnaires were used to collect data from patients and entered in to Microsoft excel. Data cleaning was done to check for any missing or abnormal values, logical errors and misplaced values. Information sheets were consulted again for any discrepancy. Statistical analysis was carried out using SPSS 22. The normality of the data was tested using Kolmogorov-Smirinov and Shapiro-Wilk tests and histograms. Non normal data (ALT, AST, Urea and triglycerides) were log transformed for analysis. Descriptive statistics (percentages, mean ± SD) were used to describe the data. . Independent T-test was performed to compare continuous variables (age, weight, BMI, Hb, ALP, ALT, AST, Urea, Creatinine, Cholesterol, HDL, LDL,TGs, FBS, RBS and HbA1c) between groups specified by fatty liver status, sex and age. P values less than 0.05 was accepted as significant. Chi-square test was performed to check if NAFLD had any association with gender and age groups

RESULTS

The current study included 100 non-obese T2DM participants in our study, out of which 55 (55%) were male and 45 (45%) were female. Data of all the parameters were normally distributed except for ALT, AST, Urea and triglycerides which were log transformed for analysis. Mean age of the patients was 46.78 ± 8.39 years as summarized in Table 1. Out of all the participants fifty eight (58%) subjects were found to have fatty liver on ultrasonography (58.6% male and 41.4% female). When the sample was split according to presence or absence of NAFLD, patients with NAFLD were significantly heavier (p= <0.001) with a mean weight of 73.2 ± 10.7 vs. 65.3 ± 10.1, and BMI 26.7 ± 2.8 vs. 24.5 ± 3.4, (p= 0.001) for those without NAFLD (Table 1). In biochemical analysis urea [34.18(31.37-37.25) vs. 29.83(27.47-32.39), p= 0.028] and creatinine (0.93 ± 0.20 vs. 0.84 ± 0.16, p= 0.024) were also significantly raised in NAFLD patients (Table 1).

Chi-square test was performed to check if prevalence of NAFLD had any association with gender and different age groups. NAFLD had no significant association with gender [(p= 0.392) and age [(< 45 years and ≥ 45 years, p= 0.847), however, males had higher prevalence (61.8%) compared to females (53.3 %). Comparison between male and female showed that males were significantly heavier (74.2 ± 10.7 vs. 64.4 ± 9.1, p= 0.000). Males were observed to have significantly high Hb, urea, ALT and creatinine as shown in Table 2.

Table 1 - Comparison of anthropometric and metabolic parameters between patients with and without NAFLD

Variables	NAFLD		P
	YES (n=58)	NO (n=42)	
Age (ys)	46.6 ± 8.2	47 ± 8.8	0.843
Weight (kg)	73.2 ± 10.7	65.3 ± 10.1	<0.001
BMI (kg/m2)	26.7 ± 2.8	24.5 ± 3.4	0.001
Hb (gm/dl)	12.6 ± 1.6	12.4 ± 1.5	0.389
ALP (U/l)	198.5 ± 59.1	201.8 ± 53.2	0.773
Creatinine (mg/dl)	0.93 ± .20	0.84 ± .16	0.024
Cholesterol (mg/dl)	182.8 ± 33.3	181.5 ± 39	0.856
HDL (mg/dl)	35.4 ± 9.8	37.8 ± 9.3	0.232
LDL (mg/dl)	109.2 ± 24.8	108.5 ± 22.9	0.879
FBS (mg/dl)	156.8 ± 46.2	159.6 ± 52.7	0.778
RBS (mg/dl)	214.6 ± 70.6	218 ± 75.8	0.817
HbA1c (%)	7.7 ± 1.3	7.7 ± 1.1	0.826
ALT † (IU/l)	41.98 (37.80-46.60)	40.70 (36.84-44.96)	0.678
AST † (U/l)	31.28 (27.90-35.07)	30.23 (27.34-33.43)	0.668
Urea † (mg/dl)	34.18 (31.37-37.25)	29.83 (27.47-32.39)	0.028
TGs † (mg/dl)	162.52 (145.38-181.68)	151.77 (129.48-177.87)	0.466

Values are mean ± SD. P for independent sample T test comparison between presence and absence of NAFLD. † Log transformed for analysis, values as geometric mean (confidence interval)

Table 2 - Comparison of anthropometric and metabolic parameters between males and females

Variables	Sex			p
	Total (n=100)	MALE (n=55)	FEMALE (n=45)	
Age (ys)	46.78 ± 8.39	46.2 ± 9.3	47.5 ± 7.1	0.435
Weight (kgs)	69.82 ± 11.13	74.2 ± 10.7	64.4 ± 9.1	<0.001
BMI (kg/m2)	25.81 ± 3.28	25.5 ± 3.3	26.2 ± 3.2	0.279
Hb (gm/dl)	12.57 ± 1.58	13.4 ± 1.2	11.6 ± 1.4	<0.001
ALP (U/l)	199.86 ± 56.47	201.6 ± 43.9	197.8 ± 69.2	0.751
Creatinine (mg/dl)	0.89 ± .19	0.96 ± 0.18	0.81 ± 0.18	<0.001
Cholesterol (mg/dl)	182.22 ± 35.66	179.7 ± 33	185.2 ± 38.8	0.442
HDL (mg/dl)	36.42 ± 9.7	35.4 ± 8.9	37.6 ± 10.4	0.237
LDL (mg/dl)	108.93 ± 23.89	110.9 ± 25.9	106.6 ± 21.1	0.371
FBS (mg/dl)	157.99 ± 48.82	160.1 ± 51.8	155.3 ± 45.2	0.628
RBS (mg/dl)	216.04 ± 72.45	220.3 ± 63.9	210.8 ± 82	0.518
HbA1c (%)	7.71 ± 1.22	7.7 ± 1.3	7.7 ± 1.1	0.966
ALT † (IU/l)	41.43 (38.53-44.54)	44.61 (40.41-49.23)	37.91 (34.14-42.09)	0.026
AST† (U/l)	30.84 (28.54-33.32)	32.52 (29.17-36.26)	28.89 (25.87-32.28)	0.133
Urea† (mg/dl)	32.28 (30.37-34.32)	34.48 (31.96-37.20)	29.78 (27.02-32.82)	0.017
TGs† (mg/dl)	157.91 (144.12-173.01)	153.50 (135.11-174.38)	163.49 (142.86-187.07)	0.498

Values are mean ± SD. P for independent sample T test comparison between males and females. †Log transformed for analysis, values as geometric mean (confidence interval)

Table 3 - Comparison of anthropometric and metabolic parameters between patients with age < 45 years and≥ 45 years

Variables	Age		P
	< 45 YEARS(n=37)	≥ 45 YEARS(n=63)	
Weight (Kgs)	69.7 ± 11.8	69.9 ± 10.8	0.921
BMI (kg/m2)	25.8 ± 3.3	25.8 ± 3.2	0.902
Hb (gm/dl)	12.8 ± 1.5	12.4 ± 1.6	0.199
ALP (U/l)	194.4 ± 54.1	203 ± 57.9	0.462
Creatinine (mg/dl)	0.85 ± 0.17	0.92 ± 0.20	0.073
Cholesterol (mg/dl)	178.2 ± 30.3	184.6 ± 38.4	0.386
HDL (mg/dl)	36.43 ± 9.75	36.41 ± 9.70	0.992
LDL (mg/dl)	110.5 ± 19.7	108.03 ± 26.12	0.626
FBS (mg/dl)	162.3 ± 53.7	155.5 ± 46.0	0.504
RBS (mg/dl)	224.03 ± 73.5	211.3 ± 72.03	0.401
HbA1c (%)	7.5 ± 1.2	7.8 ± 1.2	0.246
ALT† (IU/l)	44.94 (39.96-50.54)	39.55 (36.06-43.37)	0.092
AST† (U/l)	33.21 (28.98-38.06)	29.52 (26.86-32.45)	0.146
Urea† (mg/dl)	31.07 (28.19-34.26)	33.01 (30.48-35.75)	0.347
TGs† (mg/dl)	142.82 (122.60-166.38)	167.49 (149.35-187.85)	0.095

Values are mean ± SD. P for independent sample T test comparison between different age groups. †Log transformed for analysis, values as geometric mean (confidence interval)

The population was also split according to age (<45 years and ≥ 45 years), to show if anthropometric, clinical and other parameters were significantly different within the diabetic population (Table 3). There was no age related difference and prevalence of fatty liver was almost same in <45 years (56.8%) and ≥ 45 years (58.7%), p=0.847.

DISCUSSION

The present study showed 58% prevalence in T2DM population, ranging from age of 30 to 60 years. Our study showed that the prevalence of NAFLD was not affected by the advancement of age. In spite of having same age range and mean, people with NAFLD were heavier and had higher BMI although within overweight range (as obesity was an exclusion criterion). Moreover, NAFLD was almost equally prevalent between males and females in our study. However, Frith et al. showed higher prevalence of NAFLD in males than females with in age limit of 40 to 65 years.[29] In contrast, Lee reported that NASH was found to be more common among females in middle age group who were mostly obese and diabetic.[30] This difference from our study may be due to differences in age and metabolic groups (our study population was non-obese), or different ethnic population as racial and ethnic differences have effect on the risk of NAFLD development and prevalence.[31]

The present study found out that other parameters (age, Hb, ALT, AST, ALP, cholesterol, TGs, HDL, LDL, FBS, RBS and HbA1c) were not significantly different between the two groups. Clinical parameters related to NAFLD were not significantly different between the groups characterized by presence or absence of NAFLD. Various trials have shown that age, gender, systemic hypertension, and parameters including ALT, AST, TGs, GGT, haptoglobin, total bilirubin and FBS were associated with NASH.[3]

Insulin resistance (IR) and high hepatic fat content are interlinked, as IR along with increased level of insulin in blood; may lead to hepatic steatosis.[32,33] Similarly, this accumulation of hepatic fat may also result from increased lipid supply which then leads to hepatic IR.[22] In addition to hepatic IR, NAFLD is also associated with peripheral IR i.e. muscle and adipose tissue IR.[9,22] IR has been shown to be involved in the development of diabetes mellitus and so NAFLD has a pathophysiological link with the development of diabetes mellitus.[34,36]

In our study we did not find an independent association of NAFLD with fasting and random blood sugar and HbA1c. One of the reasons may be that NAFLD is associated with development of IR,[37] but in addition to IR; development of diabetes is due to insufficient secretion of insulin which is the cause of hyperglycemia.[38,39] So, NAFLD may be associated with IR but not associated with insulin secretion. Giorgio et al. conducted a study on young obese pediatric patients with NAFLD and tried to evaluate the association of NAFLD with relationship between insulin secretion (ISEC) and insulin sensitivity (ISEN). They showed that NAFLD was not associated with the ISEC and ISEN.[40]

In people with diabetes who do not have NAFLD in the beginning may develop it after the development of diabetes. The reason may be that initially in development of diabetes there is hyperinsulinemia which can increase liver fat[41] and same hyperinsulinemia, due to drugs or insulin; may be the cause of development of NAFLD after development of diabetes.[42] However, in later stages of diabetes there is decrease in insulin secretion due to decline in β- cells function or number.[43]

Our study have some limitations, including cross sectional nature of study, as the progress of disease assessed can be better evaluated in a longitudinal study. NAFLD was assessed through USG, but the gold standard is still biopsy and histopathology. There was no previous record for the presence of NAFLD as USG was performed for the first time in most of the participants after development of diabetes.

In conclusion, nonalcoholic fatty liver disease (NAFLD) is a highly prevalent (58%) disorder in T2DM patients. NAFLD was also found to have no significant association with different age and sex groups.

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