Frequency of Non-Alcoholic Fatty Liver Disease in patients withDiabetes Mellitus in a Tertiary care Hospital from Southindia

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Abstract:
Introduction: Nonalcoholic fatty liver disease (NAFLD) prevalence is high, especially in patients with obesity and type 2 diabetes, and is expected to rise steeply in the coming decades. **Objective:** We estimated NAFLD prevalence in patients with type 2 diabetes and explored associated characteristics and outcomes.

Material and Method: This is a prospective and cross-sectional study was conducted in the Department of Medicine at Tertiary care Teaching Hospital over a period of 6 months. The study population comprised of male and female patients who were diagnosed as having type 2 diabetes mellitus based on their fasting and random blood sugar levels. Frequency and percentages were calculated for categorical variables like sex and non-alcoholic fatty liver diagnosed on abdominal ultrasound. Non-alcoholic fatty liver was stratified among the age, sex and duration of type-2 diabetes mellitus to see the effect modifiers.

Results: Out of 320 participants, 200 patients (62.5%) had NAFLD on ultrasound. A total of 320 participants were enrolled for the study, of whom there were 140 (43.75%) males and 180 (56.25%) females. The mean age and mean duration of T2DM are presented. Overall, NAFLD was present in 200 (62.5%) study participants. Moreover, patients having NAFLD

were compared with patients having no ultrasonographic evidence of NAFLD. There was no statistically significant difference between the two groups regarding mean age and gender distribution. Though, there was a statistically significant difference amongst the two groups in terms of HbA1c, triglycerides, total cholesterol, LDL and HDL cholesterol and serum uric acid.

Conclusion: This study reported an increased frequency of NAFLD in our diabetic population and evaluated in depth the risk factors associated with NAFLD, underpinning the significance of carrying further large-scale studies to assess the effects of lifestyle modification in the form of physical activity and dietary modifications on the status of NAFLD and glycemic control. Taking in to account the results of this study, patients and their treating physicians should emphasize on the modification of the associated factors and it is also advisable to screen diabetic patients for this condition in routine clinical practice. Early detection and timely management will help promote healthy lifestyle and prevent long term complications of the condition.

Keywords: Non alcoholic fatty liver disease, Diabetes mellitus, HbA1c.

Introduction

Nonalcoholic fatty liver disease (NAFLD) encompasses a spectrum ranging from the relatively benign isolated hepatic steatosis (HS) to the more harmful problems of nonalcoholic steatohepatitis (NASH), hepatic fibrosis, and cirrhosis. NAFLD, by definition, can be diagnosed only in the absence of other causes of liver disease. For diagnosis, many different modalities are used, ranging from laboratory tests, to imaging, to the gold standard, liver biopsy. [1]

Over the past years, the clinical and economic burden of NAFLD have become apparent and are expected to rise steeply in the coming decades as a consequence of the increased prevalence and incidence of obesity and type 2 diabetes mellitus. [2] Global prevalence rates of NAFLD in the general population are estimated at 25%. [3] NASH prevalence in the general population is estimated at 1.5% to 6.45%, and 41% within the NASH group develop fibrosis progression. [4] Since 2013, NASH cirrhosis is the second leading etiology for liver transplantation (LT) in the United States and in Europe it is anticipated to become the leading indication for LT within the next decade. [5] Moreover, mortality due to liver disease is increased in patients with NAFLD. [6] The high NAFLD burden is caused not only by these hepatic complications but also by the associated increased cardiovascular morbidity and mortality in patients with NAFLD. [7]

NAFLD and type 2 diabetes are closely associated phenomena. [8] NAFLD may be considered as a hepatic manifestation of metabolic syndrome. [9] In contrast to the knowledge about NAFLD and type 2 diabetes, there are limited and inconsistent data on NAFLD prevalence in patients with type 1 diabetes mellitus. [10] Type 1 diabetes and type 2 diabetes show major pathophysiological differences, but share certain similarities as well. Insulin resistance and systemic hyperinsulinemia are seen both in type 1 diabetes and type 2 diabetes. [11] Obesity, a well-known NAFLD risk factor clearly related to type 2 diabetes and insulin

resistance, is becoming more prevalent in the type 1 diabetes population. [12] Taking into account these similarities and adding the generally long lifetime exposure to type 1 diabetes, the spectrum of NAFLD and its long-term sequelae might be clinically relevant in patients with type 1 diabetes as well.

The aim of the present study is to estimate the prevalence of NAFLD in patients with diabetes. We performed a study on the prevalence of NAFLD and distribution of its different stages in patients with diabetes. Moreover, we explored associated characteristics and outcomes of NAFLD in the diabetes population.

Material and Methods

This is a prospective and cross-sectional study was conducted in the Department of Medicine at Tertiary care Teaching Hospital over a period of 6 months. All non-alcoholic patients above 30 years of age, and having Type II diabetes mellitus of any duration were included in the study.

Persons consuming alcohol for any duration (by history), patients with positive viral markers (detected by HCV Ab and HBs Ag in blood), already diagnosed cases of hemochromatosis, Wilson's disease and autoimmune hepatitis (by clinical record), use of certain drugs like steroids, oral contraceptive pills use in females, amiodarone and T2DM patients using statins and pioglitazones (clinical record and history), pregnancy (by history), insulin dependent diabetes mellitus (history of insulin injections) were excluded from the study. After getting approval from the hospital ethical committee to conduct the study, data was collected from all those patients with Type 2 diabetes mellitus (fasting glucose of ≥l26mgldl, random glucose ≥200mgldl) of any duration presenting to medical OPD.

Patients with type 2 diabetes mellitus of any duration were worked up with detailed history and clinical examination. Imaging examination of all patients was done with abdominal ultrasound (Sonoline 450(siemens), B-mode, Probe 3.5Mhz) for non-alcoholic fatty liver disease. All information was recorded in a pre- designed proforma. Exclusion criteria were followed strictly to control bias in the study results.

Statistical Analysis

All the data collected through design proforma was entered and analyzed in SPSS version 25. Frequency and percentages were calculated for categorical variables like sex and Non-alcoholic fatty liver. Mean ±SD was calculated for continuous variables like age. Non-alcoholic fatty liver was stratified among the age, sex and duration of type-2 diabetes mellitus to see the effect modifiers. All the results were presented as tables and graphs.

Result

A total of 320 participants were enrolled for the study, of whom there were 140 (43.75%) males and 180 (56.25%) females. The mean age and mean duration of T2DM are presented in Table-I. Overall, NAFLD was present in 200 (62.5%) study participants. Moreover, patients having NAFLD were compared with patients having no ultrasonographic

evidence of NAFLD. There was no statistically significant difference between the two groups regarding mean age and gender distribution. Though, there was a statistically significant difference amongst the two groups in terms of HbA1c, triglycerides, total cholesterol, LDL and HDL cholesterol, serum uric acid and ALT. The baseline demographic and laboratory characteristics along with the comparison of clinical and laboratory findings between the two groups of patients are presented in Table-I and Table-II, respectively.

Table-I: Comparison of baseline clinical characteristics of Type-2 DM patients with and without NAFLD.

Patient Characteristic		***All Patients (n= 320)***	***Without NAFLD (n=120)***	***With NAFLD (n=200)***	***p-value***
Age (years)		55.22 ± 7.75	54.41 ± 7.40	55.73 ± 7.93	0.1
Gender	Male	140(43.25%)	55(45.8%)	90(45.0%)	0.2
	Female	180 (56.75%)	65(54.2%)	110 (55.0%)	

Table-II: Duration of Type-2 DM patients with and without NAFLD.

Duration of Type-2 DM categories (years)	***All Patients***	***Without NAFLD***	***With NAFLD***	***p-value***
< 5	30(9.4%)	10(8.3%)	12 (6.0%)	
5–10	90 (28.2%)	25(20.9%)	20(10.0%)	
10–15	100(31.2%)	45(37.5%)	73(36.5%)	
> 15	100(31.2%)	40 (33.3%)	95(47.5%)	0.004

Table-III: Comparison of laboratory parameters of Type-2 DM patients with and without NAFLD.

Lab Parameter		*All patients (n=320)*	*Without NAFLD (n=120)*	*With NAFLD (n=200)*	*p-value*
FBS (mg/dl)		175.23 ± 66.201	150.25 ± 56.231	180.63 ±70.268	<0.0001
RBS (mg/dl)		240.23 ± 81.549	220.31 ± 70.216	260.23± 85.641	<0.0001
HbA1c (%)		8.234 ± 1.9748	8.424 ± 1.8541	10.234± 1.8153	<0.0001
HbA1c Categories (%)	7-8.9	45 (14.1%)	38 (31.6%)	18 (9.0%)	
	9-10.9	85 (26.5%)	45 (37.5%)	27 (13.5%)	
	≥ 11	190(59.3%)	37 (30.8%)	155 (77.5%)	<0.0001

Table-IV: Comparison of Lipid profile of Type-2 DM patients with and without NAFLD

Lipid Profile	***All patients***	***Without NAFLD***	***With NAFLD***	***p-value***
Total cholesterol (mg/dl)	160.21± 110.365	130.83 ± 27.468	185.62 ± 130.465	<0.0001
Triglycerides (mg/dl)	195.01± 90.862	125.21 ± 37.642	240.20± 90.301	<0.0001
LDL (mg/dl)	90.64± 29.721	70.01 ± 16.615	100.22± 30.916	<0.0001
HDL (mg/dl)	25.40± 8.719	30.26 ± 8.472	20.26+± 6.154	<0.0001
VLDL (mg/dl)	113.902± 1.0129	112.902 ± 0.9+94124	133.701 ± 1.1125	<0.0001

The results of the multivariate logistic regression analysis are shown in Table-III. These results showed that smoking, hypertension, central obesity, obesity, higher HbA1c (≥ 11%), were having independent association with the presence of NAFLD on ultrasound in T2DM patients. High triglyceride level and low HDL levels were the variables with the strongest association, conferring 11.6- and 11.5-fold increased likelihood of NAFLD in T2DM patients, respectively.

Discussion

Previous research has identified NAFLD in 19% to 74% of patients suffering from DM2. [13] The present study diagnosed NAFLD in 62.5% of the patients with DM2, although this frequency may have been even higher, given that lower grade of NAFLD may go unnoticed by US. US is around 90% sensitive and 100% specific in diagnosing NAFLD. [14]

Neither age nor sex presented any relation to the occurrence of NAFLD, neither did smoking or exercise, as confirmed by other studies. Other interesting data was the diagnosis of hypertension in the majority (85%) of patients with NAFLD, very probably related to the BMI, which was also higher in patients with NAFLD. It is worth noting that when NAFLD is associated with DM2, obesity, hypertension and dyslipidemia there is an increased risk of it progressing to end-stage liver disease, and a greater chance of cardio vascular diseases. [15]

In the present study, increased weight, BMI and waist measurement were more prevalent in patients with NAFLD, situations in which the insulin Resistance is a predominant factor. It should be highlighted that the researcher who diagnosed NAFLD using US may have been influenced by the biotypes of the patients, thus identifying liver disease with greater frequency in overweight or obese patients. Despite this unavoidable bias, the examiner followed all ultrasonographic criteria in order to diagnose fatty liver disease. [16]

Although MS was diagnosed in the majority of patients within this study, it was more frequent in those with NAFLD than those without ($p = 0.015$), as described above. These findings suggest that NAFLD constitutes the hepatic expression of MS and that when associated with visceral obesity and hypertension it may be employed as one of the diagnostic criteria for this syndrome. [17]

In our study, no significant differences were encountered amongst patients with or without NAFLD in relation to levels of pre- and post-prandial glucose, HbA1c, cholesterol profile, AP, bilirubin and INR. Similar se-rum levels of blood glucose may be because all the patients included in the two groups presented DM2. In addition, evaluating patients with non-alcoholic steatohepatitis (NASH), Poynard and cols. [18] did not also find differences in serum levels of cholesterol and bilirubins among patients with and without NASH.

Higher levels of triglycerides were more observed in patients with NAFLD, which may possibly reflect a greater accumulation of fatty acid into the liver, higher IR and a greater tendency to develop into NASH. In fact, patients with DM2 present a particular type

of dyslipidemia with higher triglycerides and lower HDL-c levels, and patients with NAFLD also present the same lipid profile. [19]

The blood levels of uric acid were higher in 30 patients with NAFLD, and there were also described higher levels of uric acid in patients with visceral adiposity and IR. A recent study described the association of NAFLD with hyperuricemia and a higher risk of coronary disease. [20]

Also encountered in this study were significantly higher levels of ALT, AST and γ-GT in patients with NA-FLD, which could be a consequence of hepatic aggression resulting from the infiltration of fatty acids, bringing about inflammatory stimulus. Data published in the "Third National Health and Nutrition Survey" illustrate a significant association between high levels of ALT and IR, and DM2 and MS. It should be noted that the levels of aminotransferases in patients with NAFLD were higher than in those patients without, but the mean levels of both groups were below the upper limits of normality. These findings suggest that the normal limits of amino-transferases need to be reduced. It should be remembered that when the current limits were originally established, around 25 years ago, it is probable that certain individuals must have been considered "healthy" when in fact they were patients suffering from undiagnosed NAFLD. [21]

On the other hand, the mean serum levels of γ-GT were above the normal limits in those with NAFLD. In effect, γ-GT is linked to sedentarism, obesity, hypertension, hyperinsulinemia, dyslipidemia, oxidative inflammation and stress. [22] High concentrations of γ-GT were also found in association with hypertension and central adiposity, suggesting a potentially pathogenic relationship between NAFLD, endothelial dysfunction and cardiovascular risk. [23]

When the AST/ALT ratio is greater than the unity, it is indicative of more advanced liver disease, with more intense fibrosis. No significant difference in the AST/ALT ratio of either group was encountered in this study, although there was a tendency for this ratio to alter, above the unity, in those without NAFLD, probably because of higher levels of ALT in patients with NAFLD (Table 4).

No differences were observed in the serum levels of ferritin between the two groups, although there was a more frequent tendency ($p = 0.045$) for altered levels in patients with NAFLD (Table 4). Higher levels of ferritin were discovered in patients with increased visceral fat or MS and, when the higher levels of protein were associated to advanced age, BMI > 30 kg/m2 and DM2, there was a greater risk of developing liver fibrosis. [24]

HOMA-IR was used in this study as a method to evaluate the degree of IR. Its values were significantly higher in patients with NAFLD compared to those without NAFLD ($p = 0.005$). This finding reflects a more accentuated degree of IR in patients with NAFLD, as has already been described in the literature. [25]

Even in patients without DM2, the IR determined by HOMA-IR is associated to a higher grade of fat ac-cumulation in patients with NAFLD. [26] IR exacerbates NAFLD by means of two mechanisms. Peripherally, through the compromised distribution of fatty acids, thus overloading the liver, and in the liver itself because of the alteration to lipid metabolism, where these fatty acids undergo extra-mitochondrial (abnormal) oxidation, leading to oxidative stress. [27]

The release of reactive particles of oxygen generated in oxidative stress may increase mitochondrial damage in hepatocytes and expand extra-mitochondrial oxidation of fatty acids. They may also stimulate neighboring macrophages (Kupffer cells) and release TNF-α which interferes with insulin sensitivity and increases its resistance. For the progression of NAFLD an alteration in the balance between two antagonism adipokines is important, in other words, TNF-α and the adiponectin, a hormone which acts to sensitize insulin, increases smooth muscle glucose uptake, and free fatty acid oxidation, decreases hepatic glucose production and decreases intracellular triglycerides. [29] TNF-α is the cytokine that most contributes to liver damage. In NAFLD, serum levels of adiponectin are low, however, in some studies TNF-α serum levels are not elevated. [30] In this study, levels of TNF-α were slightly higher in patients with NAFLD ($p = 0.040$).

Conclusion

This study reported an increased frequency of NAFLD in our diabetic population and evaluated in depth the risk factors associated with NAFLD, underpinning the significance of carrying further large-scale studies to assess the effects of lifestyle modification in the form of physical activity and dietary modifications on the status of NAFLD and glycemic control. Taking in to account the results of this study, patients and their treating physicians should emphasize on the modification of the associated factors and it is also advisable to screen diabetic patients for this condition in routine clinical practice. Early detection and timely management will help promote healthy lifestyle and prevent long term complications of the condition.

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