Hepatobiliary dysfunction in Type-2 diabetes mellitus

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ABSTRACT

Objective: Nonalcoholic fatty liver disease (NAFLD) and gallstones are frequently present in diabetics, but its exact prevalence is not well studied in India. We have done a prevalence study of hepatobiliary involvement in Type-2 diabetes mellitus (T2DM) and also studied the other risk factors of NAFLD. Materials and Methods: Two hundred diabetics and 200 controls underwent anthropometric measurements, abdominal ultrasonography, (USG) and biochemical tests at a tertiary care hospital. Univariate and multivariate logistic regression analyses were done. Results: One hundred and thirty (65%) diabetics and 47 (23.50%) controls were having USG evidence of fatty liver (odds ratio [OR] = 6.046, 95% confidence interval [CI]: 3.904–9.363 [P < 0.0001]). Raised liver enzymes were present in 42 (21%) diabetics and 16 (8%) controls [OR = 3.057, 95% CI: 1.654–5.648 [P < 0.004)]. Gallstones were present in 32 (16%) diabetics and 10 (5%) controls (OR = 2.825; 95% CI: 1.850–4.315 P < 0.0001). In addition, waist circumference (WC) and body mass index (BMI) were significantly more in diabetics, but lipid profile was not significantly deranged as compared to controls. Then, all patients with fatty liver were compared with patients with normal liver, and we found that fatty liver group was having raised BMI, WC, liver enzymes, and more dyslipidemia. Multivariate analysis was done which shows the presence of T2DM, elevated liver enzymes, obesity, and elevated WC as independent risk factors of fatty liver. Conclusion: The prevalence of NAFLD and gallstones was higher in diabetics as compared to healthy population. In addition, the presence of T2DM, elevated liver enzymes, obesity, and elevated WC are independent predictors of NAFLD.

Keywords: Dyslipidemia, fatty liver, gallstone, nonalcoholic fatty liver disease, Type-2 diabetes mellitus

Introduction

Nonalcoholic fatty liver disease (NAFLD) which develops in the absence of alcohol abuse is now recognized as one of the major health burden. The increasing prevalence of obesity, insulin resistance, and the metabolic syndrome has significant implications for the development of chronic liver disease which is characterized by abnormal fat accumulation in liver cells and histologically resembling alcohol-induced liver damage. With increasing incidence and prevalence of NAFLD, the perception of it being a benign condition is rapidly changing. It includes steatosis (increased liver fat without inflammation) and nonalcoholic steatohepatitis (NASH) (increased liver fat with inflammation). Simple steatosis is not dangerous for the patient, but NASH may progress to end-stage liver disease (cirrhosis), liver failure, and hepatocellular carcinoma.[1] The pathogenesis of NASH is poorly understood, but lipid peroxidation and oxidative stress are the leading culprits.[2] Patients with NAFLD appear to have a higher all-cause mortality in addition to liver-related cause of death and an increased risk for cardiovascular disease.[3] There is an epidemic of Type-2 diabetes mellitus (T2DM) affecting 285 million people worldwide in 2010, and this number is expected to rise to 439 million by 2030.[4] Numerous studies show that NAFLD is the hepatic component of metabolic syndrome. NAFLD is becoming the most common liver disorder worldwide with a median prevalence of around 20% in general population[5] and 42%–70% in T2DM.[6] Its prevalence in India is studied in only few studies. Gallbladder emptying abnormalities are also found in diabetic patients who may predispose them to
cholelithiasis, but obesity and hyperlipidemia may be contributing factors. Thus we conduct our study to know the prevalence of NAFLD and gallstones in patients with T2DM coming to our institute and to compare it with normal healthy individuals. In addition, we have compared anthropometric measurements and various biochemical tests between the two. We have also compared anthropometric and biochemical variables between patients having fatty liver with patients not having it.

**Materials and Methods**

A total of 200 patients of Type-2 DM attending the Outpatient Department of Medicine for routine checkup at a tertiary care institute were studied for the presence and type of hepatobiliary dysfunction between January 2012 and September 2013. Patients with alcohol intake more than 20 g/day, positive hepatitis B surface antigen, positive anti-hepatitis C virus, having significant comorbidities (sepsis, organ failure, malignancy, etc.), positive antinuclear antibody and taking hepatotoxic drug for more than 3 consecutive months were excluded from the study. A total of 200 non-diabetic volunteer age- and sex-matched healthy controls were also recruited for comparison. Exclusion criteria for controls were same as that for patients. All subjects gave informed consent, and the study protocol was approved by the Ethics Committee of Institute.

A thorough medical history and physical examination were performed including measurement of height, weight, and waist circumference (WC). Body mass index (BMI) was calculated by the formula weight (kg)/height (m²) and WC was measured midway between the uppermost border of iliac crest and lower border of the rib cage. The normal BMI for Asian Indians is 18–22.9 kg/m², and normal WC is <90 cm for men and <80 cm for women. BMI between 23 and 24.9 kg/m² are overweight and >25 kg/m² are obese.

Ultrasound (USG) abdomen was done in all the patients as well as controls for any evidence of hepatobiliary involvement. In ultrasonography, the right kidney echogenicity was used for the determination of liver parenchyma echogenicity. With the kidney cortex and liver parenchyma echogenicity being the same, it is evaluated as normal. Fat infiltration in liver is described in 3 ultrasonographic stages. Mild (Grade 1): Minimal diffuse increase in hepatic echogenicity. Diaphragm and intrahepatic vessel contours seem normal. Medium (Grade 2): Medium grade diffuse increase in hepatic echogenicity. There was mild deterioration in the image of diaphragm and intrahepatic vessels. Severe (Grade 3): Apparent increase in echogenicity. Posterior segment of the right hepatic lobe is difficult to display. Intrahepatic vessel structure and diaphragm contours are vague or not seen.

Five milliliters venous blood (2 ml in ethylenediaminetetraacetic acid and 3 ml in plain vacutainer) was drawn under aseptic condition from all patients and controls after minimum of 8 h of fasting. Lipid profile and liver enzymes were analyzed using automated biochemistry analyzer. HbA1C was measured using chemiluminescence analyzer (Elecsys 2010 Systems, Hitachi, Japan).

**Statistical analysis**

All data were stored in Microsoft Excel format and were analyzed using IBM SPSS version 20.0 software (SPSS, Chicago, Illinois, USA). P <0.05 was considered statistically significant. Comparison of continuous variables was carried out using unpaired Student’s t-test. Chi-square test was applied to compare between the categorical variable. Statistical tests were based on two-tailed probability. Multinomial logistic regression was also done.

**Results**

Baseline characteristic of cases and controls are shown in Table 1. The mean age of cases was 55.75 ± 14.53 years, of which 113 were male and 87 were female. The mean age of controls was 53.71 ± 13.42 years, of which 115 were male and 85 were female. Diabetic patients were at significantly higher risk for the occurrence of NAFLD with odds ratio of 6.046, (95% confidence interval = 3.904–9.363, P < 0.0001) [Table 1]. Out of 130 diabetic with NAFLD patients, eighty patients had Grade I NAFLD, thirty patients had Grade II NAFLD whereas Grade III

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### Table 1: Clinical and laboratory characteristics of cases and controls

| Variables                  | Diabetics (n=200) | Controls (n=200) | 95% CI       | OR       | P       |
|----------------------------|-------------------|------------------|--------------|----------|---------|
| Mean age                   | 55.75±14.53 years | 53.71±13.42 years| 0.646–1.426  | 0.960    | 0.144   |
| Sex (male/female)          | 113/87            | 115/85           |              |          |         |
| Fatty liver                | 130               | 47               | 3.904–9.363  | 6.046    | <0.0001 |
| Obesity (BMI >25 kg/m²)    | 135               | 89               | 1.725–3.890  | 2.590    | <0.0001 |
| Elevated liver enzymes     | 42                | 16               | 1.654–5.648  | 3.057    | 0.004   |
| Elevated liver enzymes + fatty liver | 40   | 12               | 1.987–7.722  | 3.917    | <0.0001 |
| Elevated waist circumference| 174             | 154              | 1.179–3.388  | 1.999    | 0.0134  |
| Raised cholesterol         | 90                | 70               | 1.016–2.273  | 1.519    | 0.525   |
| Raised LDL                 | 113               | 101              | 0.8857–1.888 | 1.273    | 0.272   |
| Low HDL                    | 139               | 116              | 1.093–2.491  | 1.650    | 0.221   |
| Raised TG                  | 102               | 84               | 0.9686–2.133 | 1.437    | 0.083   |
| Gallstones                 | 32                | 10               | 1.850–4.315  | 2.825    | <0.0001 |

CI: Confidence interval; OR: Odds ratio; BMI: Body mass index; LDL: Low-density lipoprotein; HDL: High-density lipoprotein; TG: Triglyceride.
NAFLD was observed in twenty diabetic patients [Table 2]. Patients having more duration of diabetes have more incidence of Grade III NAFLD as compared to patients with lesser duration \((P < 0.0001)\) [Table 3]. Diabetic patients were also found to have higher risk of obesity, high WC, elevated liver enzymes, and occurrence of gallstones. Among the lipid profile, no variable was found to be significantly altered in diabetic patients as compared to nondiabetic.

All the subjects were also grouped according to the presence of NAFLD or not, and we observed that patients with NAFLD had higher incidence of obesity, high WC, elevated liver enzymes and gallstones as compared to non-NAFLD group. Lipid profile was also significantly deranged in NAFLD group as compared to non-NAFLD [Table 4].

Multinomial logistic regression analysis was performed to see the independent risk factors for NAFLD. The presence of T2DM, obesity, high WC, and elevated liver enzymes were observed as independent risk factor for the development of NAFLD [Table 5].

### Discussion

The result of our study showed that patients with T2DM have significantly higher prevalence of NAFLD on abdominal ultrasonography as compared to healthy controls \((130/200 \text{ vs. } 47/200 \ [P < 0.0001])\). Worldwide prevalence of NAFLD ranges from 6.3% to 33% with a median of 20% in the general population.\(^{[9]}\) In Indian population, its prevalence is around 9%–32%\(^{[6,12]}\). In Dallas Heart study, one-third of the population had hepatic steatosis, whereas 62% of subjects who were known diabetics or fasting glucose >110 mg/dl had hepatic steatosis.\(^{[13]}\) Forlani et al. in 2016 done a nationwide study in 160 diabetic clinics of Italy and observed a 59.6% prevalence of NAFLD in T2DM patients.\(^{[14]}\) The overall prevalence of NAFLD in T2DM in Indian population is reported to be in range of 12.5%–87.5%.\(^{[8,10,12,13]}\) Hence, the results of our study are consistent with the studies done previously.

Similar to our study, elevated transaminases have been found in 2.8%–13.3% in general population\(^{[16,17]}\) and 7.8%–31.5% in T2DM.\(^{[18‑23]}\) The extent of elevated liver enzymes in Indian T2DM patients is not clear.\(^{[20]}\)

Obesity is a well-known risk factor for NAFLD which was also observed in our study. The study done by Wanless and Lentz\(^{[24]}\) in 351 patients showed that 70% of obese patients have liver steatosis and degree of steatosis was proportional to degree of obesity. An Italian study\(^{[25]}\) also showed that steatosis was 4.6-folds more common in obese persons. Also in persons undergoing bariatric surgery, its prevalence can exceed 90%\(^{[9]}\). Kalra et al.\(^{[26]}\) have found that in Indian T2DM patients, obese individuals have 14% higher risk for NAFLD.

In our study, dyslipidemia was more common in NAFLD patients. NAFLD is present in around 50% of dyslipidemia patients attending lipid clinics.\(^{[26]}\) Hyperlipidemia and specifically high levels of triglycerides and low levels of high-density lipoprotein (HDL)-cholesterol are strongly associated with NAFLD. In a study done by Marchesini et al.,\(^{[27]}\) hypertriglyceridemia and low HDL-cholesterol level were present in 64% and 30%–42% of NAFLD patients, respectively. In a study done by Duseja,\(^{[28]}\) in Indian subjects, abnormal cholesterol, TG, and HDL were present in 36%, 53%, and 66%, respectively. Silaghi et al. showed that sex, ALT, low-density lipoprotein (LDL) cholesterol were significantly and independently associated with the presence of NAFLD in T2DM subjects.\(^{[29]}\)

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**Table 2: Grading of fatty liver in cases and controls**

| Fatty liver | Diabetics | Controls |
|------------|-----------|----------|
| Grade I    | 80        | 31       |
| Grade II   | 30        | 12       |
| Grade III  | 20        | 4        |
| Total      | 130       | 47       |

**Table 3: Relationship of duration of diabetes mellitus with grading of fatty liver**

| Fatty liver | Duration of DM (years) | <5 | 5-10 | 10-20 | >20 | \(P\) |
|------------|------------------------|----|------|-------|-----|------|
| Grade I    | 23                     | 18 | 30   | 9     |     | 0.0001 |
| Grade II   | 1                      | 5  | 12   | 12    |     |       |
| Grade III  | 0                      | 2  | 9    | 9     |     |       |

**Table 4: Clinical and laboratory characteristics of patients with fatty liver and normal liver**

| Variables                      | Fatty liver \((n=177)\) | Normal liver \((n=223)\) | 95% CI | OR   | \(P\) |
|--------------------------------|--------------------------|--------------------------|--------|------|------|
| Diabetics                      | 130                      | 70                       |        |      |      |
| Obesity (BMI >25 kg/m\(^2\))   | 150                      | 103                      | 3.977–10.534 | 6.472 | <0.0001 |
| Elevated liver enzymes         | 52                       | 6                        | 6.282–36.036 | 15.045 | <0.0001 |
| Elevated waist circumference   | 172                      | 161                      | 5.194–33.784 | 13.247 | 0.0001 |
| Raised cholesterol             | 75                       | 85                       | 0.798–1.875 | 1.194 | 0.388 |
| Raised LDL                     | 108                      | 106                      | 1.158–2.578 | 1.728 | 0.007 |
| Low HDL                        | 123                      | 128                      | 1.116–2.562 | 1.691 | 0.013 |
| Raised TG                      | 95                       | 82                       | 1.108–2.456 | 1.650 | 0.013 |
| Gallstones                     | 28                       | 14                       | 1.428–5.511 | 2.805 | 0.0028 |

CI: Confidence interval; OR: Odds ratio; BMI: Body mass index; LDL: Low-density lipoprotein; HDL: High-density lipoprotein; TG: Triglyceride
Table 5: Multivariate regression analysis of variables to predict nonalcoholic fatty liver disease

| Variables                          | B    | OR   | 95% CI          | P     |
|-----------------------------------|------|------|-----------------|-------|
| T2DM                              | 1.569| 4.800| 2.836-8.124     | <0.0001|
| Elevated liver enzymes            | 2.796| 16.377| 5.638-47.574   | <0.0001|
| Obesity (BMI >25 kg/m²)           | 1.964| 7.127| 3.897-13.035     | <0.0001|
| Elevated waist circumference      | 2.387| 10.884| 3.997-29.641     | <0.0001|

Conflicts of interest

There are no conflicts of interest.

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