Non-alcoholic fatty liver disease assessment in Nepal

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ABSTRACT

Background: Non-alcoholic fatty liver disease (NAFLD) is a common entity seen in routine ultrasonography of the patients. The study also aims to demonstrate its relationship with biochemical markers and anthropometric measurement.

Methods: It was a retrospective observational study of the patients who had come for general health check-up where routine clinical history and examination, ultrasound of the abdomen and biochemical tests for liver function and lipid profile were performed. Exclusion made in the study includes alcoholic patients and other secondary causes of the fatty changes in the liver. Ultrasound staging was done for control (no steatosis), mild, moderate and severe fatty changes of the liver. Software named SPSS Statistics version 16.0 used for the proforma and analysis of data.

Results: In a total of ninety-nine patients, NAFLD was seen higher in women with 57.1%. The mild fatty liver was observed in 55.1%, moderate fatty liver in 32.7% and severe fatty liver in 12.2% of the patients. There were significantly higher triglycerides (TG) and alanine aminotransferase (ALT) levels as the severity of fatty liver increased. Cholesterol high density lipoprotein (HDL) was significantly lower as the severity of fatty liver increased. When compared to normal counterparts, overweight and obese patients had 4.2 and 5.1 times increased the risk of having NAFLD. Elevated ALT (OR=2.5, CI=1.8-3.6), triglyceride (OR=2.3, CI=1.6-3.4) and very low density lipoprotein (VLDL) (OR=1.6, CI=1.1-2.3) levels were associated with NAFLD.

Conclusions: Increased body mass index had more risk of having non-alcoholic fatty liver disease. HDL significantly decreased as the severity of fatty liver increased. ALT, TG, and VLDL concentrations showed strong association of being raised in non-alcoholic fatty liver disease patients.

Keywords: Biomarkers, Body mass index, Non-alcoholic fatty liver disease, Ultrasonography

INTRODUCTION

Nonalcoholic fatty liver disease (NAFLD) is a condition defined by significant lipid accumulation (5-10%) in hepatic tissue in the absence of significant chronic alcoholic consumption.¹ It includes no more than 30 gram of alcohol per day in men and 20 gram per day in women.² It is a broad terminology which encloses accumulation of fat cells in the liver without inflammation or fibrosis called simple hepatic steatosis to hepatic steatosis with histological evidence of inflammatory changes that may or may not proceed to liver cirrhosis. The latter condition is also known as non-alcoholic steatohepatitis (NASH).³ ⁴ It has been noticed that up to 20% of patients with NASH may develop to cirrhosis of the liver.⁵ NASH is presently known to be one of the common chronic liver disease and foremost cause of cryptogenic cirrhosis.⁶ ⁷ In fact, the etiology of NAFLD is multifactorial, and has not been explained fully.⁸ Primary NAFLD strongly linked with metabolic syndrome and the risk for hepatic steatosis rises...
exponentially with the joining of each component of metabolic syndrome: type II diabetes mellitus, hyperlipidemia, visceral obesity and hypertension. Also, secondary NAFLD is caused by the use of some drugs, by toxins, surgeries for obesity, and total parenteral nutrition. The gold standard method for diagnosis and prognosis of NAFLD is liver biopsy; however, the possible risks and invasiveness have limited its use. Liver biopsy is not obligatory for the diagnosis of NAFLD and is not always necessary to differentiate simple fatty liver from the most severe form. Radiological imaging techniques are safe, reliable, and acceptable methods for the diagnosis of NAFLD. They are becoming popular for estimating the severity of NAFLD and the diagnosis of patients in the preclinical stage of disease, even before the liver function tests show any abnormality. Because of the cost-effectiveness and availability, ultrasonography (USG) is widely used to detect NAFLD. This research is first of its type in the country mainly evaluated the association of biochemical markers with the NAFLD. We used liver ultrasonography for the diagnosis of NAFLD and the evaluation of disease severity in this study.

**METHODS**

It was a retrospective observational study done in Manmohan Memorial Hospital, Kathmandu. Medical records from January 2015 to December 2015 were collected for those patients who had visited hospital for general health check-up where routine laboratory investigations (lipid profile and liver function tests) as well as abdominal ultrasound was performed along with short medical history and examination reports. Demographic profiles and findings were recorded. Ethical approval from Institutional Review Board (IRB) was also obtained.

**Inclusion and exclusion criteria**

Exclusion in the study includes patients with any of the following criteria listed below:

- Chronic alcohol abuse.
- Hepatobiliary diseases
- Severe chronic illnesses
- Medications are known to cause hepatic steatosis
- Familial hyperlipidemia.
- Focal fatty changes in the liver as it sometimes mimic metastases, and resolve as early as six days.

**Biochemical analysis of serum parameters**

**AST**

It is an enzyme found in liver and other organs like heart and muscle cells. It increases in liver diseases and stress condition. The abnormal level is >40 U/L.

**ALT**

It is a liver enzyme and rises in hepatic injury. The abnormal level is >40 U/L.

**TG**

TG are packed into chylomicrons and VLDL particles, and its level is measured in fasting state. Its normal range is <200 mg/dl.

**Cholesterol**

It is a fat-like substance, presence in cell membranes and steroid hormones, and is transported by lipoproteins in the blood. More than 200 mg/dl is abnormal. Lipoproteins are particles that contain triglycerides, phospholipids and cholesterol and amphipathic proteins called apolipoproteins. The measure of lipid in a lipoprotein affects its density. The density is inversely proportional to the degree of lipid, the lower the density of a lipoprotein, then more lipid it contains relative to protein. The main four types of lipoproteins are chylomicrons, VLDL, LDL, and HDL. Chylomicrons and VLDL (<40 mg/dl) deliver TG to cells in the body. LDL (<100 mg/dl) delivers cholesterol to cells in the body. HDL (<55 mg/dl) is involved in reverse cholesterol transport. It carries excess cholesterol from cells to the liver, which secretes cholesterol in bile or converts it to bile. Total bilirubin (TB) <1.0 mg/dl and Direct bilirubin (DB) <0.3 mg/dl, Alkaline phosphatase (AP) <40-130 U/L and serum albumin 3.9-4.9 g/dl were considered as normal values.

**Ultrasound measurements**

An ultrasound device with a speed of 3.5 MHz, the convex-type transducer was used to demonstrate the fatty liver infiltration. Sonography of fatty infiltration varies depending on the amount of fat diffuse steatosis may appear as mild, moderate, and severe. Figure 1 illustrate ultrasound photograph of the fatty liver.

**Mild**

Minimal diffuse increase in hepatic echogenicity with normal visualization of the diaphragm and intrahepatic vessel borders.

**Moderate**

Moderate diffuse increase in hepatic echogenicity with slightly impaired visualization of intrahepatic vessels and diaphragm.

**Severe**

Marked increase in echogenicity of liver with poor penetration and visualization of the posterior segment of the right lobe of the liver, hepatic vessels, and diaphragm.
Anthropometric measurement

According to the World Health Organisation (WHO) classification, BMI <18.5 categorized as ‘underweight’, BMI = 18.5–24.9 as ‘normal’, BMI = 25.0–29.9 as ‘overweight’, and BMI ≥30 as ‘obese’.

Alcohol unit

UK guideline reports, one alcohol unit is equal to 8 grams of alcohol.22 The UK units of alcohol are calculated by multiplying the volume of the drink in milliliters with its percentage alcohol by volume (ABV), and dividing the result by 1000.23

Statistical analysis

Analysing data using SPSS PC software package for our statistical calculation. Descriptive tests were used to assess the difference in general characteristics of the patients. Chi-square test was used to compare categorical variables. In the case of more than two groups, one-way ANOVA test was done. Calculation executed for Odds ratios (ORs) with their 95% confidence intervals (CIs) for each independent variable.

RESULTS

General characteristics of the study group

General characteristics of the 99 participants showed that 57 were men, and 42 were women. However, NAFLD was seen higher in women (57.1%) than men (42.9%). The result we observed in NAFLD patient is that 27 persons (55.1%) had mild fatty liver, 16 persons (32.7%) moderate fatty liver, and 6 persons (12.2%) had the severe fatty liver. The average age was 44±11.96 years for patients with NAFLD. The mean BMI of the patients diagnosed with NAFLD was 28±4.41 kg/m² indicated a tendency to be overweight as compared to the normal groups without having NAFLD.

Table 1: Baseline characteristics of the participants.

| Parameter                        | Value                  |
|----------------------------------|------------------------|
| Number of patients (N)           | 99                     |
| Number of fatty liver patients   | 49                     |
| Number of non-fatty liver patients | 50                   |
| Weight: mean±SD (kg/m²)          |                        |
| BMI of fatty liver               | 28±4.1kg/m²            |
| BMI of no fatty liver            | 21.8±1.7 kg/m²         |
| Age (years)                      |                        |
| Fatty liver                      | 44±11.96 years         |
| No fatty liver                   | 35±10.9 years          |
| Male (Number of patients)        |                        |
| Fatty liver                      | 21 (42.9%)             |
| No fatty liver                   | 36 (72%)               |
| Female (Number of patients)      |                        |
| Fatty liver                      | 28 (57.1%)             |
| No fatty liver                   | 14 (28%)               |
| USG diagnosed fatty liver grade  |                        |
| Mild                             | 27 (55.1%)             |
| Moderate                         | 16 (32.7%)             |
| Severe                           | 6 (12.2%)              |

Comparison of gender, BMI, lipid profile and hepatic serum parameters with ultrasonographic severity of fatty liver

There was higher triglyceride and ALT levels as the severity of fatty liver increased. However, the HDL level was getting lower as the severity of fatty liver increased. Statistically significant result within three groups was seen in ALT, TG and HDL (p<0.001). The examination of varying weight categories showed that BMI was high in all three types of severity of fatty liver. Its proportion increased as the severity of fatty liver increased (p<0.001). The comparison is listed in Table 2.

Analysis of risk factors for ultrasound diagnosed nonalcoholic fatty liver disease

The male is less likely to have fatty liver changes as compared to female OR=0.553 (CI=0.360-0.826). Overweight and obese groups had 4.2 and 5.1 times the risk of having fatty liver, respectively, as compared to their normal counterparts. ALT is likely to be 2.5 (CI:1.8-3.6) times the risk of having raised values among those with fatty liver changes. Another most important significant factor associated with NAFLD was TG, with a 2.3 (CI:1.6-3.4) fold increase in risk for among those with having NAFLD. Similarly, VLDL also showed OR=1.6 (CI:1.1-2.3) which was also statistically significant (Table 3).
Table 2: Gender, BMI and biochemical parameters of ultrasound diagnosed severity of fatty liver.

| Parameter       | Mild            | Moderate        | Severe           | p-value |
|-----------------|-----------------|-----------------|------------------|---------|
| Male/Female     | 11/16           | 8/8             | 2/4              | 0.027   |
| BMI (kg/m²)     | 26.4±4.3        | 28.5±4.6        | 30±1.7           | <0.001  |
| Cholesterol (mg/dl) | 190±21.3       | 186.3±43.1      | 210±90.6         | 0.06    |
| ALT (U/L)       | 38.7±14         | 47.4±24.4       | 81.8±29.1        | <0.001  |
| AST (U/L)       | 37±7.9          | 36±21.5         | 39±12.4          | 0.09    |
| TG (mg/dl)      | 217±101.8       | 242±66          | 348±150          | <0.001  |
| HDL (mg/dl)     | 58±5.6          | 56±4.8          | 43±9.3           | <0.001  |
| LDL (mg/dl)     | 80±23           | 87±33           | 87±17            | 0.15    |
| VLDL (mg/dl)    | 45±16           | 44±25           | 54±22.3          | 0.08    |
| AP (U/L)        | 79±30           | 75±30           | 80±36.5          | 0.8     |
| Albumin (gm/dl) | 4.0±0.4         | 4.2±0.3         | 4.0±0.1          | 0.6     |
| TB (mg/dl)      | 0.4±0.5         | 0.25±0.44       | 0.5±0.50         | 0.66    |
| DB (mg/dl)      | 0.18±0.39       | 0.25±0.44       | 0.3±0.51         | 0.86    |

Table 3: Risk factors analysis for variables associated with non-alcoholic fatty liver.

| Parameter       | OR (CI) | p-value |
|-----------------|---------|---------|
| Sex (male versus female) | 0.55 (0.37-0.82) | 0.003   |
| BMI Overweight versus normal | 4.2 (1.83-9.37) | <0.001  |
| Obese versus normal       | 5.1 (2.76-12.96) | <0.001  |

DISCUSSION

This retrospective study mainly evaluated the association between lipid profile and hepatic serum parameters with ultrasound-diagnosed NAFLD excluding patients with possible causes for secondary hepatic steatosis.

The study population demonstrated slightly higher proportion of female patients with NAFLD, which could be due to sedentary lifestyle and limited mobility than the male population. It also revealed sonographic mild fatty liver in 55.1%, moderate and severe fatty liver in 32.7% and 12.2% respectively in NAFLD group. It is similar to a study performed by Razavizade et al and Chen-chung Fu et al. Compared with normal counterparts; overweight had four times, and obese groups had five times the risk of having NAFLD. The severity of fatty liver positively associated with how overweight or obese the subject findings in our study was similar to other studies. So as the body mass index increased, increased risk of fat accumulation in liver occurred, causing more severe NAFLD.

Significantly higher ALT, triglyceride levels and lower HDL as the severity of fatty liver increased indicated that a higher ALT and above-mentioned lipid levels had a connection with the ultrasound-diagnosed severity of NAFLD. This finding of elevated ALT correlated with other researchers in that raised ALT was a predictor of NAFLD. Razavizade et al demonstrated increased ALT, TG and decreased HDL in the severity of fatty liver. Ghamar-Chehreh et al however, showed elevated LDL and hypercholesterolemia rather than hypertriglyceridemia associated with severity of NAFLD. ALT is considered to be a specific marker for liver injury. Raised level of ALT in NAFLD is still not clear. There have been several hypothesis are being proposed. One of them is that in obesity, there is an accumulation of excess fat in liver parenchyma. Since adipose tissue also exhibits an endocrine function as it secretes adipocytokines that have a potential role in insulin resistance as well as reactive oxygen species activation, the latter inducing necroinflammation in hepatic tissue.

Triglycerides are fats synthesized in the liver or, in the case of those derived from dietary sources, are ingested by the liver; the triglycerides subsequently transports throughout the circulation by triglyceride-rich lipoproteins (mainly chylomicrons and VLDL). Insulin resistance induces lipolysis in adipose depots, producing increased free fatty acid, thus a state of energy excess, causing increased formation of hepatic triglycerides. The liver then secretes triglycerides mainly in the form of chylomicrons and VLDL particles for transportation to peripheral tissues, including, cardiac muscle, skeletal muscle, and adipose tissue. So significantly raised the level of TG and VLDL in this study was justifiable. AST is an intracellular enzyme that is discharged into serum
when tissue injury and cell death occur. It is not a precise marker of hepatic cell damage and could elevate in many other conditions independent of liver disease.32 No significant association of AST level and ultrasonographic grading in this study was reasonable.

The study comprised of small sample size of one hospital so there was a lack of generalization. It was a cross-sectional study so the cause and progression of NAFLD could not be revealed in our research. Nonalcoholic steatohepatitis (NASH) is an aggressive liver disease that can lead to advanced fibrosis, cirrhosis, and even hepatic failure. Therefore additional longitudinal study would be more useful. Ultrasonographic grading was based on the visual classification. Overlapping usually between the moderate and severe fatty liver might occur. Sometimes patients with the borderline ultrasonographic findings of moderate or severe might be misclassified as to either group.33

**CONCLUSION**

This research strongly revealed the association of obesity, elevated TG, VLDL and ALT levels with NAFLD, and showing dominance for female population in Nepal. Routine ultrasonography of the liver thus recommended for obese patients. Therefore, this study helped to add further knowledge of the understanding of anthropometric, biochemical and radiologic associations in NAFLD, a very common entity of growing importance.

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