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BIOCHEMICAL CHARACTERISTICS OF NON-ALCOHOLIC FATTY LIVER DISEASE

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Abstract
Nonalcoholic fatty liver disease (NAFLD) is one of the most common causes of chronic liver disease. It encompasses a spectrum of conditions associated with lipid deposition in hepatocytes. It ranges from nonalcoholic steatohepatitis to advanced fibrosis and cirrhosis. The disease is mostly silent and is often discovered through incidentally elevated liver enzyme levels. This study was to analyze the distribution of non-alcoholic fatty liver disease (NAFLD) with reference to age, gender, and socioeconomic status, to look for clinical features A total of 3762 residents were included in the present study including 2328 males and 1434 females with a mean age of 46.37 ± 14.28 years (range 20–92 years). Measurements were taken for assessment of BMI and blood samples were collected for estimation of fasting blood glucose, Triglycerides, Cholesterol, Alanine transaminase, Aspartate transaminase, Total bilirubin, HDL and LDL. All the nonalcoholic fatty liver disease patients studied are either overweight or obese and 92% of them are glucose intolerant or diabetics. The fasting blood glucose, serum triglyceride, serum cholesterol, serum uric acid, ALT and ALT/AST ratio values are significantly increased in NAFLD patients compared to controls. Uric acid and ALT/AST ratio are showed best overall discriminatory capacity among all biochemical parameters for NAFLD. NAFLD can be seen in both male and female patients associated with Insulin resistance syndrome and oxidative stress. The ALT/AST ratio is the better biochemical marker for diagnosis of NAFLD. More large-scale prospective studies can validate our observations, help physicians in early identification of patients who may benefit from therapeutic interventions, and even help them formulate more effective treatment algorithms.
KEYWORDS: Non alcoholic fatty liver disease, steatohepatitis, Insulin resistance.

INTRODUCTION
Non-alcoholic fatty liver disease (NAFLD) is the most common cause of liver disease in the Western world [1]. The spectrum of abnormalities includes a simple accumulation of triglycerides (TG) in the hepatocytes (hepatic steatosis), hepatic steatosis with inflammation (steatohepatitis), fibrosis, and cirrhosis. The prevalence of NAFLD is around 9–32% in the general Indian population [2]. It is present in up to 90% of obese patients [3]. NAFLD is also the most common cause of elevated liver enzymes [4]. It can progress to hepatitis and hepatocarcinoma. Understanding the epidemiology and possible correlations of dyslipidemia, glycemic status and other risk factors with NAFLD are essential for developing effective treatment and prevention strategies, especially for people of this part of eastern India.

Hence, this study was undertaken to evaluate the risk factors and biochemical characteristics of NAFLD with the aim to analyze the distribution of NAFLD with reference to age, gender, and socioeconomic status, to look for clinical features and biochemical parameters in patients of NAFLD, and to analyze statistically significant differences in biochemical parameters of patients and controls. Such a study has not been documented in this part of Chennai residence.

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MATERIAL AND METHODS

The present study was carried out in the Sixth People' Hospital of Tamilnadu .3762 cases of 1177 Non Alcoholic fatty liver disease patients were studied. 309 subjects who were clinically healthy were taken as controls. The study was conducted with the approval of the institutional ethical committee. The clinical diagnosis of cases of NAFLD was based on the presence of insulin resistance, in the absence of alcohol abuse, viral, autoimmune, genetic and induced liver disease and was correlated by further investigation (ultrasound abdomen showing fatty liver). Most of NAFLD patients were asymptomatic while a few complained of discomfort in the right upper quadrant of the abdomen.

In addition, blood tests were performed to assess liver function and to exclude other causes of liver disease. The exclusion of significant alcohol intake was essential. Presence of abnormal fat accumulation in the liver found by X-rays and ultrasound images confirmed the diagnosis.

Monthly income as indicator of socioeconomic status was recorded as follows: Upper socioeconomic status - 30,000/- and above per month, middle socioeconomic status - 10,000–30,000/- per month, and lower socioeconomic status - <10,000/- per month. Background was recorded as urban or rural.Weight, height, waist circumference (WC), and blood pressure (BP) including systolic BP (SBP) and diastolic BP (DBP) were measured. Body mass index (BMI) was calculated according to the World Health Organization (WHO) [5]. Lean patients had BMI 18.5–24.9 kg/m2, overweight patients had BMI 25–29.9 kg/m2, and obese had a BMI >30 kg/m2.Waistline was measured at midpoint between lower chest wall and iliac crest with the patient standing. WC >90 cm in male and >80 cm in female would indicate central obesity.

NAFLD was diagnosed by USG of liver using the standardized criteria of increased echogenicity of liver texture compared to the right kidney, lack of transmission of sound to the posterior diaphragmatic interface, lack of visibility of vascular structure due to ill-defined portal walls invaded by fat, and increased liver size as measured in midclavicular line [6]. Fatty liver was diagnosed in the presence of two of the three observations of bright hepatic echotexture compared to kidney and spleen, blurring of hepatic veins, and loss of deep echodiscontinuous diaphragm.

All respondents donated blood samples after over 10 h of overnight fasting for biochemical measurement including fasting blood glucose, total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), TG, aspartate aminotransferase (AST), alanine aminotransferase (ALT), γ-glutamyl transferase, alkaline phosphatase, and C-reactive protein (Siemens Dimension RXL Max Biochemistry Analyzer). Other tests done were complete blood count (Sysmex KX 21), prothrombin time (Sysmex CA50 Analyzer), serology for antinuclear antibody, markers for viral hepatitis A, B, C, and E by ELISA, serum ferritin, ceruloplasmin, thyroid-stimulating hormone (TSH) (chemiluminescence, immunoassay on Siemens Advia Centaur XP), and glycosylated hemoglobin (HbA1C) by HPLC (Transasia Hb Vario).

We have used NAFLD fibrosis score (www.nafld score.com) which is a validating scoring system comprising of six parameters (age, hyperglycemia, BMI, platelet count, albumin, and AST/ALT ratio). This helps to classify patients into groups with low, intermediate, and high

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probability/risk for liver-related complications [7].All values are reported as the mean and standard deviation for continuous variables and the number (percent) for categoric variables. Comparison of groups was performed using analysis of variance and Fisher tests. Statistical significance was set at $p<0.05$. All statistical calculations were performed using Microsoft Excel.

RESULTS AND DISCUSSION

Majority of the studies have shown that NAFLD patients are diagnosed during routine investigation of suspected patients or patients with mild vague symptoms. It has been reported that majority of the patients are shown to have mild abnormal liver function tests.

With the increased incidence of diabetes mellitus, obesity, and insulin resistance in India in the past two decades, it is only logical to expect an increase in the incidence of NAFLD in India. Several diagnostic modalities have been used for the diagnosis of fatty liver, including ultrasound, liver enzymes, computed tomography, magnetic resonance imaging spectroscopy (MRS), and liver biopsy. Ultrasound was the preferred method for our study due to the relatively low cost, fair accuracy, and non-invasive nature. Liver enzymes may not be elevated in NAFLD patients. Liver biopsy through the gold standard is not feasible in all suspected cases. The accuracy of MRS is overshadowed by its huge cost.

In this study, 31.25% of 1177 patients who had undergone USG evaluation were diagnosed as NAFLD. About two-third (62%) patients were from rural areas. 62% of NAFLD patients were middle socioeconomic class. Our study shows that weight and BMI were significantly higher in cases compared to controls ($p<0.05$). Mean age of the patients is 45 years. More males were affected as has been depicted in Table 1.

We classified cases and controls into lean, overweight, and obese with BMI ranging 18.5–25 kg/m2 for lean, 25–30 kg/m2 for overweight, and >30 kg/m2 for obese [5]. In the control group, 77% were lean, 20% overweight and 3% obese. In NAFLD patient group, 48% were lean, 40% overweight and 12% obese. Lean and overweight together constituted 88% of the total patient population. Our study shows that lipid profile was significantly higher in cases compared to controls. Mean TSH is significantly higher among cases when compared with that of control group. Mean serum glucose level is significantly higher among cases. All these biochemical findings are summarized in Table 2.

Mean total serum cholesterol, triglyceride, and LDL cholesterol level of control group were significantly lower than patient group. A study [8] showed significant association of elevated total serum cholesterol and NAFLD as when compared with controls. However, other authors [9,10] showed no significant association of total serum cholesterol and NAFLD. Singh *et al*. [11] found that serum triglyceride level was significantly higher in NAFLD group when compared to controls. Nigam *et al*. [12] showed significant association of serum triglyceride level and NAFLD. Mean serum HDL level is low among NAFLD patients when compared with control group, and this is statistically significant ($p<0.005$). Odds ratio as per another study [13] of low HDL-C (<40 mg/dl in men or <50 mg/dl in women) is 1.61–2.0. Although most patients of NAFLD are obese or overweight, lean NAFLD may represent a different pathophysiology, rather than merely a by-product of different BMI [14]. Our lean patients' lipid profile did not differ significantly from overweight and obese patients.

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As per a study [15], the entire histologic spectrum of NAFLD can be seen in subjects with normal ALT. Our study, like others' [12], showed a significant association of ALT with NAFLD. However, the study of Gupte *et al.* [17] showed no significant association between the two.

Plasma glucose level and HBA1C were higher among NAFLD cases when compared with control group, as were observed by other authors [16]. Mean HBA1C level in our study was 4.9±0.5 among control group and 4.8±0.9 among NAFLD cases. When we divided the patient group into lean, overweight, and obese as per the WHO classification, we found that HDL-C was significantly different in obese compared to lean. AST/ALT ratios were significantly different on comparing lean with overweight and lean with obese.

In a recent study [18], it was observed that BMI, SBP, DBP, TC, HDL cholesterol, LDL cholesterol, and triglyceride were significantly different in lean patients with NAFLD compared to control group. Furthermore, WC in men and hip circumference in women were higher in lean patients with NAFLD. Feng *et al*. [13] noted that total free fatty acid (FFA) profiles were insignificantly different between lean (2093.33±558.11 μg/ml) and overweight (2420.81±555.18 μg/ml) NAFLD patients, obese NAFLD (2739.01±810.35 μg/ml) presented most significantly elevated ($p<0.05$) total FFA profiles.

Obesity is known to be a good predictor of hepatic steatosis and disease progression [14]. Steatosis is at least 2 times more frequent in overweight than in lean subjects. The lipids which are elevated inside the hepatocytes may be TG, FFA, diacylglycerol, cholesterol, cholesterol esters, phospholipids, and ceramides. In a study in India [15], it was reported that a statistically significant difference between the BMI, waist–hip ratio, mean triglyceride, and AST and ALT values exists between the obese and non-obese patients of NAFLD.

TSH levels showed statistically significant difference when we compared lean with overweight and lean with obese patients. The thyroid gland is significantly involved in energy homeostasis, lipid and carbohydrate metabolism, and adipogenesis. In a clinical setting, subclinical hypothyroidism has been associated with metabolic syndrome, cardiovascular mortality, and disturbance of lipid metabolism. The prevalence of hypothyroidism was reported to a range from 15.2% to 36.3% [16] among patients with NAFLD or non-alcoholic steatohepatitis.

To clarify the mechanism of thyroid dysfunction and NAFLD, studies mention elevated leptin and visfatin, both adipocytokines seen in NAFLD . Leptin is involved in the regulation of appetite. Elevated levels are seen in obesity. They induce collagen synthesis in the liver and stimulate hepatic insulin resistance. Patients with NAFLD have abnormal lipid profiles. Thyroid hormones induce their effects on lipid metabolism through thyroid hormone receptor β, which is expressed in liver [17]. Thyroid hormone receptor activation results in a reduction in body weight and fat as well as a decrease in cholesterol and triglyceride levels, which takes place only in hepatocytes [18]. Stress also elevates leptin levels and can be an indirect cause for NAFLD. While one study [19] advocates the use of Ezenus in the treatment of stress, another study [20] mentions that flavonoids present in *Vitis Vinifera* seeds have powerful antioxidant and anti-inflammatory properties, maintain hepatocytes structure integrity, and decrease ALT levels in NAFLD.

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We found that 70% of patients had NAFLD score <1.5. They are at low probability of progressing to liver fibrosis. 10% of NAFLD patients had a score >0.67 and were at high probability of progressing to fibrosis while 20% of patients with score ranging from >1.5 to <0.67 are at intermediate probability of progressing to liver fibrosis [21]. Compared to lean patients, overweight and obese showed significantly higher fibrosis score ($p<0.05$).

CONCLUSION

A simple non invasive predictive model that incorporates both clinical and biochemical parameters of metabolic syndrome can identify patients at risk with NAFLD at an early stage and the condition can be reversed by taking proper therapeutic measures, avoiding routine liver biopsy.

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Table 1: Anthropometric measurement of cases and controls (mean±SD)

Parameter	Control (n=30)	Case (n=50)	p value
Age	31.2±8.8	42±12	NS
Sex (M/F)	21/9	30/20	Sex (M/F)
Height (m)	1.7±0.1	1.7±0.1	NS
Weight (kg)	67.8±10.5	70.5±15.4	<0.05
BMI (Kg/m2)	23.6±2.9	25.6±4	<0.05
Waist/hip ratio	0.9±0.1	0.9±0.3	NS

NS: Not significant, BMI: Body mass index

Table-2: Biochemical characteristics of cases and control (mean±SD)

Parameter	Control (n=30)	Case (n=50)	p value
Cholesterol (mg/dl)	151.4±18	181.2±46	p<0.001
Triglyceride (mg/dl)	115±17.4	147.4±56.7	p<0.005
HDL cholesterol (mg/dl)	49.9±7.7	43.3±10.5	p<0.005
LDL cholesterol (mg/dl)	78.7±10.1	98.1±30.9	p<0.001
AST (U/L)	21.8±8	46.6±22.7	NS
ALT (u/l)	30±8.2	87.7±51.5	p<0.05
Albumin (g/dl)	4.3±0.2	4.3±0.5	NS
TSH (mIU/L)	2.3±1.2	3.6±1.9	p<0.001
Fasting glucose (mg/dl)	102±5.7	115.2±45	p=0.001
Ferritin (ng/ml)	117.6±87.3	146.7±97.3	NS
Ceruloplasmin (mg/dl)	26±4.2	30.4±6.4	NS

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HBA1C (%)	4.9±0.5	5.0±0.9	p=0.001
Platelet (103/mm3)	2.9±0.7	3.1±1	NS

HBA1C: Hemoglobin, TSH: Thyroid-stimulating hormone, ALT: Alanine aminotransferase, AST: Aspartate aminotransferase, LDL: Low-density lipoprotein ;HDL: High density lipoprotein, NS: Not significant