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Study of Lipid Profile in Non-Alcoholic Fatty Liver Disease.

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Abstract:Non-*alcoholic fatty liver disease (NAFLD) is a major cause of chronic liver disease worldwide occurring when fat is deposited (steatosis) in the liver not due to excessive alcohol use. The present study was carried out in the Department of Biochemistry, Gandhi Medical College and Hospital, Secunderabad, to evaluate the utility of body mass index, serum triglycerides, total cholesterol, high-density lipoprotein cholesterol, low-density lipoprotein cholesterol in the characterization and diagnosis of non-alcoholic fatty liver disease. A total of 100 subjects were selected in this study, of which 75 cases were nonalcoholic fatty liver disease patients and 25 clinically healthy subjects as controls. The body mass index of all the cases (n=75) had above normal BMI values (> 25 kg/m^2), among them n=22 subjects (29.33%) were overweight, while 53 (70.67%) were obese. The average BMI for the cases was significantly high 32.15±2.47 compared to controls 22.96±4.64. Lipid profile abnormality was observed in NAFLD cases. 82.67% and 60% patients were found to have hypertriglyceridemia and hypercholesterolemia respectively. The mean serum triglycerides, total serum cholesterol, LDL cholesterol were significantly ($p \leq 0.05$) high in 65.33% fatty liver cases 248.09±168.37, 210.99 ± 43.25 and 116.86 ± 43.53 mg/dl respectively compared to controls which were 173.6±55.43, 167.4±25.88 and 168 ± 21.38 mg/dl respectively. HDL cholesterol was significantly decreased in 65.33% of fatty liver patients compared to controls 40.24 ± 9.15 vs 32.44 ± 8.22 mg/dl*

Key words:*HDL cholesterol, LDL cholesterol, Non-alcoholic fatty liver disease, serum triglycerides, total serum cholesterol.*

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I. Introduction

Non-alcoholic fatty liver disease (NAFLD) is a major cause of chronic liver disease worldwide [1], occurring when fat is deposited (steatosis) in the liver not due to excessive alcohol use. Non-alcoholic fatty liver disease is a clinic-histopathological entity with histological features that resemble alcohol-induced liver injury, but by definition, it occurs in patients with little or no history of alcohol consumption. It encompasses a histological spectrum that ranges from fat accumulation in hepatocytes without concomitant inflammation or fibrosis (simple hepatic steatosis) to hepatic steatosis with a necro-inflammatory component (steatohepatitis) that may or may not have associated fibrosis. The latter condition, referred to as non-alcoholic steatohepatitis (NASH), may progress to cirrhosis in up to 20% of patients [2] and is associated with liver cancer [3, 4]. Non-alcoholic fatty liver disease (NAFLD) is one of the most common liver diseases in developed countries; about 20-50% of the total populations are suffering from this condition. The important risk factors associated with this disease are obesity, diabetes mellitus and dyslipidemia [5]. The major risk factors for NAFLD, central obesity, type 2 diabetes mellitus, dyslipidemia, and metabolic syndrome are common in western societies. NAFLD is the most common liver disorder in Western industrialized countries, affecting 20 to 40% of the general population [6]. NAFLD are now being increasingly recognized as a major health burden. The prevalence of fatty liver in India has been shown to be as high as 15%-30% [7], which is similar to that reported from some of the western countries [8, 9]. Estimates of prevalence of NAFLD in Asia-Pacific regions range from 5 to 30% [10].

According to the earlier reports majority of NAFLD cases are relatively mild and have a benign course. However, it has now been documented that number of these cases can progress to fibrosis, cirrhosis, liver failure and hepatocellular carcinoma and thus contributes to liver related mortality and morbidity [11].

At the time of diagnosis most NAFLD patients had no symptoms or signs of liver disease, although many patients report fatigue or malaise and a sensation of fullness or discomfort on the right side of the upper abdomen. Hepatomegaly is the only physical finding in most patients.

The present study was aimed to investigate the clinical patterns and serum lipid profile in the subjects selected for the study. Therefore, the study was designed to evaluate the utility of body mass index (BMI), serum triglycerides (TGL), serum total cholesterol (CHOL), high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C) in the characterization and diagnosis of nonalcoholic fatty liver disease. This will help to know the association of lipid profile abnormalities in the patients; which will help for the earlier identification of dyslipidemia and better management in future.

II. Material And Methods

The present study was carried out in the Department of Biochemistry, Gandhi Medical College and Hospital, Secunderabad. A total of 100 subjects were selected in this study. Of which 75 cases were nonalcoholic fatty liver disease patients and 25 subjects that were clinically healthy without NAFLD were treated as controls. The approval from institutional ethical committee was duly taken before start of this research. 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.

Consent from the subjects was taken to participate after explaining the objective of the study. All the 100 subjects (n=100) included in the present study were subjected to a detailed history taking and systematic examination. All patients were investigated for serum lipid profile. Subjects were considered as cases if they have fatty liver according to the standard criteria accepted by the American Gastroenterology Association i.e., an increase in hepatic echogenicity as a reference, the presence of enhancement and lack of differentiation in the periportal intensity and the vascular wall due to great hyperechogenicity in the parenchyma.

Enzymatic methods were used for determination of serum cholesterol [12] using Bio-Diagnostic Kit and triglyceride [13] by Human triglyceride kit. Estimation of HDL-chol was done by precipitation method [14]. HDL precipitant and subsequent determination of cholesterol by enzymatic method [13]. LDL-chol was calculated according to the formula given by Friedwald et al. [15].

Exclusion Criteria For Both NAFLD Patients And Controls Included:

1. Daily alcohol intake > 30gm in men or > 20gm in women.
2. Use of corticosteroids, tamoxifen, methotrexate or high dose estrogen.
3. Jejunoileal by pass or extensive bowel resection.
4. liver cirrhosis and other known liver diseases.
5. Malignancy.
6. Pregnancy.

Systematic data collection was carried out from cases as well as controls after obtaining the consent. Age, gender, height and weight were documented. Body mass index (BMI) was calculated and defined as weight/height ((kg/m^2). The blood samples were collected for the estimation of following parameters in the serum triglycerides, serum total cholesterol, HDL-C and LDL-C.

III. Statistical Analysis

The results of controls and cases were recorded on Microsoft excel sheet using windows XP. Mean values and standard deviation in controls and cases were calculated using Microsoft excel sheet. Analysis of variance test (ANOVA), p-value was calculated and $p \leq 0.05$ was considered statistically significant.

IV. Results And Discussion

The present study was undertaken to assess lipid profile parameters in the diagnosis and progression of nonalcoholic fatty liver disease. The study participants consisted of 100 (n=100) subjects, of which seventy five (n=75) subjects were ultrasonographically diagnosed NAFLD cases and twenty five (n=25) were normal and treated as controls. The parameters such as BMI, serum cholesterol, LDL and HDL were analyzed in 100 subjects. Among 75 (n=75) cases selected in this study, n=28 (37.33%) were males and the rest n=47 (62.67%) were females. Among n=25 controls, n=10 (40%) were males and the remaining n=15 (60%) were females.

The mean age and SD values of cases (n=75) were 48.28±9.26 and 40.40±11.67 for controls (n=25). In the previous studies on Indian population the mean age was reported to be 42.90 ± 10.54 years [16], 55.4 years [17],

49.14 ± 9.65 years [18], 46.65 ± 15.06 years [19]. Most of the western studies have reported the mean age of NAFLD cases between 41-45 years.

In our study the body mass index (BMI) of all the cases (n=75) had above normal BMI values (> 25 kg/m^2). Among them n=22 subjects (29.33%) were overweight, while 53 (70.67%) were obese. The average BMI for the cases was 32.15 ± 2.47, while that of controls was 22.96 ± 4.64. The differences were statistically significant ($P < 0.05$). Our results are contradictory to the earlier findings. Body mass index (BMI) of participants varied from 18.14 to 44.75 kg/m^2 with mean value of 27.97 ± 4.28 kg/m2. In a population-based study from rural India, 52% of individuals with NAFLD were lean with BMI < 23 Kg/m^2 [20]. Das et al. [21] reported that individuals with normal BMI had two-fold increases in the risk of NAFLD than those with a BMI < 18.5 Kg/m^2.Obesity is considered to play an important role in the development of NAFLD, and the majority of patients with NAFLD are either obese or overweight. However, NAFLD has been reported in lean subjects residing in developing countries as well as developed countries [21], Kumar et al. [20], Alam et al [22]. Some epidemiological studies reported direct correlation between BMI and the probability of having fatty liver. In this study, all the cases (100%) had above normal BMI values, 70.67% of patients were found obese and the remaining 29.33% subjects were overweight.

In this study, lipid profile abnormalities was observed in NAFLD cases (Table 1). 62 (n=62) patients (82.67%) were found to have hypertriglyceridemia. The mean serum triglycerides in the controls was 173.6 ± 55.43 mg/dl, while that of the cases was significantly high 248.09 ± 168.37 mg/dl (Table 1). The difference was statistically significant ($P < 0.05$). 45 patients (60%) had hypercholesterolemia in this study. The mean serum cholesterol of NAFLD patients was significantly high recording 210.99 ± 43.25 compared to controls (167.4 ± 25.88 mg/dl). The increase in mean serum cholesterol levels of NAFLD cases was statistically significant ($P < 0.05$). Our results are comparable with earlier findings [2]. LDL cholesterol compared to normal controls was significantly high in fatty liver cases, 116.86 ± 43.53 vs 168 ± 21.38 ($p < 0.05$) and was observed in 65.33% of the cases. HDL cholesterol was significantly reduced in fatty liver patients 40.24 ± 9.15 vs 32.44 ± 8.22 mg/dl ($p < 0.05$) compared to controls. Roli Agrawal et al [16] reported hypertriglyceridemia in 63.7%, hypercholesterolemia in 50%-80% patients and elevated LDL in 25% of NAFLD patients.

In our study significantly decrease in HDL was seen in 65.33% of the cases. Roli Agrawal et al [16] reported low HDL in 45.16% of NAFLD patients.

Table 1. Mean ± SD of all parameters studied in both cases and controls

Parameters	**Cases (Mean ± SD) (mg / dl)**	**Controls (Mean ± SD) (mg / dl)**
Triglycerides	248.09 ± 168.37	173.60 ± 55.43
Cholesterol	210.99 ± 43.25	167.40 ± 25.88
LDL-C	168 ± 21.38	116.86 ± 43.53
HDL-C	32.44 ± 8.22	40.24 ± 9.15

On comparison of lipid changes in patients with NAFLD, it was observed that there was significant increase in the levels of serum total cholesterol ($p < 0.05$), LDL ($p < 0.05$) and triglycerides ($p < 0.05$) and significantly decreasing HDL ($p < 0.05$) in accordance with study made by Mahamoud et al. [23] and Kumar et al [20] but differs from the observations made by Alam et al [22]. Several studies demonstrated variable results. Our results showed correlation of lipid profile abnormalities with the presence of fatty liver. Subjects with NAFLD were more likely to have hypertension, hypercholesterolemia, hyperlipidemia, metabolic syndrome, diabetes, asthma, arthritis, ischemic heart disease, congestive heart failure, stroke, COPD, and cancer. Subjects with NAFLD were also more likely to have higher BMI, waist circumference, triglycerides.

NAFLD appears to be common in some ethnic groups like Philipinos, Indians and aboriginals of Australia / Malaysia. NAFLD can cause end stage liver disease including some cases of 'cryptogenic cirrhosis' and has been proposed to lead to hepatocellular carcinoma. Prevalence of nonalcoholic fatty liver disease is observed to be rising in the Asia-Pacific region as the society becomes affluent and changes in traditional lifestyles (increasing fat in the diet, less physical activity, increasing prevalence of type 2 diabetes).

In this study, we observed 62 (82.67%) patients presented with hypertriglyceridemia and 45 (60%) patients with hypercholesterolaemia. Almost all NAFLD patients in this study were asymptomatic or presented only with mild symptoms. The disease is discovered either incidentally during routine laboratory examination or when the patient is investigated for conditions like hypertension, diabetes or obesity. This supports the above consideration. Amarapurkar and Amarapurkar [17] reported 69.23% symptomatic patients having right hypochondrial pain as the presenting complaint. 64% patients were reported symptomatic and right upper quadrant pain, fatigue and malaise were the main symptoms [24]. NAFLD is characterized by the excessive accumulation of various lipids where hepatocytes are occupied by triglycerides. Triglycerides constitute the majority of lipids stored in the livers of NAFLD patients where alterations of triglycerides mediated pathways have been demonstrated.

To summarize majority of the NAFLD subjects in our study were asymptomatic. All the nonalcoholic fatty liver patients studied were either obese or overweight. BMI elevated values were most universal abnormalities in the cases. The serum triglycerides, serum cholesterol and LDL-C values were significantly increased, whereas HDL-C was significantly decreased in NAFLD patients compared to normal controls. Ultrasonography offers promising role to diagnose NAFLD which is supported by significantly increased lipid profile values in our study. Ultrasonography can be used for the early detection ofNAFLD. Sonographically diagnosed NAFLD patients showed statistically significant association with serum lipid profile parameters. It may be possible to say that ultrasound is the least expensive modality for detecting changes associated with NAFLD and minimizes the exposureof unnecessary, expensive, complicated and tedious investigation in these patients and asymptomatic cases.

V. Conclusion

NAFLD is a mild disease which affects both female and male. Raised serum triglycerides, total cholesterol and LDL were seen in 82.67%, 60% and 65.33% cases respectively and significantly low HDL in 65.33% of NAFLD patients.

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