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ORIGINAL RESEARCH

STUDY OF CLINICAL, BIOCHEMICAL, HISTOLOGICAL PROFILE OF PATIENTS WITH NON-ALCOHOLIC FATTY LIVER DISEASE

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ABSTRACT

Background: Non-alcoholic steatohepatitis is a clinicopathological syndrome, characterized by the development of histological features comparable to those induced by excessive alcohol intake without alcohol abuse. This study is an attempt to evaluate the clinical, biochemical and histological profile of non-alcoholic steatohepatitis in this tertiary hospital in Rayalasema.

Material and Methods: Present study was single-center, prospective, observational study, conducted in patients of all ages, either gender, who are found to have increased echo texture of liver on routine ultra-sound scan.

Results: The present study was conducted on 60 patients, comprised of 36(60%) males and 24(40%) females. Majority were from the age group of 41 to 50 years there were 22 (36.66%) cases of which 12 (54.54%) were males and 10 (45.46%) were females. The commonest symptom was fatigability and malaise (66.66%), followed by right upper abdominal discomfort (46.66%), Jaundice (10%), ascites (10%), UGI Bleed (8.3%) patients and 20 (33.33%) patients were asymptomatic at the time of diagnosis and diagnosis was established due to abnormal sonographic finding and abnormal liver function tests during investigations for other causes. Hyperlipidemia (61.67%), diabetes mellitus (58.33%), obesity (46.67%) & overweight (50%) were the most commonly associated risk factors noted. 7 patients underwent biopsy. Of these 4.28% patient showed steatohepatitis, 42.86% patients showed simple fatty change and 14.28% patient had cirrhosis. Other 28.57% had no specific changes.

Conclusion: This study demonstrates that features suggestive of the metabolic syndrome are observed more frequently in patients with non-alcoholic steatohepatitis, share many of the systemic disorders that constitute insulin resistance syndrome, hyperlipidemia, hypertension, obesity, type 2 diabetes and hepatic steatosis.

Keywords: Metabolic Syndrome, Non-Alcoholic Steatohepatitis, Insulin Resistance Syndrome, Hyperlipidemia, Hypertension, Obesity, Type 2 Diabetes.

INTRODUCTION

Non-alcoholic steatohepatitis is a clinicopathological syndrome, characterized by the development of histological features comparable to those induced by excessive alcohol intake without alcohol abuse.[1] Nonalcoholic steatohepatitis is an increasingly recognized chronic liver disease because of its potential to progress to end stage liver disease. The pathological features resemble that of alcohol-induced liver injure, but it occurs in obese, diabetic patients who do not abuse alcohol.[1,2,3]

There is dearth of literature about the magnitude of non-alcoholic steatohepatitis in Indian subcontinent. The available reports (Aggarwal etal.[2]; Amarapurkar etal.[4]) confirms that non-alcoholic steatohepatitis equally prevalent in Indian population. Progression to cirrhosis has been documented in 7-15% of patients with non-¬alcoholic steatohepatitis. It is however, generally accepted that progression occurs slowly over several years. Ballooning degeneration was shown to be associated with a relatively accelerated progression to advanced fibrosis.[5,6,7]

This emerging disease must be tackled at many levels and from many directions to limit the morbidity and mortality associated with non-alcoholic steatohepatitis. The practicing physician must be able to identify these cases to institute appropriate therapy. Recent importance given to the non-alcoholic steatohepatitis is due to the observation that it can occasionally progress over the years to liver cirrhosis and liver failure. Hence early recognition and steps to halt and reverse the process are advisable. This study is an attempt to evaluate the clinical, biochemical and histological profile of non-alcoholic steatohepatitis in this tertiary hospital in Rayalasema.

MATERIALS & METHODS

Present study was single-center, prospective, observational study, conducted in Department of General Medicine, at Kamineni Institute of Medical Sciences & Hospital, Narketpally, Nalgonda, Telangana, India. Study duration was of 2 years (September 2012-october 2014). Study was approved by institutional ethical committee.

Inclusion criteria

- Patients of all ages, either gender, who are found to have increased echo texture of liver on routine ultra-sound scan, willing to participate in this study.

Exclusion criteria:

- Alcoholics as defined by Powell etal.
- Viral hepatitis B and C.
- Sepsis, Total parenteral nutrition, Jejuno-Ileal by-pass, Auto-immune disease.
- Rapid weight loss.
- Drugs - Steroids, Tamoxifen, Hormone replacement therapy, Nifedipine, Diltiazem, Methotrexate, Amiodarone, Warfarin, Pentoxifylline, Chloroquine.

All cases included into the study underwent a complete clinical, anthropometric and laboratory evaluation after a screening ultra-sound scan of liver. Brightness and posterior

attenuation were considered indices of the extent of fatty infiltration and fibrosis and were graded appropriately by a single radiologist. All patients with elevated liver enzymes and fulfilling the criteria for liver biopsy underwent a percutaneous liver biopsy after an informed consent on a short stay hospital admission. Liver biopsy was scored according to scoring system devised by Brunt et al by a single pathologist. Serum insulin levels along with F.B.S. were measured in fasting state and after a complex formula derivation, insulin resistance was calculated. Also correlation between serum ferritin levels and non-alcoholic fatty liver disease was derived.

All data was compiled in Microsoft access and excel database. Statistical analysis was done using descriptive statistics.

RESULTS

The present study was conducted on 60 patients, comprised of 36(60%) males and 24(40%) females. Majority were from the age group of 41 to 50 years there were 22 (36.66%) cases of which 12 (54.54%) were males and 10 (45.46%) were females.

Table 1: Age & gender distribution

	Male	**Female**	**Total**
<20	0	0	0
21-30	1	1 (1.66 %)	2 (3.33%)
31-40	0	9 (100%)	9 (15%)
41-50	12 (54.54%)	10 (45.46%)	22 (36.66%)
51-60	7 (38.89%)	11 (61.11%)	18 (30%)
61-70	4 (44.45%)	5 (55.55%)	9 (30%)
Total	36(60%)	24(40%)	
Mean age	52.25 ± 8.67 years	49.94 ± 9.66 years	50.86 ± 9.27 years

The commonest symptom was fatigability and malaise (66.66%), followed by right upper abdominal discomfort (46.66%), Jaundice (10%), ascites (10%), UGI Bleed (8.3%) patients and 20 (33.33%) patients were asymptomatic at the time of diagnosis and diagnosis was established due to abnormal sonographic finding and abnormal liver function tests during investigations for other causes. The commonest clinical sign detected in this study was of hepatomegaly or palpable liver found in 54 (90%) subjects.

Table 2: Clinical features

Characteristic	**Frequency**	**Percentage**
Fatigability	40	66.67%
Malaise	40	66.67%
Right upper abdominal discomfort	28	46.67%
Jaundice	6	10.00%
Ascites	6	10.00%
UGI Bleed	4	6.67%
Asymptomatic	20	33.33%
Signs		

hepatomegaly or palpable liver	54	90.00%
Edema	21	35.00%
Abdominal distension	11	18.33%
Splenomegaly	10	16.67%
Icterus	8	13.33%

In this study out of total number of 60 cases, out of 24 male patients, 11(45.83%) patients were obese and 13(54.16%) patients were overweight. Out of 36 female patients, 17(47.22%) were obese & 17(47.22%) were overweight. In this study, out of total 60 patients, 35(58.33%) patients were found diabetic. Among males 17 (70.83%) & females 18 (50%) had type 2 diabetes mellitus. The mean duration of disease was 11.53±7.88 years. Total 32 (53.33%) patients were hypertensive, in the male group, 15 (62.50%) were hypertensive and female patients, 17 (47.22%) were hypertensive. Dyslipidemia was noted in 37 (61.67%) patients. 24 (40%) patients gave history suggestive of coronary artery disease; among them 11 (45.83%) were males and 13 (36.11%) were females.

Table 3: Risk factors

Risk factors	**Male (n=24)**	**Female (n=36)**	**Total (n=60)**
Obese	11(45.83%)	17 (47.22%)	28 (46.67%)
Overweight	13 (54.16%)	17 (47.22%)	30 (50%)
Diabetes Mellites	17 (70.83%)	18 (50%)	35 (58.33%)
Hypertension	15 (62.50%)	17 (47.22%)	32 (53.33%)
Dyslipidemia	17 (70.83%)	20 (58.72%)	37 (61.67%)
Coronary Artery Disease	11 (45.83%)	13 (36.11%)	24 (40%)

The mean hemoglobin level was 11.46±2.55 gm/dl (Male: 12.79±2.57; Female: 10.58±2.14 gm/dl). It ranged from 3 to 20 gm/dl. 21 (35%) had a level in the range of 7-10 gm/dl; 26 (43.33%) had in the range between 10-13 gm/dl and 11 (18.33%) had a level in the range of more than 13 gm/dl. Mean total leukocyte count was 8335±4804.54. TLC ranged from 2800 to 36000 per cu.mm.

The mean neutrophil count was 67.23±11.06. The mean lymphocyte count was 27.35±9.97. The mean eosinophil count was 3.183±2.803. The mean monocyte count was 1.48±1.79. The mean platelet count was is 2.33±0.93.

The mean erythrocyte sedimentation rate was 35.96±20.68. The range was between 10-104 mm/ 1st. Of the study group 8 (13.33%) had less than 10, 9 (25%) had in the range of 10-20, 12 (33.33%) in the range of 20-30, 10 (16.66%) in the range of 30-40, 8 (13.33%) in the range of 41-50 and 13 (21.66%) above 50 mm/l8 hr.

Table 4: Hematological investigations

Characteristic	Mean ± SD/ Frequency (%)	Range
mean hemoglobin level	11.46 ± 2.55 gm/dl	3 to 20 gm/dl
Male	12.79±2.57 gm/dl	
Female	10.58±2.14 gm/dl	
hemoglobin level		
less than 4 gm/dl	1 (1.66 %)	
4-7 gm/dl	1 (1.66 %)	1.66 %
7-10 gm/dl	21 (35.00 %)	
10-13 m/dl	26 (43.33 %)	
>13 gm/dl	11 (18.33 %)	
Mean total leukocyte count	8335±4804.54	2800 to 36000 per cu.mm.
Mean neutrophil count	67.23±11.06	42-95%
Mean lymphocyte count	27.35±9.97	1-5%
Mean eosinophil count	3.183±2.803	0-10%
Mean monocyte count	1.48±1.79	0-6%
mean platelet count	2.33±0.93	1-5 lakh per cu.mm.
Mean erythrocyte sedimentation rate	35.96±20.68	10-104 mm
<10	8	
10-20	9 (15%)	
21-30	12 (20 %)	
31-40	10 (16.67 %)	
41-50	8 (13.33 %)	
>50	13 (21.67 %)	

Among diabetics mean fasting blood glucose was 171.48 ± 74.67 and post prandial blood glucose was 260.08 ± 69.8. Among non-diabetics mean fasting blood glucose was 88.6 ± 21.71 and post prandial blood glucose was 111.9 ± 45.99.

Table 5: Blood Glucose

	FBS (Mean ± SD)	**PPBS (Mean ± SD)**
Diabetic	171.48 ± 74.67	260.08 ± 69.8
Non Diabetic	88.6 ± 21.71	111.9 ± 45.99

All patients were negative for HIV, HbSAg, HCV and other markers of autoimmune hepatitis (ANA, AMA, etc.). Blood urea level ranged from 11-214 mg/dl with a mean level of 27.10±27.66 mg/dl. Serum creatinine level ranged from 0.6-6.7 md/dl with a mean level of 1.89±1.01 mg/dl. The mean prothrombin time (FT) was 14.18±2.28 sec and ranged from 10-20 seconds. The mean partial thromboplastin time was (APTT) was 34.11 ±6.89 sec and ranged from 20 - 60 seconds. The total serum proteins ranged from 5 - 8.2 gm/dl and the mean was 6.56±0.75 gm/dl. The serum albumin ranged from 1.9 - 5.2 gm/dl and the mean

was 3.63±0.71 gm/dl. The total bilirubin ranged from 0.3-5.4 mg/dl and the mean was 1.17±1.03 mg/dl. The conjugated fraction was 0 - 1.6 mg/dl and the mean was 0.21±0.44 mg/dl. Alkaline phosphatase levels ranged from 115 - 653 IU/L and the mean was 234.781115.30 IU/L. The mean serum glutamate pyruvate transferase (SGPT) was 57.47151.39 IU/L and ranged from 11- 263 1U/1. The mean serum glutamate oxaloacetate transferase (SGOT) was 46.88131.95 IU/L and ranged from 13-200 IU/1.

Table 6: RFT, LFT & clotting function

Characteristic	Mean ± SD	Range
Blood urea	27.10 ± 27.66 mg/dl	11-214 mg/dl
Serum creatinine	1.89±1.01 mg/dl.	0.6-6.7 mg/dl
Clotting Parameters		
mean prothrombin time (PT)	14.18±2.28 sec	10-20 seconds
mean partial thromboplastin time (APTT)	34.11 ±6.89 sec	20 - 60 seconds.
Liver function tests		
total serum proteins	6.56±0.75 gm/dl.	5 - 8.2 gm/dl
serum albumin	3.63±0.71 gm/dl.	1.9 - 5.2 gm/dl
Total bilirubin	1.17±1.03 mg/dl.	0.3-5.4 mg/dl
conjugated	0.21±0.44 mg/dl.	0 - 1.6 mg/dl
Alkaline phosphatase	234.781115.30 IU/L.	115 - 653 IU/L
Serum glutamate pyruvate transferase (SGPT)	57.47151.39 IU/L	11- 263 1U/1
Serum glutamate oxaloacetate transferase (SGOT)	46.88131.95 IU/L	13-200 IU/1.

The mean total cholesterol level was 187.43±91.88 mgm/dl, mean HDL cholesterol level was 34.9± 12.79 mgm/dl, mean LDL cholesterol level was 112.53±39.31 mgm/dl, mean VLDL cholesterol level was 41.92±37.30 mgm/dl, mean triglyceride level was 205.96± 109.25 mgm/dl.

Table 7: Lipid Profile

Serum Lipid	**Mean ± SD**	**Range**
total cholesterol	187.43±91.88 mgm/dl	94 -819 mgm/dl.
HDL	34.9± 12.79 mgm/dl	7-62 mgm/dl.
LDL	112.53±39.31 mgm/dl	30 - 300 mgm/dl.
VLDL	41.92±37.30 mgm/dl	3-230 mgm/dl.
triglyceride level	205.96± 109.25 mgm/dl	46-652

7 patients underwent biopsy. Of these 4.28% patient showed steatohepatitis, 42.86% patients showed simple fatty change and 14.28% patient had cirrhosis. Other 28.57% had no specific changes. Of these 2 patients one had glycogen deposits in the hepatocytes and the other patient had a normal liver biopsy.

Table 8: Liver biopsy

Liver Biopsy	Frequency (N=7)	Percentage
Steatohepatitis	1	14.29%
Cirrhosis	2	28.57%
Simple fatty change	4	57.14%
• No specific changes	2	28.57%
• Normal liver biopsy	2	28.57%

DISCUSSION

Most of our patients 36 (60%) were females and this high female prevalence in our study is similar to the study of Aggarwal etal. 2001,[2] and may have several potential explanations. They may either reflect social attitude patterns in the local population or alternatively they may reflect referral bias.

The patients in the study had a variety of complaints or symptoms. The commonest symptom was fatigability and malaise which was present in 40 (66.66%) patients. The second commonest symptom was right upper abdominal discomfort which was present in 28 (46.66%) patients. These findings were contrary to the findings Lee RG,[8] Aggarwal etal. 2001.[2] The predominant population studied was females who might be having associated nutritional anemia which is a common coexisting problem in many females in India, which was not analyzed further.

Most patients with non-alcoholic steatohepatitis have no symptoms or signs of liver disease at the time of diagnosis. 30 (50%) patients were asymptomatic at presentation and Aggarwal etal.[2] Found asymptomatic cases in series only 9%. The commonest clinical sign detected in this study was of hepatomegaly or palpable liver found in 54 (90%) subjects. No patient had oral/mucosal candidacies, skin pigmentation, gynecomastia, testicular atrophy, clubbing, koilonychia or engorged neck vein. This disease commonly progresses without much clinical symptoms and signs. These findings were similar to a study by Angulo etal,[5] were hepatomegaly was the only physical finding.

In present study, obesity (46.67%), overweight (50%), hyperlipidemia (61.67%) and diabetes mellitus (58.33%) were the most commonly associated risk factors for the development of nonalcoholic steatohepatitis. Truncal obesity seems to be an important risk factor for non-alcoholic steatohepatitis, 52 (86.67%) patients were viscerally obese in our study. These findings were similar to the findings from Agarwal etal,[2] and Marchesini G etal,[9] In this study, 35 patients (58.33%) were found diabetic. These were similar to the findings of Marchesini G etal.[9]

Mildly to moderately elevated serum levels of SGOT, SGPT or both are the most common and often the only laboratory abnormality found in patients of non-alcoholic steatohepatitis. In our study the mean serum glutamate pyruvate transferase (SGPT) was 57.47±51.39 IU/L and ranged from 11 - 263 IU/1. The mean serum glutamate oxaloacetate transferase (SGOT) was 46.88±31.95 IU/L and ranged from 13 -200 IU/1. 31 (51.67%) had SGOT elevation more than 40 IU/L and 37 (61.67%) had SGPT elevation more than 40 IU/L. These findings were

similar to the findings of Mathieson NL etal,[10] where the most common abnormality in liver Function test was two to five fold elevation of transaminases.

As evident from the study of Pinto TK etal.[11] serum alkaline phosphatase were above the normal range in many patients. In this study also 25 (41.67%) patients had level of alkaline phosphatase above normal. Alkaline phosphatase levels ranged from 115 - 653 IU/L and the mean was 234.78±115.30 IU/L. The total bilirubin ranged from 0.3-5.4 mg/dl and the mean was 1.17±1.03 mg/dl. The conjugated fraction was 0-1.6 mg/dl and the mean was 0.21 ±0.44 mg/dl. Hyper bilirubinemia in cases of nonalcoholic steatohepatitis was found in a study of Francis J etal.[12]

In this study, hepatomegaly was found in 54 (90%) of the patients on ultrasonography and cirrhosis was found in 6 (10%) patients. 2 (3.33%) patients had mild steatosis (grade 1), 34(56.67%) had moderate steatosis (grade 2) and 24(40%) patients had severe steatosis (grade 3). The sensitivity and specificity of detecting steatosis by ultrasonography is 89% and 93% respectively on ultrasonography examination and this finding is consistent with Angulo P et al 2000.[5]

Liver biopsy remains the best diagnostic tool for confirming non-alcoholic steatohepatitis and diagnosis of non-alcoholic steatohepatitis can be established by a liver biopsy.[13] Liver biopsy was done on those patients who satisfied the criteria (Transaminases > 40 IU/L for a period of 6 months). Only 7 patients underwent biopsy. Of these 1(14.28%) patient showed steatohepatitis, 3 (42.86%) patients showed simple fatty change and 1 (14.28%) patient had cirrhosis. Other 2 (28.57%) had no specific changes. Of these 2 patients one had glycogen deposits in the hepatocytes and the other patient had a normal liver biopsy.

Treatment strategies for non-alcoholic fatty liver disease have revolved around (1) identification and treatment of associated metabolic conditions such as diabetes and hyperlipidemia; (2) improving insulin resistance by weight loss, exercise, or pharmacotherapy; (3) using hepato-protective agents such as antioxidants to protect the liver from secondary insults.[14,15]

This study had some limitations such as paucity of liver biopsies; serum Insulin levels could not be done all the patients for comparing insulin resistance for both diabetics and non-diabetics. Follow up of these patients over a period of time was also difficult since this would have helped in assessing the improvement and response to different treatment modalities used i.e. Urso deoxycholic acid, Vit E, etc.

CONCLUSION

This study demonstrates that features suggestive of the metabolic syndrome are observed more frequently in patients with non-alcoholic steatohepatitis and patients of non-alcoholic steatohepatitis share many of the systemic disorders that constitute insulin resistance syndrome, hyperlipidemia, hypertension, obesity, type 2 diabetes and hepatic steatosis. Our observation emphasizes the need to consider non-alcoholic steatohepatitis in patients with abnormal transaminases with negative serological work for HBV and HCV and alcoholic liver disease.

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