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Research Article

TO DETERMINE THE BIOCHEMICAL, RADIOLOGICAL AND HISTOLOGICAL CHANGES IN NON ALCHOLIC FATTY LIVER DISEASE COMPARE WITH DIABETES AND OBESE PATIENTS

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A B S T R A C T

Background: NAFLD is considered the most common cause of chronic liver disease worldwide. In the general US population, the prevalence of NAFLD is estimated to be approximately 30%, but much higher estimates are reported in selected high-risk populations, such as Hispanics, obese persons, and patients with type 2 diabetes mellitus (T2DM) or with metabolic syndrome. The purpose of this study to correlate the biochemical, radiological & histological changes in non alcoholic fatty liver disease patients compare with obese and diabetic patients.
Material & Methods: 41 cases out of which 25 were male and 16 females recruit for this study. In this study biochemical, radiological and histological parameters were determined.
Statistical Analysis: Comparison of radiological grade of fatty liver and biochemical parameters we used Pearson Chi square test.
Results: All the biochemical parameters were increased. When the radiological grade was compared with the biochemical findings (FBS, TG and SGPT) individually through Pearson's formula the p value (>0.05) thus found were not statistically significant for any of the biochemical tests. We found statistically significant correlation between radiological grading of fatty liver and histological steatosis.
Conclusion: In this study we concluded that Non-alcoholic fatty liver disease (NAFLD), radiological, biochemical and histological findings give a good correlation and are equally helpful in assessing the severity of NAFLDS.

INTRODUCTION

Non alcoholic fatty liver disease (NAFLD) is the most common liver disease since its prevalence is estimated to be 20-30% in general population of Western countries[(1)]. Studies introduced that NAFLD may progress to cirrhosis, liver failure, and hepatocellular carcinoma [(2)]. It has been shown that NAFLD is strongly associated to the features of metabolic syndrome. Insulin resistance is a key pathogenic factor in both NAFLD and metabolic syndrome. Available data from clinical, experimental and epidemiological studies indicate that NAFLD may be the hepatic manifestation of metabolic syndrome [(3)]. The prevalence rate of NAFLD increases with body mass index (BMI) [(4)]. An analysis of liver histology obtained from liver donors,[(5)] automobiles crash victims, [(6)] aytopsy findings [(7)] and cilinical liver biopsies [(8)] suggest that the prevalence rates of steatosis and steatohepatitis are approximately 15% and 13%, respectively, in non-obese persons, 65% and 20%, respectively, in persons with class I and II obesity(BMI30.0-39.9 kg/m^2), and 85% and 40%, respectively, in extremely obese patients (BMI ≥40 kg/m2).

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The relationship between BMI and NAFLD is influenced by racial/ethnic background and genetic variation in specific genes.[(9,10,11)]. The natural history of NAFLD is not well defined. It is clear that some patients with steatosis follow a progressive clinical course whereas others remain stable. It appears that 50% of cases with steatosis do not show any significant change in histology on follow-up biopsies, whereas 27% show fibrosis and 19% cirrhosis.[(12)] It has been suggested that age above 45 years, obesity, diabetes and an AST/ALT ratio >1 are associated with an increased risk of fibrosis. These are criteria which may guide clinicians towards undertaking a liver biopsy[(13)]. Histologically proven ballooning degeneration of hepatocytes with fibrosis and Mallory's hyaline appears to indicate the aggressive form of NAFLD. Since there was a variety of presentation and findings (biochemical, radiological and histological) in various studies done, this study was taken in order to find a correlation between biochemical , radiological and histopathological changes in patients with non-alcoholic fatty-liver having obesity and diabeties.

MATERIAL AND METHODS

Non-alcoholic fatty liver disease was considered in those patients with clinical characteristics, laboratory findings suggestive of NAFLD, Ultrasound/CT scan showing fatty

changes, and no findings on investigations suggestive of viral, metabolic or other specific etiologies of liver diseases. Apart from alcoholic patients, certain patients were excluded from the study even though they showed one or more features suggesting fatty changes in liver. The exclusion criteria were as follows.

1. Recent gastrointestinal surgery.
2. Pregnancy.
3. Usage of drugs known to cause steatosis including glucorticoids, synthetic estrogens, aspirin, tamoxifen, amiodarone, calcium channel blockers and methotrexate.
4. Investigations suggestive of viral, metabolic or other specific etiologies of liver diseases.
5. Malignancy

Method and evaluation

Medical History: A detailed medical history was taken of patients attending the OPD (Out Patient Department), Department of Gastroenterology and Hepatology, S.R.N Hospital, Allahabad. This include name, age, sex, clinical history (chief complaint especially right hypochondrial pain), history of alcohol intake and other drugs, family history (especially of diabetes and hypertension), past history of any viral infections especially hepatitis virus, and other relevant history.

Physical examination*:* A thorough physical examination was undertaken, which included height, weight, waist/hip ratio, blood pressure and general well being.

Body mass index (BMI): BMI is a gross estimate for the amount of fat in the body. It was calculated by taking weight in kg and dividing it by height in m^2.

$$\text{BMI} = \frac{\text{Weight (kg)}}{\text{Height (m}^2\text{)}}$$

Waist was measured by a taking the circumference at the level of the navel in case of males and midway between the bottom of the ribs and top of the hip bone in case of females.

Hip was measured by taking the circumference at the tips of the hip bones in case of males and the widest point between the hips and buttocks is taken as hip circumference in cases of females. Again grading was done as mentioned in the table below.

Investigations

Ultrasound abdomen- Ultrasound abdomen was one of the most important investigations of the study. Grading of fatty liver was done according to the following findings:

Grade I- Echogenecity of the liver is mildly increased.

Grade II-Echogenecity of the liver is moderately increased, mild blurring of blood vessels.

Grade III- Echogenecity of the liver is severely increased marked blurring of blood vessels, diaphragm not visualized properly.

Haematological test-Haemoglobin, total leucocyte count and differential leucocyte count.

Biochemical tests

- Fasting blood sugar (FBS) done by glucose oxidase method.
- Liver function tests(SGOT, SGPT, ALP and Serum bilirubin),
- Lipid profile(triglyceride)
- Plasma insulin estimation.

Statistical Method: Comparison of radiological grade of fatty liver and biochemical parameters (FBS, TG and SGPT) was done by Pearson Chi square test.

RESULTS

The study included 41 cases out of which 25(61%) were male and 16(39%) females. The male: female ratio was thus 1.5:1. Maximum no of patients were between 31 to 40 years of age followed by patients of age group 50-60 yrs and 41-50 yrs. The mean age was found to be 42.5 years (range 18-70 yrs). The presenting symptoms in most of the patients (85%) in decreasing order of frequency were mild abdominal discomfort (85%), bloating (70%), anorexia (70%) and lethargy (65%). The rest 15% cases had other additional problems.7% of the cases were known diabetic. Most of the patients (68%) had BMI>40(grade III obesity).

Table 1 This table shows the variation in BMI in NFLD patients. Most of the patients (68%, had BMI>40(grade III obesity).

BMI	Males	Females	Total
< 18.5	0	0	0
18.5- 24.9	0	1(6%)	1(2.5%)
25-29.9	0	0	0
30-34.9	1(4%)	2(12)	3(7.5%)
35-39	6(24%)	3(19)	9(22%)
<40	18(72%)	10(63)	28(68%)
Total	25	16	41

The waist/hip ratio was calculated60% of the males had W/H ratio of 0.96-1, therefore were in moderate risk group which was followed by 20% who were at low risk (w/h ratio of 0.91-0.95). Regarding the females almost all of them were in severe risk group. Ultrasonography (USG) of the abdomen is an integral part of the diagnosis of fatty liver disease. All the patients were advised for an USG abdomen. We found that among the 41 patients studied 32 (78%) had grade I fatty liver, and only 5% with grade III while the rest had grade II fatty liver.

FBS was within normal limits in most (83%) of the cases. Only 17% had fasting hyperglycemia as shown in table 2.

Table 2 This table shows the levels of fasting blood sugar in patients group. FBS was within normal limits in most (83%) of the cases. Only 17% had fasting hyperglycaemia.

FBS levels in mg/dl	Male	Female	Total
<110	22(88%)	12(75%)	34(83%)
110-126	1(4%)	3(18.5%)	4(9.5%)
>126	2(8%)	1(6.5%)	3(7.5%)
Total	25	16	41

Liver function tests- Assessment were done for bilirubin, ALT and the most important being AST (SGOT), ALT (SGPT) and SGOT/SGPT ratio. 44% of the patients had normal SGOT levels irrespective of the gender. Rest had elevated SGOT levels out of which 5% had more than 200 IU/L. Compared to the SGOT levels only 32.5% of patients had normal SGPT

levels.67.5% had elevated SGPT out of which 7% had more than 200 IU/L.

Table 3 This table shows the correlation between biochemical and radiological changes.

	Biochemical finding	FBS >110mg/dl	TG >160mg/dl	SGPT >40 IU/L	SGOT/SGPT ratio>1
Radiological grade:	Grade I (32)	5(15.5%)	9(28%)	22(69%)	5(15.5%)
	Grade II (7)	2(28.5%)	4(57%)	6(86%)	4(57%)
	Grade III(2)	0	0	0	1(50%)

The percentages are shown in table 6 which depicts that SGPT levels are elevated in higher number of cases as compared to SGOT levels. When the ratios of SGOT/SGPT were calculated the value was less than 1 in about 75.5% of patients and was more than 1 in only 24.5% of patients which shows a marked contrast to that of patients with alcoholic liver disease, in which the SGOT/SGPT levels are more than 1.Serum ALP was elevated (>100IU/L) in73%with the mean being 143.73 IU/L and total bilirubin was raised(>1.2mg/dl) only in17% of the patients with mean being 0.82. Thus, as the grade of fatty liver in radiology increased the levels of biochemical parameters (FBS, TG and SGPT) also increased. Triglyceride (TG)-68% of the patients were having normal triglyceride level and out of the patients having elevated levels 25% had triglyceride levels more than 200 IU/L.

Table 4 Correlation between radiological and histological steatosis

Radiological grade		Grade I	Grade II	Grade III	Total
Steatosis	Grade I	3(18%)	0	0	
	Grade II	11(64%)	3(100%)	0	
	Grade III	3(18%)	0	0	
	Total	17	3		20

Correlation between clinical, radiological, biochemical and histological parameters

When radiological findings and BMI of these patients were taken together about 97% of patients with fatty liver grade I were obese, out of which 62% showed grade III obesity. Similarly 100% of the patients with grade II and grade III fatty liver in radiology were obese.

When a correlation between Waist/ Hip ratio and radiological fatty liver were done we found that among males about 61 % and 80% of patients with grade I and grade II fatty liver showed moderate risk respectively but almost all the grade III patients demonstrated low risk. This was because of low number of grade III cases. However 100% females showed high risk irrespective of the grade of fatty liver on ultrasound.

When a correlation between biochemical and radiological grade of fatty liver were made among 32 grades I fatty liver patient only 15.5% showed elevated fasting sugar levels whereas 28.5 % of grade II patient showed elevated sugar levels. Elevated triglyceride levels were seen in 28% of grade I and 57% of grade II patients. Similarly 69% of patients with grade I fatty liver showed elevated SGPT levels whereas 86% of grade II had increased SGPT levels. SGOT/SGPT ratio which is usually > 1 in patients with alcoholic liver disease was seen in only in 15 % of grade I patients. 50% of both grade II and III patients had SGOT/SGPT ratio >1, may be because of lesser number of cases as compared to grade I patients. Thus it showed that as the grade of fatty liver increases the biochemical parameters (FBS, TG, and SGPT) also increased.

When the radiological grade was compared with the biochemical findings (FBS, TG and SGPT) individually through Pear sons formula the p value (>0.05) thus found were not statistically significant for any of the biochemical tests.

When correlation between Radiological steatosis and different histological finding were made we found that 64% of patients showing grade I fatty liver in radiology had grade II steatosis and grade I ballooning degeneration in histology and rest 18% each had grade I and grade III steatosis.

We found a rather significant correlation between radiological grading of fatty liver and histological steatosis when the Spearman's Rank Correlation Coefficient was applied to calculate the r^2 value as well as the Z value. Thus the correlation between radiological fatty liver and histological steatosis was statistically significant.

DISCUSSION

The present study was conducted to study the biochemical, radiological and histological correlation in patients with non-alcoholic fatty liver disease.

A total of 41 cases were included in the study. The male: female ratio was 1.5:1. The mean age was 42.5 years (18- 80 yrs). Recent changes in life style like high fat in the diet and lack of exercise to which an individual is exposed right from childhood along with higher prevalence of diabetes, hypertension and genetic predisposition is responsible for occurrence of NASH at an earlier age. It was almost similar with the recent study done by Singh *et al,* (2010) [13] which showed a mean age of 35.07+ 8.06 years for NASH.

The physical examination included height, weight, BMI and W/H ratio. About 68% of patient had grade III BMI (> 40), which clearly shows that patients with high BMI are prone to develop NAFLD compared to people with normal BMI. Waist/Hip ratio when calculated showed that 60% of males had moderate risk and all the females of the study group had severe risk. Dixon *et al* (2004) [13] in their study demonstrated convincingly that steatosis, NASH and fibrosis were at least partially reversible after losing weight. A study by Lazo *et al* (2008)[14], showed a high prevalence of NAFLD in patients with BMI > 35kg/m^2.Yet another study by Bellentani *et al,* (2004)[15] found that NAFLD was present in 94% of obese patients(BMI>/= 30kg/m^2), 67% in overweight patients(BMI>/= 25kg/m^2) and 25% in normal weight patients. Taking all these studies in account we can surely say that BMI indeed plays a role in the development of NAFLD.

Of 40 patients 32(78%) had grade I fatty liver, 7(17%) had Grade II and only 2(5%) grade III fatty liver in radiology. Maximum cases comprised of grade I fatty liver because most of the patients included in the study were the ones who had come with the initial symptoms and were in their preliminary stage of the disease.

When biochemical parameters were analysed 17% had elevated fasting glucose and32% had hyper triglyceridemia. The mean fasting blood glucose was 88.41mg/dl and mean triglyceride level was 151.3mg/dl. This finding shows that most of the patients with NAFLD need not have elevated blood sugar or triglyceride level in contrary to various studies previously done which shows a strong association between

NAFLD, obesity, type II diabetes and hyperlipidemia. Huang *et al,* (2008) [16] in their study found that metabolic syndrome, high fasting glucose and high blood pressure were independently related to increased risk of NASH. Bajaj *et al*(2009) [18], in their study showed a close relationship of NAFLD with multiple features of metabolic syndrome which comprises of central obesity, hypertriglyceridemia, hyperglycemia along with hypertension and low levels of HDL cholesterol. In contrast to the many studies, Singh *et al* (2010) also showed a similar finding of only 16.67% of NASH patients with hypertriglyceridemia as seen in our case.

SGPT was increased in about 67% patients, the mean being 78.9 IU/L. whereas SGOT levels were raised in 56%, the mean being 67.3 IU/L. SGOT/SGPT ratio in 24.5% of patients showed a ratio of >1, which was incontrast to that found in alcoholic patients with fatty liver where SGOT/SGPT ratio is usually >1. Thus it is a useful index in distinguishing NASH from ASH. The hypertransaminasemia in NASH was found to be SGPT dominant in our study. Similar findings were reported by Sugimoto *et al* (2003)[17]. Singh *et al*,(2010) in their comparative study between NASH and ASH clearly showed SGPT levels were more elevated in NASH as compared to ASH(mean being110.82IU/L in NASH and 79.69IU/L in case of ASH).Also showed a higher AST/ALT ratio in ASH as compared to NASH (mean of 1.24+0.81 in ASH and 0.68+0.33 in NASH) This study strongly supports our finding that SGPT is a more relevant marker than SGOT in NASH patients and AST/ALT ratio Is >1 in ASH rather than NASH, which indeed helps in distinguishing between ASH and NASH. This was also reported by Sorbi *et al* (1999)[18]In contrary to our findings Uslusoy *etal,*(April 2009)[19], in their study showed that amino transferase levels and AST/ALT ratio do not seem to be a reliable predictor for NASH as patients with normal and high amino transferases level had almost the same prevalence of NASH metabolic syndrome.

Among these patients 16 of them had their insulin levels checked. All the patients had normal insulin levels (<26 micro IU/L).When HOMA IR was calculated 11/16 (68%) of the patients showed insulin resistance. These values were compared with that of controls of a previous study conducted by Bajaj S*et al*, 2009 in our college itself. We found that the range and median for HOMA values of the patients were higher [1.1(0.3-2.2)] than controls [0.6(0-2.2)] The difference was also statistically significant. This finding showed that despite normal insulin levels, 68% patients had insulin resistance, thus we can say that HOMA is a more sensitive test than plasma insulin for detecting insulin resistance. Caballerial *et al* (2010)[20] in their study showed that Insulin resistance was one of the important factor associated with NAFLD.

When BMI and grade of fatty liver in ultrasonography were compared then62% of patients with grade I fatty liver had BMI> 40(grade III obesity)and 43% of grade II had BMI >40. The disparity in percentage were because of the lesser number of grade II patients in the study group. Patients with grade III fatty liver couldn't be assessed as the number was too small. When W/H ratio were compared with grade of fatty liver in radiology we found that all the female patient were at high risk group, irrespective of grade of fatty liver while in the case of males 61% of grade I and 80% of grade II had moderate risk. Dixon *et al* (2001)[21], Clark *et al*(2002)[22], Ong *et al*(2005)[23] in their studies established the association of severe obesityleading to severe NAFLD.

When a Comparison was made between biochemical and radiological grade of fatty liver we found out that as the grade increases there was an increase in all the biochemical parameters (FBS, TG and SGPT). Despite the increasing percentages of the biochemical parameters (FBS, SGPT, and TG) with increase in grade of fatty liver in ultrasonography, we failed to establish any statistical significance, probably due to smaller number of cases studied. Further larger studies with adequate follow up may help in elucidating this failure.

When radiological fatty liver grade were compared with histological steatosis we found that liver biopsies of patients with grade I fatty liver in ultrasonography showed12% with grade I steatosis, 70.5% with grade II steatosis and 17.5% with grade III steatosis. 100% of patients with grade II fatty liver in ultrasonography showed grade II steatosis in histology.The correlation was also statistically significant. Kojima *et al*(2005)[24] and Morita *et al* (2005)[25] also reported similar findings. Almeida *et al* (2008)[26]in their study found that preoperative abdominal ultrasonographyto be accurate method for diagnosis of hepatic steatosis in severe obese patients.The histological prevalence of steatosis was 89.5%. The sensitivity and specificity of ultrasonography in the diagnosis of hepatic steatosis were 64.9% and 90.9% respectively. The presence of steatosis in ultrasonography was associated to advanced grades of steatosis on histology (p=0.016). Histological findings (ballooning degeneration, lobular inflammation, and fibrosis)are more as the grade of fatty liver in radiology increases.75% of patients with grade I fatty liver in ultrasonography showed ballooning degeneration. 80% of patients with grade I fatty liver in radiology showed lobular inflammation of grade I in histology and 100% of grade II fatty liver in radiology showed grade Iinflammation. The inflammatory cells mainly comprised lymphocytes. 35% of patients with grade I fatty liver in radiology had grade I fibrosis. Apart from these findings there was evidence of glycogenated nuclei in 70% of the cases. None of the cases showed evidence of ductular proliferation, sclerosing hyaline necrosis and cholestatic hepatitis which are usually a feature of alcoholic fatty liver disease. Singh *etal* (2010) in their very recent study showed that steatosis was present in 100% of cases with NASH. Ballooning degeneration was severe in ASH as compared to NASH (71.7%-ASH, 28.3%-NASH). Ductular proliferation, sclerosing hyaline necrosis and cholestatic hepatitis were present (92.1%, 34.1% and 60.1% respectively) in ASH cases while none of the NASH cases showed this finding as seen in our cases. All these findings together were helpful in distinguishing ASH from NASH.

Liver biopsy was performed only in 20 patients either due to refusal by the patients to undergo an invasive procedure or some of them didn't turn up for regular follow up. Among the biopsies examined 100% showed steatosis(10% grade I, 75% grade II, 15% grade III),75% showed grade I ballooning degeneration, 80% lobular inflammation grade I and portal fibrosis of grade I in 35% of patients. Apart from these findings there was evidence of glycogenated nuclei in 70% of the cases.

When a Comparison was made between biochemical, radiological and histological grade of fatty liver we found out

that as the grade increases there was an increase in all the biochemical parameters (FBS, TG and SGPT). Despite the increasing percentages of the biochemical parameters (FBS, SGPT, and TG) with increase in grade of fatty liver in ultrasonography, we failed to establish any statistical significance, probably due to smaller number of cases studied. Further larger studies with adequate follow up may help in elucidating this failure.

CONCLUSION

NAFLD is strongly associated with obesity but body fat distribution appears to play a more important role in the pathogenesis of NAFLD. Excess of intra abdominal fat in particular may be a key determinant in the pathogenesis of NAFLD, because of its strong association with insulin resistance and possibly as a source of FFAs. From this study it can be concluded that even though liver biopsy is the gold standard for grading of Non-alcoholic fatty liver disease (NAFLD), radiological, biochemical and histologcal findings give a good correlation and are equally helpful in assessing the severity of NAFLD its management if a liver biopsy is not possible.

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