

Nonalcoholic fatty liver disease - Non-invasive markers of fibrosis or liver biopsy for determining liver fibrosis

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Abstract

Non-alcoholic fatty liver disease (NAFLD) is a common cause of chronic liver disease & liver transplantation and its incidence is increasing even in Asian countries. The gold standard for assessing hepatic fibrosis is liver histology but due to its limitations, noninvasive tests to assess hepatic fibrosis which can be used as alternative to liver biopsy have been developed. In a cross-sectional study, 25 diagnosed NAFLD patients underwent detailed laboratory investigations including the specific non-invasive markers of fibrosis namely, Haptoglobin, Alpha-2 macroglobulin, Apolipoprotein- A1 levels and Insulin Resistance was calculated by Homeostasis Model Assessment (HOMA), then patients were subjected to liver biopsy. Out of 25 patients, 19(76%) were male and 6(24%) were females. Nine patients (36%) were diabetic and 21 (84%) were dyslipidemic. Metabolic syndrome was present in 18(72%), body mass index was increased in 22 patients and waist hip ratio was altered in 22 patients (88%). Using SPSS 10, p value was significant ($p= 0.04$) for correlation between steatosis and waist/hip ratio and HOMA-IR with inflammation and fibrosis ($p=0.03$) but non-invasive markers were not significant in predicting hepatic fibrosis on histology.

Keywords: Non-alcoholic fatty liver disease, Liver fibrosis, Liver biopsy, Diabetes Mellitus, Metabolic Syndrome

Introduction

Non alcoholic fatty liver disease (NAFLD) is a disease of our generation (Geoffrey et al. 2005). It was rarely recognized as a distinct clinical entity before 1980 (Ludwig et al. 1980) but now has emerged significantly all over the world and has a definite but uncertain rate of progression to cirrhosis and end stage liver disease (Falck et al. 2001). It is very essential to understand the pathophysiologic mechanisms involved in NAFLD, so that rationale therapeutic strategies can be developed (Matteoni et al. 1999). NAFLD has a wide spectrum ranging from simple steatosis to non alcoholic steatohepatitis (NASH); the later is believed to predispose

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to cirrhosis and hepatocellular carcinoma (McCullough 2005). Insulin resistance (IR) plays a key role in the pathogenesis of NAFLD (Duseja 2004a, 2004b). A recent summary of available data indicate that prevalence rates for NAFLD and NASH have increased from previous estimates of 17 to 33% for NAFLD and 5.7 to 17% for NASH (Geoffrey et al. 2005, Brown et al. 2004).

The significance of these categories rests not only on the fact that the prevalence varies histologically, but also that clinical outcomes vary by histologic category. Once NASH has set in, it leads to cirrhosis in 15% to 25% of patients (Falck et al. 2001). Once cirrhosis is developed, 30% to 40% of these patients succumb to liver related deaths over a ten year period (Geoffrey et al. 2005). The mortality rate is similar to (Hui et al. 2002) or worse than (Pratziv et al. 1999) cirrhosis associated with hepatitis C. NASH is also now considered to be the major contributor to the basket of disease manifesting as cryptogenic cirrhosis (Caldwell et al. 1999). NASH associated cirrhosis also can decompensate into subacute liver failure (Caldwell and Hespshire 2002), progress to hepatocellular carcinoma (Bugiones et al. 2002), and reoccur post transplantation (Ong et al. 2001). More recent studies indicate that although fibrosis progresses in 32% to 53% of patients who have NASH, fibrosis may also regress (Ong et al. 2001; Harisson et al. 2003). In contrast, steatosis alone is reported to have a benign clinical course, although cirrhosis has occurred in 3% of patients who have steatosis alone. (Pratziv et al. 1999; Teli et al. 1995; Dom et al. 2002). The current understanding of the pathophysiology of NAFLD remains incomplete and it involves both hepatic and nonhepatic mechanisms. The nonhepatic process includes obesity, diabetes, sedentary lifestyle, type of food consumption, increased body fat mass, abnormal fat distribution, insulin resistance and bacterial overgrowth (Flegal et al. 2002).

The hepatic mechanism of non alcoholic fatty liver disease is explained on basis of insulin resistance, which leads to hyperinsulinemia and increased free fatty acid influx to the liver, which further causes hepatic steatosis by increased lipogenesis. A two hit hypothesis is proposed for development of NAFLD. The first hit or step is insulin resistance, resulting

in hepatic steatosis and second hit is oxidative stress caused by compounds such as endotoxin, cytokines and environmental toxins (Edmisson and McCullough 2007). It may be defined as a condition in which higher than normal insulin concentrations are needed to achieve normal metabolic response (Kahn 1978). The various causes of insulin resistance are abnormal sedentary life style, obesity, diabetes haemochromatosis, polycystic ovarian syndrome, hypercortisolism, drugs, genetic causes, trauma, sepsis, surgery, pregnancy, puberty etc (Pham et al. 2007). The various methods used to assess IR include: Euglycemic Hyperinsulinemic Clamp studies, frequently sampled intravenous glucose tolerance test (FSIGT), Homeostasis Model Assessment for Insulin Resistance (HOMA-IR), Quantitative Insulin Sensitivity Check Index (QUICKI) and Insulin Tolerance Test clamp studies, but most of these tests are difficult to perform (Neuschwander and Caldwell 2003).

Most patients are suspected to have NAFLD because of characteristic appearance on hepatic ultrasonography and / or elevation of liver enzymes. USG examination for bright hepatic echotexture (comparing with kidney or spleen), deep attenuation and vascular blunting (Chitturi et al. 2007) has adequate threshold for the detection of steatosis when more than 33% of hepatocytes contain fat, as shown by liver histology (Saadeh et al. 2002; Sanyal et al. 2002). Liver biopsy, the gold standard for the assessment of necro-inflammatory activity and fibrosis, is invasive and is associated, even in expert hands, with rare but severe side effects such as bleeding or pneumothorax (James and Lindol 1993). Moreover, it carries a sampling error of one or two stages in a third of the cases, as demonstrated by Regev et al (2002). The single markers including platelet count, prothrombin time, hyaluronic acid, type IV collagen, N-terminal propeptide of type III procollagen (PIII-P), age, hyperglycemia, BMI, albumin, AST/ALT ratio have been evaluated and found not to be sufficiently accurate to predict amount of liver fibrosis (Renou et al. 2001). Therefore several markers combining multiple factors have been tried which include Fibrotest- Actitest, APRI (Aspartate aminotransferase / Platelet ratio index) (Poynard 2004), Forns score (Forns et al. 2002), ELF scores (Rosenberg et al. 2004), Hepascore (Adams et al. 2005) and Fibrometer score (Cales et al. 2005). The most extensively used test is the Fibrotest- Actitest which has been validated in more than 1500 patients (hepatitis B, C and NAFLD) in clinical trials.

The aim of the study was to evaluate efficacy of non-invasive markers of fibrosis in determining liver fibrosis vis-à-vis liver biopsy, in patients of nonalcoholic fatty liver disease.

Materials and methods

This cross sectional study was conducted from 1st September 2010 to 1st September 2013, in the department of Gastroenterology at PGIMS, Rohtak, India.

Patient selection

Inclusion criteria

Patients who were detected to be having Nonalcoholic Fatty Liver Disease on radiological imaging and/or laboratory investigations (transaminasemia i.e increased AST or ALT) after ruling out other causes of fatty liver like alcohol intake, viral infections, autoimmune diseases, metabolic liver diseases and drugs were enrolled in the study.

Exclusion criteria

Patients having history of any ethanol intake (currently or in the past) Patients who have autoimmune or viral hepatitis (B, C, HIV). Patients who have any history of drug intake on a chronic basis including indigenous drugs. Patients who have metabolic or genetic liver diseases e.g. Wilson disease, haemochromatosis, Alpha-1 antitrypsin deficiency.

Study design

At the time of enrolment into the study, detailed history and clinical examination was done for every patient and after that, patients were included once they consented for liver biopsy and sampling for noninvasive fibrosis markers, then detailed laboratory investigations were done.

Statistical analysis

All the data was analyzed and mean, median, and standard deviation were calculated. The correlation between different variables was calculated by Fisher's exact test. SPSS-10 was used to calculate p value (<0.05 was considered significant).

Results

A total of 110 patients were screened for non alcoholic fatty liver disease but 45 patients were excluded .Out of these excluded 45, 25 were alcoholic, 5 each had chronic hepatitis B and C infection, 6 were abusing indigenous medications and 4 refused to get enrolled in the study. Out of 65 patients who were enrolled into the study over this period of time, only 25 patients consented for both liver biopsy and detailed laboratory investigations, hence, data pertaining to cohort of these 25 patients was analysed. The overall mean age of cohort was 41.08 yrs while median age was 45 yrs. Nineteen (76%) of subjects were male while 6 (24%) were female. Male to female ratio was 3.1. Nine patients (36%) were diabetic, 8(89%) were male and one (11%) was female. Hypertension was recorded in 5(20%) patients out of which 3(60%) were male and 2(40%) were female. Twenty-one patients (84%) were found to be dyslipidemic, 17(81%) were males and 4 (19%) were females. The mean Serum triglycerides level was 156.7 mg/dl. In case of HDL cholesterol, the mean level was 36.1 mg/dl. Metabolic syndrome was present in 18 (72%) subjects in the cohort, with male preponderance (13 males (72%) and 5(28%) females). The overall mean body mass index was 28.2 Kg/m². In males the mean body index was 27.8 Kg/m² and in females the mean body mass index was 32.3 Kg/m². Body mass index (BMI) was increased in 22 patients, out of which 12(54.5%) were obese and 10 (45.5%) were overweight. In obese patients, out of 12, 7(58%) were males and 5(42%) were females whereas in overweight patients, out of 10, 9(90%) were males and 1 (10%) were females. BMI was deranged in all eight patients in which histopathology revealed any degree of fibrosis after applying Metavir scoring system. Out of these eight, 4 had F1 score, 4 had F2 score but there was no significant correlation between BMI and liver biopsy findings i.e., fatty infiltration, inflammation, fibrosis, ballooned hepatocytes or Mallory's hyaline (p value was > .05, Fisher's exact test).

In our cohort, majority of patients i.e., 22 (88%) had an abnormal waist/hip ratio. The overall mean waist/hip ratio was 0.96. In males the mean waist/hip ratio was 0.95 whereas in females mean waist/hip ratio was 0.9. On applying Kliner's

histopathology fatty score, patients with abnormal waist/hip ratio had score range from 0-3 with and 2(9%) had 0 score, 8(36.5%) had score of 1, 11(50%) had score of 2 and 1 (4.5%) had score of 3. In out of three patients who had normal waist/hip ratio, 2 had score of 1 and one had 0 score. The waist/hip ratio had statistically significant correlation with fatty infiltration (p value= 0.04). The overall mean value of ALT was 25.1 IU/ml. The mean value in males was 49.6 IU/ml whereas in females, the mean was 17.1 IU/ml. Only 7 patients (all males) had ALT elevation above the upper limit of normal and out of them 4 had metabolic syndrome. On histopathology, 3 had steatosis, and two each had portal fibrosis (F1) and inflammation (A1). Out of all 25 patients in our study group, only one (4%) had ballooned hepatocytes and Mallory's hyaline on liver histology. This patient had an ALT value of 212 IU/ml. In rest of 18 patients in whom ALT was within normal limits, 13(72%) showed inflammation or fibrosis on histopathology. Out of these 13, 9(69%) were male and 4(31%) were female. In males, out of 9, 3(33.33%) had fibrosis (F2-3) and 6(66.66%) had inflammation (A1) on histopathology whereas in females, out of 4 patients, 3(75%) had fibrosis (F1-2, F2-1) and 1(25%) had inflammation (A1). The overall mean value of GGT was 24.9 IU/ml. In males, the mean was 33.8 IU/ml, whereas in females, the mean was 25.1 IU/ml. Only one patient had increased GGT levels. This patient had metabolic syndrome (diabetes, dyslipidemia) and had increased BMI 30 Kg/m², waist/hip ratio 1, HOMA 2.3 levels and had fatty infiltration on both USG abdomen and histopathology. The normal value of platelets was taken from 1.5 -4.5-lakhs/ mm³ and overall mean was 2.2 lakhs/mm³. In males, the mean value was 2.1 lakh/mm³, whereas in females, the mean was 2.8 lakh/mm³. Only in two patients, platelet counts were decreased (1 and 1.4 lakhs/mm³) and both of them were having portal fibrosis on histopathology. One each of them was female and male with Metavir score of F1 and F2 respectively. Moreover only three patients (all male) had lower normal range of platelets (1.7, 1.7 and 1.8 lakhs/mm³), one each of them had fibrosis (F1-1), inflammation (A1) and steatosis (fatty score- 2). In rest of twenty patients, platelet counts were within normal range. The overall mean HOMA value was 1. In males, the mean HOMA was 2.99, and in females, the mean value was 1.1. Out of 25 subjects in our study group, 12(48%) had raised HOMA levels and all of them were males. Out of these 12, 6 (50%) were diabetic, 11(92%) had increased body mass index, 9(75%) had increased waist/hip ratio, 10 (83%) were dyslipidemic, 6 (50%) had raised ALT, 7(58%) had decreased Apolipoprotein levels and two had increased fasting insulin levels. On histology, out of 12, 8(66%) had inflammation (A1), 2(17%) had fibrosis (F1-1, F2-1) and rest 2 (17%) had steatosis (Fatty score- 2 & 1 in each patient). The HOMA- IR had statistically significant correlation with liver histology in respect to inflammation and fibrosis, even with two classifications i.e. Metavir (p value = 0.03) and Matteoni (p value = 0.02) but there was no correlation with fatty infiltration, ballooned hepatocytes or Mallory's hyaline (p value = > .05). In our study, mean value for the three parameters i.e., Haptoglobin, Apolipoprotein-A1 and Alpha-2 macroglobulin was 96.3, 102.8 and 2.06 respectively. The P value was not significant (>0.05) in predicting any relation between Apolipoprotein-A1, fasting C peptide, Alpha-2 macroglobulin, Haptoglobin levels and presence of fatty infiltration, inflammation, fibrosis, Mallory's hyaline or ballooned hepatocytes (Fisher's exact test). Apolipoprotein-A1 levels were decreased in 14 (56%) patients in the present study group of 25. Out of these 14, 5(36%) had fibrosis (F1-3, F2-2), 6 (43%) had inflammation (A1) and 3 (21%) had mild steatosis only (Kliner's Fatty score-1). The sole patient who had Mallory's hyaline and ballooned hepatocytes had significantly decreased level of Apolipoprotein-A1 level (37mg/dl). Out of three i.e. Apolipoprotein-A1, Haptoglobin and Alpha-2 macroglobulin, it was seen that apolipoprotein-A1 was the most common marker to be

deranged in fibrosis and inflammation but it was also decreased in patients with simple steatosis.

In our study group, all 25 patients had inietial outside report suggestive of fatty liver. On repeating USG abdomen in our hospital, 4 patients had normal scan but their histology corroborated with other patients; hence they were included in interpretation of results. In our study group, 8(32%) had grade 1 fatty liver on USG, 11 (44%) had grade 2, 2(8%) had grade 3 and in 4(16%) it was reported to be normal but out of these 4, 2(50%) had fatty liver on biopsy (Kliner's fatty score of 1) and one had fibrosis (F2).

Table 1. Mean, Standard deviation and Range of Different variables

Variables (N=25)	Cases	Range
	Mean & Standard Deviation	
Age (Yrs)	43.48 & 12.21	18-60
Waist/Hip Ratio	0.96 & .09	0.79-1.17
B.M.I (Kg/m ²)	28.28 & 4.65	24-38
ALT (IU/L)	25.12 & 42.56	13-212
AST (IU/L)	24.63 & 21.72	71-100
GGT (IU/L)	24.98 & 16.36	12-79
Platelet (lakhs/mm ³)	2.21 & 0.6	1.03-3.66
S.Triglycerides (mg/dl)	156.72 & 89.96	70-316
HDL (mg/dl)	36.04 & 7.12	25-51
Fasting Insulin (µIU/ml)	5.29. & 12.41	1.6-15.7
Fasting C-Peptide (ng/ml)	0.72 & 0.70	0.2-2.9
HOMA-IR	1.27 & 3.13	0.33-15.17
Haptoglobin (mg/dl)	96.28 & 44.93	49-225
Alpha-2-macroglobulin (gm/l)	2.06 & 0.92	1.4-5.6
Apolipoprotein-A1 (mg/dl)	102.75 & 36	37-172

Table 2. P values among Different Variables

Variable	'P' Value				
	S	I	F	B	M
Fasting Insulin	1	0.27	0.27	1	1
Fasting- c-peptide	1	0.69	0.69	1	1
Haptoglobin	1	0.72	0.72	1	1
Alpha-2-Macroglobulin	0.64	0.41	0.41	1	1
Apolipoprotein-A1	0.61	0.72	0.72	1	1
Homa IR	0.52	0.03	0.03	1	1
ALT	0.36	0.81	0.81	0.32	0.32
GGT	1	0.72	0.72	1	1
Platelet	1	0.53	0.53	1	1
S.Triglycerides	1	0.48	0.48	1	1
HDL	0.16	1	1	1	1
Waist/Hip ratio	0.04	0.53	0.53	1	1
BMI	0.49	0.53	0.53	1	1

S-Steatosis; I – Inflammation; F –Fibrosis; B-Ballooned Hepatocytes; M-Mallory' hyaline

The positive predictive value of Ultrasound in detecting fatty infiltration was 95.45% and Negative predictive value was 66.66%. The liver biopsy revealed fibrosis in 8 patients (32%). On applying Metavir scoring system, 4(50%) had F1 and other 4(50%) had F2 score (Matteoni scoring was 4 in all of them). The portal and lobular inflammation was seen in 5 (20%) and all of them had mild inflammation (Metavir A1 and Matteoni-2). The isolated steatosis was seen in 12 (48%) patients. Out of these 12, on applying Kliner's fatty score, 6(50%) had score of 1, 5(42%) had score of 2 and 1(8%)

patient had score of 0. Three patients with fibrosis (F1-2, F2-3) and two with inflammation (A1) had increased ALT levels. Out of eight patients who had fibrosis, 2 (25%) had thrombocytopenia (1 and 1.4 lakhs/mm³) and 3 (37.5%) had borderline platelet counts ((1.7, 1.7 and 1.8 lakhs/mm³). Metabolic syndrome was seen in all five patients who had inflammation and in six (75%) patients who had fibrosis. All patients who showed inflammation and fibrosis were dyslipidemic. The waist/hip ratio was increased in 7 patients (87.5%) who had fibrosis (F1-3, F2-4) and in 4(80%) who had inflammation (A1) on liver biopsy.

Discussion

The overall mean age of cohort was 41.08 yrs (SD 17.1, range 18-60 yrs) while median age was 45 yrs. Nineteen (76%) of subjects were male while 6 (24%) were female. Male to female ratio was 3.1. The age and sex ratio in our study group is in corroboration with majority of studies done in NAFLD, which now concludes that this disease is male predominant and usually occur in fourth decade, in contrast to previous concepts of female preponderance (Westwater and Fainer et al. 1958; Uygun et al. 2004). Arun et al (2006) found that the prevalence of NASH in morbidly obese men was almost twice as high as in morbidly obese women (60.3% vs. 30.9%). This higher incidence of NASH may also reflect the higher incidence of metabolic syndrome in morbidly obese men (91.4% vs. 76.2%). In our study group of 25 patients, 22 i.e 88% had increased BMI, overall males were more obese and overweight. The overall mean body mass index was 28.2 Kg/m². There was no significant correlation between BMI and liver biopsy findings i.e., fatty infiltration, inflammation, fibrosis, ballooned hepatocytes or Mallory's hyaline (p value was > .05, Fisher's exact test). A recent study from Delhi by Madan et al (2006) also found mean BMI of 26.7% in NAFLD patients and almost 70% had obesity. Ioannov et al. (2003) used data from NHANES epidemiological studies and found that obesity was associated with a higher risk for cirrhosis related death or hospitalization. Majority of patients i.e. 22 (88%) were having altered waist/hip ratio and males predominated, may be because of overall larger number of male patients in our study group. The overall mean waist/hip ratio was 0. The waist/hip ratio had statistically significant correlation with fatty infiltration (p value= 0.04). There was no correlation between the waist/hip ratio and other histological findings such as ballooned hepatocytes, Mallory's hyaline, inflammation or fibrosis. Truncal obesity becomes a risk factor even in patients who have normal body mass index. Sabir et al (2001) showed the more importance of visceral fat than subcutaneous fat thickness in pathogenesis of fatty liver. Mesenteric fat is a specific type of visceral adipose tissue, which is drained by portal circulation and has higher lipoprotein activity and this is the basis for focus given to waist circumference in the criteria of metabolic syndrome by ATP 111 proposal (JAMA, 2001). Carr and colleagues showed that intraabdominal fat is associated independently with insulin resistance, suggesting it may have a pathologic role (Carr et al. 2004).

In our study, twenty-one patients (84%) were dyslipidemic, 17(81%) were males and 4 (19%) were female. There was no significant correlation between dyslipidemia (high serum triglycerides and low HDL levels) and liver biopsy findings i.e., fatty infiltration, inflammation, fibrosis, ballooned hepatocytes or Mallory's hyaline (p > 0.05). All the patients who were having inflammation or fibrosis on histopathology were having dyslipidemia. In corroboration with published literature for NAFLD patients, dyslipidemia was most commonly for serum triglycerides and high-density lipoprotein (HDL). Duseja et al reported that prevalence of NAFLD among patients with dyslipidemia ranged between 25% and 60% (Duseja et al. 2004). In a cross-sectional

study of 95 patients referred for the evaluation and management of hyperlipidemia, Assy et al. (2004) documented a prevalence of NAFLD in 50% of patients and showed that high triglycerides levels and mixed hyperlipidemia were associated more frequently with fatty infiltration and the extent of lipid abnormalities correlated with degree of fatty infiltration. They concluded that hypertriglyceridemia and diabetes mellitus were the most useful predictors for moderate and severe fat infiltration. Metabolic syndrome was present in 18 (72%) subjects in the cohort, with male preponderance (13 males (72%) and 5(28%) females). There was no significant correlation between metabolic syndrome and liver biopsy findings i.e., fatty infiltration, inflammation, fibrosis ballooned hepatocytes or Mallory's hyaline (p > .05). Bellentani et al (2000) and Gupte et al (2004) have shown association between the metabolic syndrome and NAFLD and many researchers like Angulo et al. (2007) now consider NAFLD to be a manifestation of the metabolic syndrome. Misra et al (2007) have hypothesized that prevalence of NAFLD would approximate prevalence of the metabolic syndrome.

The mean value for the three parameters i.e., Haptoglobin, Apolipoprotein-A1 and Alpha-2 macroglobulin were 96.3 (SD 44.5), 102.8 (SD 36) and 2.06(0.92) respectively. Apolipoprotein-A1 levels were decreased in 14(56%) patients in the present study group of 25. Out of these 14, 5(36%) had fibrosis (F1-3, F2-2), 6 (43%) had inflammatory changes (A1) and 3 (21%) had steatosis only (Fatty score-1). The sole patient with Mallory's hyaline and ballooned hepatocytes had significantly decreased level of Apolipoprotein-A1 level (37mg/dl). Out of three i.e. Apolipoprotein-A1, Haptoglobin and Alpha-2 macroglobulin (A2M), it was seen that Apolipoprotein-A1 was the most common marker to be deranged in patients with fibrosis but it was also decreased in patients with simple steatosis. However there was no significant relation between Apolipoprotein-A1, Alpha-2 macroglobulin, Haptoglobin levels and presence of fatty infiltration, inflammation, fibrosis, Mallory's hyaline or ballooned hepatocytes (p value >0.05). The reason for only mild derangement of these markers and that too only with Apolipoprotein-A1 in our group can be due to smaller size of study and majority of patients had fatty liver or minimal fibrosis whereas these markers are significantly raised usually in advanced fibrosis as supported by study conducted by Naevau et al. (2005) who also showed that compared to alcoholic liver disease the decrease of apolipoprotein A1 was not significant in patients with early NAFLD. The interpretation of this negative observation must be prudent because of the small number of patients with fibrosis included in our NAFLD study group. Various panels have demonstrated high predictive values of these non-invasive markers for predicting significant lesions in patients with chronic hepatitis C, chronic hepatitis B and alcoholic liver disease (Poynard et al. 2003; Halfon et al. 2006). Interestingly, in patients with NAFLD, there is significant association between A2M and insulin levels, a hallmark of insulin resistance (Zahnd and Scheidegger 1963) but no such association was found in our study, may be due to smaller size and presence of only early fibrosis that too in few patients. In the study by Castera et al (2005), Fibrotest was compared with transient elastography (Fibroscan), APRI and liver biopsy and the AUROC for significant fibrosis (F>2) were 0.83, 0.85 and 0.78 for the Fibroscan, Fibrotest and the APRI respectively. In another study, Cales et al (2005) compared Fibrotest with a new score fibrometer and found that the difference between them in predicting fibrosis was not statistically significant. In our study

overall mean value of ALT was 25.1 IU/ml. On the whole, there was no significant correlation between ALT and liver biopsy findings i.e. fatty infiltration, inflammation, fibrosis, ballooned hepatocytes or Mallory's hyaline ($p > .05$). In a similar study by Gupte et al (13), only 10 out of 32 patients with NAFLD and NASH had elevated ALT and there was no significant difference in mean ALT between patients with fatty liver and NASH. In another study Mofrad et al. (2003) showed that some non-alcoholic steatohepatitis (NASH) patients can have normal aminotransferase levels or conversely some patients with high liver enzymes may not have steatohepatitis and whole spectrum of histological findings of fatty liver and NASH may exist without elevation of transaminases. The overall mean value of GGT was 24.9 IU/ml. There was no significant correlation between GGT and liver biopsy findings, i.e., fatty infiltration, inflammation, fibrosis, ballooned hepatocytes or Mallory's hyaline ($p > .05$). The reason for only mild derangement of GGT level in an isolated patient in our group can be due to absolute nil intake of alcohol in our patients whereas in most of studies in NAFLD, there was certain permissible alcohol intake. The PGA index (GGT, prothrombin index and apolipoprotein A1) has been validated in patients with variety of chronic liver disease and its accuracy for detecting cirrhosis has been reported to range from 66% to 72% (27-29) (Castera et al. 2005). In our study, the overall mean HOMA value was 1.3 (SD 3.13, range 0.33-15.7). Out of 25 subjects in our study group, 12(48%) had raised HOMA levels and all of them were males. Out of these 12, 6 (50%) were diabetic, 11(92%) had increased body mass index, 9(75%) had increased waist/hip ratio, 10(83%) were dyslipidemic, 6(50%) had raised ALT, 7(58%) had decreased Apolipoprotein levels and 2(16.66%) had increased fasting insulin levels. On histology, out of 12, 8(66%) had mild inflammation (A1), 2(17%) had fibrosis (F1-1, F2-1) and 2 (17%) had steatosis (Fatty score- 2 & 1 in each patient). The HOMA- IR had statistically significant correlation with liver histology in respect to inflammation and fibrosis, even with two classification i.e. Metavir (p value = 0.03) and Matteoni (p value = 0.02) but there was no correlation with fatty infiltration, ballooned hepatocytes or Mallory's hyaline (p value was $> .05$). Marchesini et al. (1999) have reported that insulin resistance as assessed by HOMA-IR to be higher in NAFLD cases as compared to controls largely due to increased insulin concentration with normal or near-normal glucose levels but in our study the main cause of increased HOMA was increased blood glucose levels (50% were diabetic) than fasting insulin levels which were increased in only two patients. Duseja et al after analyzing the data from Marchesini et al. (1999) indicated that the clinicopathological profile of Indian NAFLD patients might be somewhat different from that seen in other ethnic groups (Duseja et al. 2007).

According to Garg et al. (2002), insulin resistance is also widely prevalent in Asian Indians and is of higher magnitude than white Caucasians. However, inter-relationships of NAFLD, insulin resistance and the metabolic syndrome have been sparsely studied in Asian Indians. In the only study from north India, 38/39 patients showed insulin resistance as assessed by homeostasis model assessment of insulin resistance (HOMA-IR), and 100 per cent patients ($n=54$) had the metabolic syndrome. However, these subjects were not assessed for detailed anthropometry and were not compared to controls without NAFLD (Duseja et al. 2007). The normal value of platelets was taken from 1.5 -4.5-lakhs/ mm^3 and overall mean was 2.2 lakhs/ mm^3 . There was no significant correlation between platelets and liver biopsy findings, i.e., fatty $> .05$). Most of the studies that have focused on platelet count (APRI test) have mainly looked at with patients of hepatitis C or co-infection with HIV and alcoholic liver disease (Forns et al. 2002). One of the first studies on APRI was a retrospective analysis, which looked at a relationship to significant hepatic fibrosis or cirrhosis.

The authors found that it was simplest and most accurate test for the detection of significant fibrosis or cirrhosis. Out of 25 patients in our study group, 8(32%) had grade 1 fatty liver on USG, 11 (44%) had grade 2, 2(8%) had grade 3 and in 4(16%) it was reported to be normal but out of these 4, 2(50%) had fatty liver on biopsy (Kliner's fatty score of 1) and one had fibrosis (F2). The positive predictive value of Ultrasound in detecting fatty infiltration was 95.45% and Negative predictive value was 66.66% which is more than in study conducted by Lee ET. Al (59). We could not validate ultrasound findings with histology in 4 patients (16%) and this finding is in corroboration with study conducted by Amarapurkar et al. (2007). In our study group, liver biopsy revealed fibrosis in 8 patients (32%). On applying Metavir scoring system, 4(50%) had F1, 4(50%) had F2 fibrosis score (Matteoni score was 4 in all of them). The portal and lobular inflammation was seen in 5 (20%) and all of them had mild inflammation (Metavir A1 and Matteoni-2). The isolated steatosis was seen in 12 (48%) patients. Out of these 12, on applying Kliner's fatty score, 6(50%) had score of 1, 5(42%) had score of 2 and 1(8%) patient had score of 0. Three patients with fibrosis and two with inflammation had increased ALT levels. Metabolic syndrome was seen in all five patients having inflammation and in six (75%) patients with fibrosis. All patients who showed inflammation and fibrosis were dyslipidemics. The waist/hip ratio was increased in 7 patients (87.5%) who had fibrosis and in 4(80%) who had inflammation on liver biopsy. Whereas laboratory test abnormalities and radiographic findings may be suggestive of NAFLD, histological evaluation remains the only means of accurately assessing the degree of steatosis, the distinct necroinflammatory lesions and fibrosis of NASH, and distinguishing NASH from "simple" steatosis, or steatosis with inflammation (Brunt 2004). Matteoni et al. (1999) showed that cirrhosis developed in 21% to 28% of patients whose index biopsies had shown the combination of lesions of steatosis, inflammation, ballooning, and Mallory's hyaline or fibrosis, whereas only 4% of patients with simple steatosis and none of the patients with steatosis and inflammation alone had evidence of cirrhosis during the 10 years of follow-up (62,63).

Conclusion

The non-invasive markers were not significant in predicting hepatic fibrosis on histology in present study. The limitation of the study was small sample size and majority of patients had steatosis only. It does not mean that non-invasive markers have no role in determining liver fibrosis but conveys the message that these markers are deranged in later stages of Non alcoholic fatty liver disease like Non alcoholic steatohepatitis (NASH). One more aspect which can be derived from this study is that if these markers are not deranged in a NAFLD patient, then there are high chances that hepatic damage is not much.

Recommendations

There is strong need for understanding the dynamics of Non alcoholic liver disease and its early detection, as it is one of the important causes of liver cirrhosis and need for transplantation. The main reason for neglect of this entity is due to Physicians and health care workers who take it lightly and pass same impression to the affected population. Hence, a dedicated approach at every step is required to curb the menace of this benign looking deadly disease.

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