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Clinical Spectrum and Diagnosis of Nonalcoholic Fatty Liver Disease (NAFLD)

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Abstract

Nonalcoholic fatty liver disease (NAFLD) is a broad term that includes patients with simple steatosis and nonalcoholic steatohepatitis (NASH). The term NASH was introduced by Ludwig *et al* (1) in 1980 to describe histological changes indistinguishable from alcoholic hepatitis in patients with no or insignificant alcohol intake of less than 20g/day. In the absence of alcohol intake some patients, who either have metabolic syndrome or any of its components with insulin resistance develop hepatic steatosis due to increased lipolysis and increased delivery of fatty acids from adipose tissue to liver. Some of these patients with hepatic steatosis develop hepatic oxidative stress and with recruitment of various cytokines, lead to hepatic inflammation and fibrosis (NASH), which can later progress to cirrhosis and HCC (2, 3). From the point of view of pathogenetic mechanisms, NAFLD has also been classified as primary NAFLD and secondary NAFLD. **Primary NAFLD** is usually associated with the insulin resistance syndrome. **Secondary NAFLD** is caused by other agents such as drugs, lipid disorders, surgery, and total parenteral nutrition.

Histological classification

NAFLD is defined as fatty infiltration of liver exceeding 5-10% of liver weight and is expressed as percentage of fat laden hepatocytes observed by light microscopy. Classically, simple steatosis involves pure fatty metamorphosis with or without

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minimal inflammation. On the other hand nonalcoholic steatohepatitis (NASH) consists of varying degrees of inflammation, hepatocyte necrosis and fibrosis in addition to fat in the liver. Histopathologically, the Cleveland group divided NALFD into four types. In this classification, types 3 & 4 correspond to what has been classically known as NASH. Types 1 & 2 are simple steatosis with no or minimal inflammation with least risk of progression (4). The spectrum of injury and fibrosis in NASH can be graded and staged according to the scheme given by Brunt *et al*, which takes into account the amount of fat, degree of inflammation, quantification of ballooning degeneration and mallory hyaline and staging of fibrotic transformation (5).

Simple steatosis

Patients with simple steatosis may be detected only during workup for asymptomatic raised transaminases. A study from New Delhi showed that 35% of patients with asymptomatic transaminitis had biopsy proven NAFLD (6). Most of these were asymptomatic and while others had features of metabolic syndrome (obesity, hypertension, hyperlipidemia). The most quoted study by Teli *et al* which investigated the natural history of simple steatosis, demonstrated that simple steatosis is a benign disease (7). Among patients who underwent a repeat liver biopsy over a median of 11 years of follow up, none had progressed to NASH. Of the 14 patients who died, none did from a liver related cause. Only one of the 26 living patients developed mild fibrosis 9.8 years after the index biopsy (7). Similar results were obtained by Matteoni *et al* (4). In a retrospective analysis of 132 patients with NAFLD the progression was very rare among patients who had type 1 or type 2 NAFLD (4).

NASH

As mentioned earlier, NASH represents a more severe spectrum of NAFLD. Type 3 and 4 NAFLD qualify to be called NASH (4). The histological findings in NASH (ballooning degeneration, mallory hyaline and fibrosis) signify a liver that has already undergone significant injury and is therefore at risk for further progression. Studies have shown that almost 30-40% patients have advanced fibrosis even at the index biopsy and up to 15% may even have established cirrhosis (8). Hence, NASH is not as benign a disease as simple fatty liver and it does progress to end stage liver disease. Powell et al followed 42 patients with NASH over a median period of 4.5 years and demonstrated that fibrosis progressed in 6/13 patients and one of them developed cirrhosis (9). Matteoni *et al* in a retrospective analysis, with a maximum follow up of 18 years, demonstrated that among patients who had either ballooning degeneration (type 3) or Mallory hyaline and fibrosis (type 4) on initial biopsies, 25% progressed to cirrhosis

and 11% died of liver related causes (4). Thus long term prognosis correlated with the histological features, with a significant proportion of those with NASH progressing on to advanced fibrosis or cirrhosis.

Risk factors for progression in NASH

Angulo *et al* studied predictive factors in 144 patients of NASH who underwent liver biopsy (10). Twenty percent patients had moderate to advanced fibrosis and 11.7% had cirrhosis on index biopsy. Age ≥ 45 years, obesity and presence of diabetes mellitus were found to be independent predictors of advanced fibrosis and /or cirrhosis. Another recent study has confirmed the relationship between the degree of obesity and the likelihood of developing advanced fibrosis (11). Other factors which may predict progression to cirrhosis are presence of >40% steatosis, ballooning degeneration and coexistence of chronic hepatitis C with NASH. Serum and hepatic iron with or without HFE gene mutations probably do not play any significant role in the pathogenesis and progression of fibrosis in patients with NAFLD at least in Indian patients (Table 1) (12-14).

Cryptogenic cirrhosis

Making a diagnosis of NASH in patients with cryptogenic cirrhosis may be difficult. Cryptogenic cirrhosis may represent burnt out NASH in which, steatosis diminishes or disappears.

Table 1 : Serum iron parameters, and HFE gene mutations in patients with NAFLD (12, 13, 14)

Parameters (n = 60)	Normal values	Abnormal-No of pts
Serum Iron	65-170 µg/dl	2 (3%)
Transferrin saturation	< 55%	5 (8%)
Serum Ferritin	20-250 ng/ml	4 (7%)
HFE gene mutations (n=30)		
Normal/normal		26 (87%)
C282Y/C282Y		0
C282Y /H63D		0
H63D/ H63D		0
H63D/Normal		4 (13%)

Therefore we only have indirect evidences to link NASH with cryptogenic cirrhosis. In a case control study, in which 49 patients with cryptogenic cirrhosis and 98 age and sex matched controls were analyzed, it was observed that diabetes (55% v/s 24%) and obesity (47% v/s 22%) were more frequent in patients with cryptogenic cirrhosis than in patients with cirrhosis of other etiologies (15). Caldwell demonstrated that, patients with cryptogenic cirrhosis resemble patients with NASH. Most of these cryptogenic cirrhotics were older, obese and diabetic females (16). Liver transplant series also lend support to the link between NASH and cryptogenic cirrhosis (17). Almost 25% of patients who were transplanted for cryptogenic cirrhosis developed recurrence of NAFLD, and 15%

developed NASH in the transplanted liver. Further, patients who developed recurrent NAFLD were more likely to be diabetic and obese and to have increased prevalence of hypertriglyceridemia. Those post transplant patients who did not develop recurrent NAFLD had a lower BMI (17). Experience from our own centre also confirms a higher BMI, low HDL and presence of diabetes mellitus in patients with cryptogenic cirrhosis as compared to controls (18).

Hepatocellular carcinoma

In recent times, evidence has been accumulating that like other cirrhosis NASH related cirrhosis may also predispose to hepatocellular carcinoma. In the series by Powell *et al*, one patient out of 42 (2%) developed hepatocellular carcinoma after a follow up of 5 years (9). Marrero *et al* studied 105 consecutive patients with hepatocellular carcinoma, of whom 29% had cryptogenic cirrhosis (19). Fifty percent of these patients with cryptogenic cirrhosis had histological and clinical features of NAFLD. Bugianesi *et al* compared 23 patients having cryptogenic cirrhosis and HCC with age and sex matched HCC patients due to alcohol and viral hepatitis (20). They observed that obesity and diabetes were more frequent in patients with cryptogenic cirrhosis with HCC. These patients had higher plasma glucose, serum cholesterol and triglycerides and increased parameters of insulin resistance but lower ALT levels. Logistic regression analysis identified hypertriglyceridemia, diabetes mellitus & normal aminotransferases as risk factors for HCC in patients with cryptogenic cirrhosis. These evidences are at best indirect, which do not establish a definite link between NASH and HCC. Only long term follow up cohort studies will be able to answer this question, but these findings at least suggest that NAFLD should not be disregarded as only a benign disease. NAFLD with aggressive features should be identified and efforts instituted to find out an effective therapy.

Diagnosis of NAFLD

Clinical presentation

Incidental raised transaminases or fatty liver on sonography

By definition NAFLD is a chronic liver disease; most patients are asymptomatic in the beginning with incidental detection of raised liver enzymes or a fatty liver on ultrasound. In fact NAFLD is now considered the most important cause of unexplained asymptomatic rise in transaminases in a nonalcoholic individual. Serum biochemistry usually shows mildly elevated AST and ALT with ALT more than AST. Other liver functions are usually preserved. Even though serum ALT does not correlate with the histological severity in patients with NAFLD (21), this may be the most common abnormality at

presentation. Another issue regarding ALT is the lack of data on the normal values of ALT in different populations. Since its levels are dependent on weight of the individual, some of the population studies have suggested lowering the upper limit of normal ALT to <30 IU/L in males and <19 IU/L in females but each population may need to have its own normograms before a definite cut off is accepted (22). Some patients are detected to have fatty liver on ultrasound and raised enzymes during work up for dyspeptic symptoms, malaise/fatigability, organ donation or executive health check (Table 2) (14).

Table 2 : Clinical presentation of 100 NAFLD patients with raised transaminases (14)

- Dyspepsia : 37
- Malaise/Fatigability : 36
- Incidentaly raised transaminases : 22
  - Organ Donation
  - Executive health check
  - Work up for other illness
- Intermittent jaundice : 5

Hyperbilirubinemia

As mentioned earlier, other than the raised transaminases, other liver function tests are usually preserved in patients with NAFLD till they have progressed to cirrhosis/HCC. Some of our patients presented with history of intermittent jaundice and on work up were found to have unconjugated hyperbilirubinemia with no evidence of ongoing hemolysis and on calorie deprivation test showed an increase in unconjugated bilirubin, all suggesting an associated Gilbert's syndrome in some of these patients (23).

Cirrhosis/HCC

Later in the course of disease, patients may come with features of compensated or decompensated cirrhosis and are negative for all the possible causes and may have positive surrogate markers to suggest NAFLD as a cause of cryptogenic cirrhosis (See above). This is true for some of the patients with hepatocellular carcinoma who are also negative for all possible etiologies but may have been obese in the past and may have personal or family history of long standing diabetes or any other atherosclerotic disease (3).

Clinical examination

Clinical examination may also be normal in majority of patients with NAFLD presenting with incidental detection of raised transaminases or fatty liver on ultrasound. Though there may be some referral bias most of the Indian patients are males, majority have both central and overall obesity as seen by Asia Pacific criteria, majority have insulin resistance but diabetes mellitus, hypertension and metabolic syndrome is not very common at presentation in patients with NAFLD and

raised transaminases (Table 3) (24). Mild hepatomegaly may be an important sign in around half of these patients but signs of liver failure are absent unless the patient has developed cirrhosis liver (14).

Table 3 : Clinical and laboratory parameters in patients with (NAFLD) (n=127) (24)

Mean age ± SD (yr)	39.2 ± 10.7
Males	84
Mean body weight (range) (kg)	71 (45-100)
Mean BMI (range) (kg/m^2)	28.7 (19-34)
Overweight	27 (21%)
Obesity	86 (68%)
Abnormal waist	104(82%)
Insulin resistance	48/58 (83%)
Diabetes mellitus	16 (13%)
Hypertension	13 (10%)
Dyslipidemia	67(53%)
Metabolic syndrome	39/81 (48%)

Imaging

The various imaging modalities used for diagnosing hepatic steatosis include ultrasound, CT, MRI, Tc -99m sulphur colloid scan and xenon-133 scintigraphy .

Ultrasound

On ultrasound the diffuse fatty liver reveals increased echogenicity (Fig. 1). This is due to the increase of intracellular triglycerides leading to increase in the

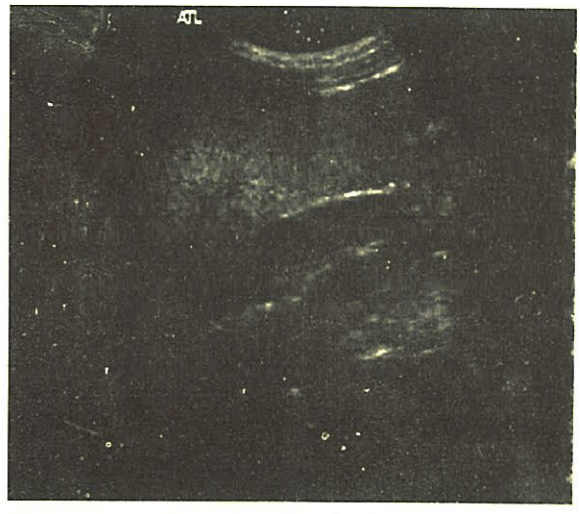


Figure 1: Longitudinal ultrasound scan of the liver showing diffuse increase in echogenicity in comparison to the renal cortex.

highly reflective surfaces. In cases of focal fatty changes, the boundaries between normal and fatty liver are angulated and geometric or there is interdigitating hyperechoic fatty tissue between relatively normal hypoechoic liver parenchyma. In cases of mild fatty infiltration there is normal visualization of diaphragm and intrahepatic vessel borders. As the severity of fatty infiltration increases (moderate fatty infiltration) there is impaired visualization of intrahepatic vessels and diaphragm with eventual non-visualization of intrahepatic vessels, diaphragm and posterior right lobe of liver (severe fatty infiltration) (25, 26). There may appear to be apparent dilatation of the biliary ducts. Sometimes due to variability

in time gain compensation and gain settings the diagnosis of mild or moderate fatty liver may be misleading. The kidney and the pancreas may be used as reference organs in this condition. Ultrasound has been reported to have a sensitivity of 83 percent and a specificity of 100 percent in diagnosing moderate or severe steatosis (fat >33%) which decreases to 77 percent in cases of mild fatty infiltration (27). However USG cannot quantify fat, differentiate fat from fibrosis or give a clue about the etiology of fatty liver.

The Doppler study is also abnormal in approximately 43% of patients with steatosis (28). There is biphasic and monophasic flow in hepatic veins which is seen in only 2 % of healthy patients (28). This may be due to compression of veins by fat deposition in hepatocytes. One study has shown significant decrease of resistive index of the hepatic artery in patients of severe steatosis as compared to healthy patients or patients with mild or moderate steatosis (29).

A newer approach is ultrasonic tissue characterization based on study of parenchymal echogenicity and attenuation of ultrasound or methods based on quantification of texture patterns (30). These physical and microarchitectural features cannot be visualized but complex computer based analysis can be used for processing ultrasound images based on texture patterns. In a manually selected interest area the grey level (echogenicity) and texture parameters (homogeneity, contrast, entropy, and variance) are automatically counted. Computerized analysis of this data may allow objective examination of hepatic steatosis and allow accurate monitoring of disease evolution. Fibroscan, a transient elastography device could predict the progression of fibrosis in NASH and could be used for long term monitoring of the disease (30). However, Munteanu et al found fibroscan unreliable in diagnosis of fibrosis in steatotic patients due to significant intraobserver variability, thereby suggesting that further work needs to be done before it can be thought of a modality to diagnose and predict progression of fibrosis in NASH (31). However, it may allow discrimination between healthy and diseased patients, between steatosis and cirrhosis and also grade severity of steatosis and cirrhosis.

CT

CT has also been used for the evaluation of hepatic steatosis. The sensitivity of NCCT for diagnosing fatty infiltration has been reported to be 93% with a PPV of 76% when one third of the liver is fatty (Fig. 2) (32). The attenuation value of normal liver is 50-60 HU. On noncontrast CT (NCCT), the normal attenuation of liver is higher than spleen and it decreases 1.6 HU for each milligram of triglycerides deposited per gram of hepatic tissue (32). Absolute attenuation

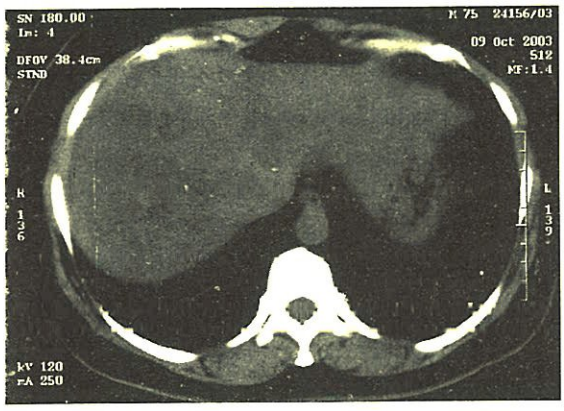


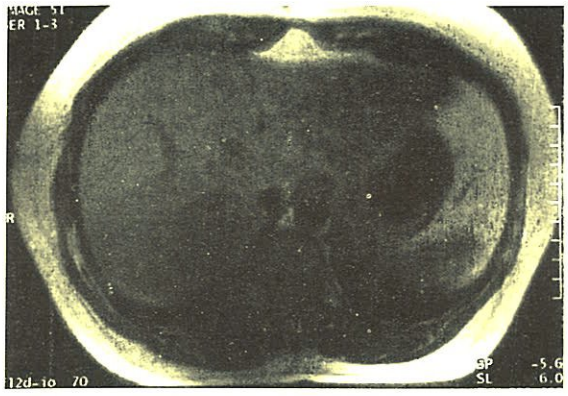
Figure 2: Axial non-contrast CT of the liver showing diffuse decrease in attenuation of the liver parenchyma with hepatic veins appearing hyperattenuated.

values of liver are of limited value in diagnosing fatty infiltration because technical variables like scanner type, tube current, method used to inject contrast, scan delay and individual variation of cardiac and renal function lead to large number of inter and intra-observer variation with poor reproducibility. The relative difference between liver and spleen attenuation are independent of these parameters, making differential liver-spleen attenuation useful for predicting presence of diffuse fatty liver. On unenhanced CT, liver attenuation minus spleen attenuation is equal to or less than -10 HU in hepatic steatosis. After contrast injection the splenic attenuation is time dependent with mean splenic attenuation initially exceeding and then approximating mean liver attenuation in normal patients. The highest sensitivity in diagnosing fatty liver on contrast enhanced CT has been found to be when the quantitative values are taken between 100-120 seconds after contrast injection and the values are taken in the inferior most aspect of the liver and spleen (33). A differential liver-spleen attenuation value of less than -20.5 HU has been found to be sensitive and specific for the diagnosis of steatosis. Dual energy CT at 90 and 120kVp has been used for diagnosing hepatic fatty infiltration with variable results (34).

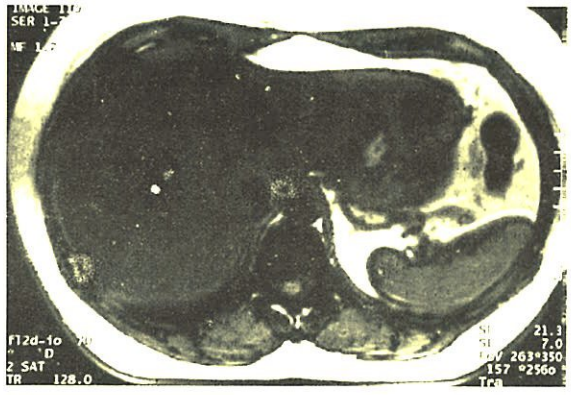
MRI

Chemical shift MR imaging takes the advantage of the difference in resonant frequencies between fat and water for detecting steatosis (35). When the signals of fat and water are in phase, the signal is maximum. Thus fatty liver has higher signal intensity on in-phase images with loss of signal on out-of phase images (Fig. 3a & b). When compared to spleen, fatty liver has a lower signal. The ratio (SI opposed-phase liver / SI opposed-phase spleen) / (SI in-phase liver / SI in-phase spleen) may be calculated. The lower the ratio, the more the signal drop and higher the fat content. The right kidney or any other organ may also be used as reference organ for signal reduction.

Proton magnetic resonance spectroscopy (1H MRS) is a noninvasive method to measure hepatic triglyceride concentration (HTGC). The spectroscopic volume of interest is positioned, avoiding



3a (In-phase MRI)



3b (Out-of-phase MRI)

Figure 3a & b: In-phase and out-of-phase chemical shift MR imaging of the liver showing drop in signal intensity in out-of- phase images consistent with diffuse hepatic steatosis.

major blood vessels, intrahepatic bile ducts, and the lateral margin of the liver. Proton spectrum from the liver parenchyma showing resonance peaks derived from hepatic water and HTG is then obtained. Using different calibration procedures, MR spectroscopy can be used to determine the hepatic fat volume fraction. A significant correlation is present between calculated liver fat concentration and the value measured by liver biopsy. As values given by 1H MRS correlate with liver biopsy results, it is widely considered to be the optimal noninvasive method to assess HTGC and diagnose hepatic steatosis. MR spectroscopy is sensitive enough to detect small amounts of triglyceride that may not even form macroscopic vesicles (36).

Recently P^{31} MR spectroscopy findings were found to correlate with severity of NASH (37). Diffusion weighted MRI has also been recently evaluated in NASH with the decrease in apparent diffusion coefficients correlating with increasing steatosis, inflammation and fibrosis (38). However till date, MRI does not offer any additional advantage over USG or CT in detecting pathological features suggestive of progression of simple fatty liver to NASH. However interobserver variability is reduced with MRI and the detection of fat is not influenced by concomitant fibrosis or inflammation. This minimal advantage should be weighed against the wider availability and lower costs of other non-invasive modalities.

Scintigraphy

99Tc sulphur colloid scintigraphy and 133 Xenon scintigraphy are useful for diagnosing focal fatty changes. Tc sulphur colloid scintigraphy shows normal photon activity in areas of fatty infiltration in

contrast to metastases which show areas of photon deficiency (39). Xenon scintigraphy, due to solubility of xenon in lipids, shows photon activity in regions of fatty infiltration and is retained even after blood pool clearing phase (39).

Thus imaging can accurately diagnose hepatic steatosis but the distinction between fatty liver and NASH which has important prognostic implications cannot be made by radiological means. With advances in MR imaging techniques including chemical shift imaging and MR spectroscopy it is possible to grade steatosis accurately. Efforts are on to estimate non-invasively the stage of fibrosis in NAFLD and to distinguish the relatively benign condition of steatosis from the more progressive form, NASH.

NAFLD and liver biopsy

Even though a definite diagnosis of NAFLD/NASH can be made only on histology, convincing these patients for a liver biopsy is difficult due to the slowly progressive nature of the disease and lack of specific treatment. We could do liver biopsy in 43 out of 127 patients of NAFLD who presented with persistently raised ALT but the clinical characteristics of these 43 biopsy proven patients were similar to 84 non-biopsy proven patients (Table 4) (14, 24). As mentioned earlier Matteoni *et al* (4) have divided NAFLD patients into four classes with class III & IV qualifying for histological NASH. Grading and staging of patients with histological NASH is usually done by Brunt *et al* (5). Typically histological features of NASH are the presence of macrovesicular steatosis, lobular neutrophilic inflammation with

Table 4

Liver histology in 43 patients with NAFLD (24)	
	n (%)
Class I	2 (5)
Class II	19 (44)
Class III	8 (19)
Class IV	14 (32)
NASH (class III + IV) on histology (n =22)	
Grade 1	10 (41)
Grade 2	12 (50)
Grade 3	0
Stage 0	6 (27)
Stage 1	7 (32)
Stage 2	5 (23)
Stage 3	4 (18)
Stage 4	0
Perls' Prussian blue staining on liver biopsy (n = 30)	
0	20 (67)
1+	6 (20)
2+	4 (13)
3+	0
4+	0

additional presence of Mallory bodies, ballooning degeneration, lipogranuloma and pericellular fibrosis. Such liver damage predominates in perivenular regions, i.e., zone 3 of hepatic acinus. The liver damage may lead to fibrogenic response that sometimes progresses from pericellular fibrosis to bridging fibrosis and ultimately to cirrhosis.

Do all patients require liver biopsy?

Performing liver biopsy in patients with NAFLD is controversial. Arguments in favour of biopsy include: (i) exclusion of alternative causes of liver disease, (ii) distinguish simple steatosis from NASH, (iii) estimate prognosis based on degree of fibrosis, and (iv) determination of progression of fibrosis over time. Arguments against biopsy include: (i) generally good prognosis of NAFLD, (ii) lack of effective therapy, and (iii) the risks and costs associated with biopsy.

Predictors of severe liver fibrosis (bridging fibrosis/cirrhosis) on liver biopsy in patients with NAFLD include old age, presence of diabetes mellitus, obesity, AST: ALT more than 1, ALT ≥ 2 times normal and triglycerides ≥ 1.7 mmol/L (10, 11). This is the sub group of patients with NAFLD who would be expected to derive the most benefit from having a liver biopsy and for considering therapy. We have also found increasing BMI and AST levels to be determinants of significant liver injury in patients with NAFLD. Overall liver histology is mild at presentation in Indian patients presenting with raised transaminases and only half of them have evidence of histological NASH (Table 4) (14, 24).

Practical approach to a patient with NAFLD

Because there are no specific tests for NAFLD, the diagnosis remains one that is established after the exclusion of other causes of chronic liver disease. Though there are no universally accepted limits for alcohol intake, an alcohol intake of < 20 g/d is unlikely to cause fatty liver, and is usually taken as a defining criterion for NAFLD (40). In a nonalcoholic patient with fatty liver on ultrasound a diagnosis of NAFLD is suggested when all other possible causes of hepatic steatosis, raised transaminases or cirrhosis and or HCC are excluded. This workup includes viral markers for hepatitis B & C, autoimmune markers for autoimmune hepatitis, serum ceruloplasmin and slit lamp examination for KF rings to rule out Wilson's disease, iron parameters including serum iron, total iron binding capacity (TIBC), serum ferritin and transferrin saturation to rule out iron over load state and alpha 1 anti trypsin levels if the clinical situation warrants. As mentioned earlier, liver biopsy is required in situations where the diagnosis is still uncertain after non invasive tests or if one wishes to prognosticate in a patient or as part of a research protocol in evaluating the efficacy of various drugs.

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