Journal of Pathology of Nepal (2016) Vol. **6**, 947 - 952

Journal of
PATHOLOGY
of Nepal

www.acpnepal.com

Review Article

Diagnosis of Non-alcoholic fatty liver disease

KC Geetika[1]

[1]*KIST Medical College, Imadole, Lalitpur, Nepal.*

Keywords:

Steatosis;
Hhepatitis;
HAIR score;
NASH test;
Fibrosis score;
BAAT score

ABSTRACT

Non Alcoholic Fatty Liver Disease is the deposition of fat in liver in absence of excessive of alcohol consumption. Non Alcoholic Fatty Liver Disease ranges from simple steatosis to Nonalcoholic steatohepatitis and cirrhosis. Most cases (90%) of Non Alcoholic Fatty Liver Disease have simple steatosis with benign prognosis. Ten to thirty percent of Non -Alcoholic Fatty Liver Disease progresses to NASH and 25-40% of Nonalcoholic steatohepatitis undergoes progressive liver fibrosis. Ultimately 20-30% of Nonalcoholic steatohepatitis will go into cirrhosis during their lifetime. Nonalcoholic steatohepatitis cirrhosis has higher chances of (2.6% per year) going into hepatocellular carcinoma. There are several risk factors noted for Non Alcoholic Fatty Liver Disease. Some of which includes increasing age, metabolic syndrome, dietary factors etc. Investigations regarding liver function test can be divided into invasive and noninvasive types. Under invasive procedures comes liver biopsy and non-invasive includes radiological tests and various biochemical tests. This article tries to analyze different scoring systems and their significance in diagnosing steatohepatitis and fibrosis.

INTRODUCTION

Fatty liver or hepatosteatosis is referred to as fat accumulation in liver that exceeds 5-10% by weight of the organ.[1] There are several causes of fatty liver, alcohol is the commonest and well known since several years. However, in recent years due to life style changes and environmental factors nonalcoholic causes of fatty liver have risen. In all cases demonstrating elevation of liver function test results, Non Alcoholic Fatty Liver Disease (NAFLD) is noted in 90% of cases.[2] It is one of the leading causes of abnormal liver function test (LFTs) in countries like UK and China.[3,4]

In general, NAFLD is the deposition of fat in liver in absence of excessive of alcohol consumption. More specifically, American Association for the Study of Liver Diseases (AASLD) has proposed that NAFLD is diagnosed when following 4 criteria are met[5]:

1) Fatty change of liver is observed by imaging or histologically,
2) No marked alcohol drinking habit is present (ethanol intake of <210 g/week for men and <140 g/week for women),
3) No presence of other factors inducing fatty change of the liver and
4) No concomitant factors causing chronic liver disease are present.

Non Alcoholic Fatty Liver Disease ranges from simple steatosis to Nonalcoholic steatohepatitis (NASH) and cirrhosis. Most cases (90%) of NAFLD have simple steatosis with benign prognosis. Ten to thirty percent of NAFLD progresses to NASH and 25-40% of NASH undergoes progressive liver fibrosis.[6] Ultimately 20-30% of NASH will go into cirrhosis during their lifetime.[7]

Correspondence:
Dr. Geetika K. C. , MBBS, MD
Department of Pathology
Kist Medical College , Imadole, Laltipur, Nepal
Email: geetikakc@hotmail.com

NASH cirrhosis has higher chances of (2.6% per year) going into hepatocellular carcinoma. In association with other liver diseases contributing to NAFLD, the disease progression is more rapid.[8] Most common cause of death associated with NAFLD is cardiovascular disease (28% of total deaths) followed by extra hepatic malignancies (25% of total deaths) and liver disease (13% of total deaths) respectively.[9] Nonalcoholic steatohepatitis has a high risk of liver disease-related deaths such as from hepatic cirrhosis and hepatocellular carcinoma.[10]

Nonalcoholic steatohepatitis can be differentiated from other NAFLD related conditions only in liver biopsy. Its microscopic findings should fulfill 3 following criteria:[10]

1) Macrovesicular fatty change of hepatocytes
2) Inflammatory cell infiltration and
3) Ballooning degeneration of hepatocytes.

Differential diagnosis of secondary Hepatic steatosis can be divided in macroversicular and mirovesicular types. Macrovesicular type which is relevant to NAFLD includes, excessive alcohol consumption, Hepatitis C (genotype 3), Wilson's disease, lipodystrophy, starvation, rapid weight loss, insulin resistance, syndrome X (obesity, diabetes, hypertriglyceridemia, hypertension), total parenteral nutrition, abetalipoproteinnemia, Medications (e.g. amiodarone, methotrexate, tamoxifen, corticosteroids) and inflammatory bowel diseases.[5]

RISK FACTORS

There are several risk factors noted for NAFLD. Some of which includes: age (higher risk with increasing age), metabolic syndrome (70-90% have NAFLD, metabolic syndrome is also a parameter having value as an independent predictor of fibrosis), gender (men>Female, however, women have higher risk of advanced fibrosis), ethnic groups (higher in Hispanics and lower in Blacks), dietary factors (High cholesterol, high saturated fats, high fructose intake and low carbohydrate intake. Caffeine on the other hand may be protective), obstructive sleep apnoea (risk of fibrosis is increased) and genetic factors (Patatin-like phospholipase domain-containing 3 (PNPLA3) gene).[6] Others with emerging association are polycystic ovary syndrome, hypothyroidism, hypopituitarism, hypogonadism and pancreato-duodenal resection.[5] Among these risk factors the metabolic syndrome has special importance. It is diagnosed when three or more of the following features are present:[6] (Table 1)

A third of NAFLD cases have full metabolic syndrome and >90% have at least one of the criteria. Also the severity of NASH is directly proportional to severity of metabolic syndrome and these patients have high changes of going into fibrosis.[6]

Table 1: Diagnostic features of metabolic syndrome

Central obesity	(waist circumference >=94 cm for men and >=80 cm for women),
Impaired fasting glucose	(>5,6 mmol/L or no treatment)
Hypertriglyceridemia	(>1.7 mmol/L or no treatment),
Low HDL cholesterol and	(<1.0 mmole/L for men or no treatment, <1.3 mmol/L for women or no treatment)
Hypertension	(>135/85 mmHg or no treatment).

However, for NAFLD, a disease (prevalence of 10-24% worldwide and as high as 57.5% to 74% in those who are obese)[2], the diagnosis to be made on an invasive procedure makes it likely to have sampling errors, costly and is not possible to be performed in all patients.

INVESTIGATIONS

Investigations regarding liver function test can be divided into invasive and noninvasive types. Under invasive procedures comes liver biopsy and non-invasive comes rest.

I. LIVER BIOPSY

Liver biopsy is usually indicated in cases where we find diagnostic uncertainty or if non-invasive staging is indeterminate. Liver biopsy remains the gold standard for the diagnosis of NAFLD.

MICROSCOPIC DIAGNOSIS

The histopathological finding of NASH includes fat deposition, inflammatory cell infiltration (neutrophil and lymphocytes) in lobules, ballooning degeneration, Mallory-Denk bodies, pericellular fibrosis, sinusoidal fibrosis, giant mitochondria, eosinophilic necrosis and iron deposition. However, all of the specific findings are not available for the diagnosis and so over the years few pathological criteria for nonalcoholic steotohepatitis have been proposed. The initial one was proposed in 1999, by Motteoni et al, which included Type 1: steotosis alone, Type 2: steatosis with inflammation, Type 3: steatosis with hepatocyte ballooning and Type 4: Type 3 with Mallory-Denk bodies or fibrosis.[15]

The stratification was further studied for several years and the prognostically two groups had different outcomes they were: type 1 plus type 2 and type 3 plus type 4. Disease related mortality ranged in former from 1.7% to 2.7% and in later from 11% -17.5%.(d) The problem lied in the subjective variation of grading the balloon degeneration, which differentiated type 2 from type 3. Hence a proposal of another grading was made in 2005, by the Nonalcoholic Steatohepatitis Clinical Research Network (NASH-CRN) which graded steotois (0-3), inflammation (0-3) and Hepatocyte ballooning (0-2) aiming at the type 2 and type 3 of Matteoni classification. The total score was divided into two groups as lower than 2 and more than 5 as Non

NASH and NASH. Cases in between were considered borderline. Validations studies later showed that it was more objective and reproducible, required no special staining, was applicable for pediatric NASH, and useful for assessing therapeutic effects in clinical studies. However, it cannot diagnosis the burnt out NASH in which inflammation has resolved after treatment and only fibrosis remains as fibrosis is not considered in the classification.[16] Motteoni et al, classification is said to have more diagnostic and prognostic significance than NAS classification. However, NAS classification is recommended for larger clinical studies and for monitoring the short term therapeutic effect of drugs.[10] Recently Younossi et al, have proposed that NASH is diagnosed as (1) any degree of steatosis along with centrilobular ballooning and/or Mallory-Denk bodies or (2) any degree of steatosis along with centrilobular pericellular/perisinusoidal fibrosis or bridging fibrosis.[17] This classification tries to eliminate the subjective variation of balloon degeneration of the Motteoni et al, study and also incorporated the burnt out NASH by including fibrosis.

Liver biopsy is usually indicated in cases where we find diagnostic uncertainty or if non-invasive staging is indeterminate

DRAWBACKS

Sampling error:

It is reported that only 1/50000 of the whole liver tissue is sampled if only one biopsy is taken.[10] Hence it is advised that 2 or more samples with thick needle and of a length approximately 15-16 mm or more.[5,10,11] In various studies done, the reporting of fatty change (78%) had higher consistency then fibrosis (41%) and balloon degeneration (18%) (needed for diagnosis of NASH). Also fibrosis stage varied by one or more stage between right and left lobe of liver.[10] Hence, AASLD had recommended the biopsy of right lobe biopsy first, and rebiopsy from the left lobe after treatment when left lobe is biopsied before treatment.[10,12]

Inter and intra-observer variation:

This inter-observer variation was measured in several studies. Efforts were made in training the histopathological observation. However, the pre and post training values did not differ much.[10]

Complications and risks:

Complications of biopsy includes: Pain (in 20-84%, depending on whether mild pain is included or not), serious complication (0.3-0.57%) to mortality (0.01%). The complication can be reduced by performance of the procedure by trained and experienced personal and using aspiration type biopsy needle.[10,13,14]

II. IMAGING

Ultrasonography is one of the common diagnostic modality used in NAFLD however its sensitivity is low when fatty changes are less than 20-30% and has subjective variation.[18,19] Various (0-3) grades of steatosis have been proposed based on visual analysis of the intensity of the echogenicity. Grade I is characterized by just increased in echogenicity. When the echogenic liver obscures the echogenic walls of portal vein branches, it is grade II. In grade III the echogenic liver not only obscures portal vein branches but also the outline of diaphragm.[20] These are however subject to inter-observer variation. The sensitivity of USG in detecting hepatic steatosis ranges from 60 to 94% and the specificity from 84 to 95%.[21-23]

Contrast enhanced ultrasound has recently been proposed to differentiate between NASH and simple steatosis.[24] Fibroscan (Transient elastography) gives an account of "liver stiffness measurement" (LSM) has a high AUROCs for detection of ≥ stage 2 fibrosis, ≥ stage 3 firborsis and cirrhosis of 0.84, 0.93 and 0.95 respectively.[6] However, the results may be invalid in older patients (>52 years) and with central obesity or type 2 diabetes.[25] CT scan is more objective and can measure visceral fat. Fatty liver is diagnosed when liver to spleen ratio is <0.9, however its sensitivity is low.[26,27] MRI has shown promising results but its use is limited to research.[10]

III. NON INVASIVE DIAGNOSTIC METHODS

Considering the risk of liver biopsy, a noninvasive diagnostic tool for the diagnosis and monitoring of NAFLD would be of great value. An ideal test should be simple, provides incremental information to currently available diagnostic tools, easy to measure and handle, cost-effective, accurate for diagnosis of NASH or stage of fibrosis, accurate for risk stratification and monitoring response to therapy and validated in multiple large and prospective trials.[27]

LIVER FUNCTION TEST

Liver enzymes are normal in up to 78% of cases of NAFLD.[7,28] The AST and ALT elevation is typically found to be mild and is usually not more than four times the upper limit of normal. As the diseases progresses from fibrosis to cirrhosis ALT falls and AST may rise.[6] The AST/ALT ratio of less than 1 can indicate no or minimal fibrosis and more than 1 that of development of cirrhosis. However, this is not very sensitive. Gama Glutamyltransferase (GGT) is increased in NAFLD. It is associated with increased mortality and also is a good predictor of advanced fibrosis. It cannot, however, be taken as NAFLD's sole diagnostic tool.[29] It has been reported that among cases with normal liver function tests (LFT), 70-80% of subjects with central obesity and around 50-80% with type 2 diabetes have evidence of NAFLD on

imaging. Hence dismissing patients as normal based on LFT will lead to mismanagement of NAFLD/NASH.

NON INVASIVE INESTIGATION IN DIFFERENTIATING SIMPLE STEATOSIS FROM NASH:

Following investigations (as shown in table 2) have been evaluated in several studies as soul modality in diagnosis and management of NAFLD. However, these studies are mostly done in western population, in severe obesity and weight loss related clinical studies and no proper validation studies done. Apart from individual tests several scores have been proposed by various groups across the world in evaluation of simple steatosis and NASH. Most of the scores have combination of investigations and clinical signs.

HAIR score: (three scored components) includes hypertension (HTN), ALT level, and insulin resistance.30 The presence of at least 2 factors have high sensitivity and specificity in predicting NASH

Mayo Clinic study: Any three of the six criteria should be met for diagnosis: 1. age ≥ 50 years old, 2. female sex, 3. BMI ≥ 30 kg/m2, 4. AST ≥ 45 IU/L, 5. ALT/AST ration ≥ 0.8, and 6. hyaluronic acid ≥ 55 ng/mL[31]

Palekar et al. studied 6 different variables: age, gender, AST, BMI, AST/ALT ratio(AAR) and serum hyaluronic acid. Sensitivity and specificity for the diagnosis of NASH was 74% and 66% respectively.[32] A recently proposed equation (2.627 × ln [AST] + 2.13 for DM)[33]

NASH clinical scoring system includes: HTN, type 2 DM, AST ≥ 27 IU/L, ALT ≥ 27 IU/L, sleep apnea syndrome, and race (other than blacks).[34]

Nice's Model: CK18, ALT, and the presence or absence of metabolic syndrome is scored.[35]

Few Japanese studies have proposed scores for the differentiation. **NAFIC score**, comprises three items – ferritin [200(female) or 300 (male) ng/ml as one point], fasting insulin [10microU/ml or higher as point one], and type 4 collagen 7S [5.0 ng/mL or higher as point one]. NAFIC score of 2 or more has higher changes of NASH. This study had an advantage of being validated by a large study and is done in an Asian population. However, the costs of these tests are high, it's utility in other Asian or non-Asian population is not known.[36]

All of these tests need more validation studies in a larger scale and in specific target population.

NON INVASIVE INVESTIGATIONS IN DIAGNOSIS OF NASH WITH ADVANCED FIBROSIS:

Table 2: Individual tests as tool for evaluation of steatosis and NASH

Biomarkers	Study results
Hepatocyte apoptosis:[10,29,30]	
Cytokeratin-18 Homocyteine levels, Serum prolidase enzyme activity catalysis, Plasma pentraxin 3 levels and Tissue polypeptide specific antigen.	Validation study done. not recommended by AASLD
Inflammation:[29]	
TNF alpha Adiponectin, C-reactive protein, IL-6,Leptin, CC-chemokine ligand 2 and Hyaluronic acid	Most of the studies not show clear utility values in investigation related to NAFLD/ NASH
Fibrosis:[29] Type IV collagen 7S, Hyaluronic acid, Platelet	Fibrosis:[29] Type IV collagen 7S, Hyaluronic acid, Platelet
Oxidative stress:[29] Lipid peroxidation products, Vitamin E levels, Copper-to-zinc superoxide dismutase, Glutathione peroxidase, Thioredoxin levels	Mixed results

Fibrosis can be staged according to **Burnt's criteria** into[37]

Stage 1- zone 3 perisinusoidal fibrosis;
Stage 2- zone 3 perisinusoidal fibrosis with portal fibrosis
Stage 3- zone 3 perisinusoidal fibrosis and portal fibrosis with bridging fibrosis; and
Stage 4- cirrhosis.

According ot **Kleiner's classification** fibrosis is classified into 4 stages with stage I subdivide into three substages: Substages 1a zone 3 perisinusoidal fibrosis with delicate collagen deposition, 1b are zone 3 perisinusoidal with dense collagen deposition and Substages 1c portal or periportal (representing the pediatric pattern of fibrosis). Stage 2 indicates Zone 3 and periportal fibrosis. Advanced fibrosis is classified as Stage 3 or 4.[38] Limitations of these classification include: no account of mixed portal/central lesions and does not evaluate remodeling. New scoring system, modified Laennec scoring system, has been proposed however it lacks validation, does not evaluate etiology and remodeling/ regression.[38]

Several scoring systems have been proposed regarding fibrosis in NASH. These include:

A French group proposed the **BAAT score** (0-4 points): 1

point each is assigned to BMI ≥ 28 kg/m2, ALT 2 or more times greater than the normal upper limit, age ≥ 50 years old, and TG ≥ 1.7 mmol/L. In this system the negative predictive value (NPV) for a score of 0-1 point was 100% in cases with fibrosis of stage 2 or higher.[10]

FibroTest takes in to account bilirubin, γGT, γ globulin, haptoglobin, and α2-macroglobulin and is proposed by the same French group.[10]

Mayo Clinic has proposed the **NAFLD fibrosis** score (NFS) [= -1.675 + 0.037 × age (year) + 0.094 × BMI (kg/m2) + 1.13 × IFG/DM (with = 1, without = 0) + 0.99 × AAR-0.013 × platelets (PLT) (× 109/L) - 0.66 × Alb (g/dL)]. The score is interpreted as low (NFS < -1.455), intermediate and high (NFS >0.676) scores.[39]

BARD score with one point to BMI ≥ 28 kg/m2, two points to AAR ≥ 0.8, and one point to DM, respectively. it is reported that with score of 2 or higher the possibility of Stage 3 or 4 is very high.[40] It is seen that with this test, even with mild disease the score is of >=2 due to obesity and diabetes.[6]

FIB-4 index, calculated as: [age (year) × AST (IU/L)]/[PLT (109/L) × ALT (IU/L)][41]

AST to platelet ratio index (APRI) {[(AST level/upper limit of normal AST)/PLT (109/L)] × 100}. Useful for predicting significant fibrosis due to NASH[42]

Combined interpretation of PLT and AAR (PAAR) is another useful parameter in that patients with platelet count of 195000 or greater along with an AAR below 0.8 have very low possibility of having Stage 3 or higher fibrosis.[43] Platelet count alone cannot be well correlated with fibrosis as the count is relatively higher with advanced fibrosis.[10]

McPherson et al made a comparison of five scoring systems, AAR, APRI, BARD, NFS, and the FIB-4 index which involved 145 English NAFLD patients.[42] They concluded that FIB-4 index was the most favorable (0.86), followed by AAR (0.83), NFS (0.81), BARD (0.77), and APRI (0.67) [within the bracket are the area under the receiver operating characteristic curve (AUROC) values]. Positive predictive values of the FIB-4 index and of NFS are promising and are 75% and 79%, respectively.

In a validation study of 827 cases with biopsy proven NAFLD fibrosis, AUROC of BARD score was 0.81 for stage 3-4 fibrosis. Similarly the validations studies done for NAFLD fibrosis score (733 cases) and FIB-4 score (541 cases) showed an AUROC of 0.88 and 0.80 respectively for stage 3-4 fibrosis.[40.41]

CONCLUSION

NAFLD is the deposition of fat in liver in absence of excessive of alcohol consumption.

Non Alcoholic Fatty Liver Disease ranges from simple steatosis to Nonalcoholic steatohepatitis (NASH) and cirrhosis. Most cases (90%) of NAFLD have simple steatosis with benign prognosis. Ten to thirty percent of NAFLD progresses to NASH and 25-40% of NASH undergoes progressive liver fibrosis.Ultimately 20-30% of NASH will go into cirrhosis during their lifetime. Various noninvasive modalities are used in the diagnosis of NAFLD and its various stages. However combination of different modalities both invasive and noninvasive is required for the ultimate diagnosis and proper management of the patient with non-alcoholic fatty liver disease.

REFERENCES

1. Szczepaniak LS, Nurenberg P, Leonard D, et al. "Magnetic resonance spectroscopy to measure hepatic triglyceride content: prevalence of hepatic steatosis in the general population". American Journal of Physiology 2005;288:E4628. Crossref
2. Angulo P. Medical progress: nonalcoholic fatty liver disease. The New England Journal of Medicine, 2002;346:1221-31. Crossref
3. Browning JD, Szczepaniak LS, Dobbins R, et al. Prevalence of hepatic steatosis in an urban population in the United States: Impact of ethnicity. Hepatology 2004;40:1387–95. Crossref
4. Wong VW, Chu WC, Wong GL, et al. Prevalence of non-alcoholic fatty liver disease and advanced fibrosis in Hong Kong Chinese: a population study using proton-magnetic resonance spectroscopy and transient elastography. Gut 2012;61:409–15. Crossref
5. Chalasani N, Younossi Z, Lavine JE et al. The diagnosis and management of non-alcoholic fatty liver disease: practice Guideline by the American Association for the Study of Liver Diseases, American College of Gastroenterology, and the American Gastroenterological Association. Hepatology 2012;55:2005-23. Crossref
6. Dyson JK, Anstee QM, McPherson S. Non-alcoholic fatty liver diasease: a practical approach to diagnosis and staging. Frontline Gastroenterology 2014;5:211-18. Crossref
7. Browning JD, Szczepaniak LS, Dobbins R et al. "Prevalence of hepatic steatosis in an urban population in the United States: impact of ethnicity" Hepatology,2004;40:1387-95. Crossref
8. Powell EE, Jonsson JR, Clouston AD. Steatosis: co-factor in other liver diseases. Hepatology 2005;42:5–13. Crossref
9. Musso G, Gambino R, Cassader M, Pagano G. Metaanalysis: natural history of non-alcoholic fatty liver disease (NAFLD) and diagnostic accuracy of non-invasive tests for liver disease severity. Ann Med 2011;43:617-49 Crossref
10. Sumida Y, Nakajima A, Itoh Y. Limitations of liver biopsy and non-invasive diagnostic tests for the diagnosis of nonalcoholic fatty liver disease/non alcoholic steatohepatitis. World J Gastroenterol. 2014;20:475-84. Crossref
11. Vuppalanchi R, Unalp A, Van Natta ML, Cummings OW, Sandrasegaran KE, Hameed T, Tonascia J, Chalasani N. Effects of liver biopsy sample length and number of readings on sampling variability in nonalcoholic Fatty liver disease. Clin Gastroenterol Hepatol 2009;7:481-6. Crossref
12. Sanyal AJ, Brunt EM, Kleiner DE et al. Endpoints and clinical trial design for nonalcoholic steatohepatitis. Hepatology 2011;54:344-53. Crossref

13. Rockey DC, Caldwell SH, Goodman ZD, Nelson RC, Smith AD. Liver biopsy. Hepatology 2009;49:1017-44. Crossref

14. Friedman LS. Controversies in liver biopsy: who, where, when, how, why? Curr Gastroenterol Rep 2004;6:30-6. Crossref

15. Matteoni CA, Younossi ZM, Gramlich T, Boparai N, Liu YC, McCullough AJ. Nonalcoholic fatt y liver disease: a spectrum of clinical and pathological severity. Gastroenterology 1999;116:1413-9. Crossref

16. Juluri R, Vuppalanchi R, Olson J, Unalp A, et al. Generalizability of the nonalcoholic steatohepatitis Clinical Research Network histologic scoring system for nonalcoholic fatty liver disease. J Clin Gastroenterol 2011;45:55-8. Crossref

17. Younossi ZM, Stepanova M, Rafiq N et al. Pathologic criteria for nonalcoholic steatohepatitis: interprotocol agreement and ability to predict liver-related mortality. Hepatology 2011;53:1874-82. Crossref

18. Hernaez R, Lazo M, Bonekamp S et al. Diagnostic accuracy and reliability of ultrasonography for the detection of fatty liver: a meta-analysis. Hepatology 2011;54:1082-90. Crossref

19. Dasarathy S, Dasarathy J, Khiyami A, Joseph R, Lopez R, McCullough AJ. Validity of real time ultrasound in the diagnosis of hepatic steatosis: a prospective study. J Hepatol 2009;51:1061-7. Crossref

20. Saadeh S, Younossi ZM, Remer EM, Gramlich T, Ong JP, Hurley M, et al. The utility of radiological imaging in nonalcoholic fatty liver disease. Gastroenterology. 2002;123:745–50. Crossref

21. Joseph AE, Saverymuttu SH, al-Sam S, Cook MG, Maxwell JD. Comparison of liver histology with ultrasonography in assessing diffuse parenchymal liver disease. Clin Radiol. 1991;43:26–31. Crossref

22. Debongnie JC, Pauls C, Fievez M, Wibin E. Prospective evaluation of the diagnostic accuracy of liver ultrasonography. Gut. 1981;22:130–5. Crossref

23. Saverymuttu SH, Joseph AE, Maxwell JD. Ultrasound scanning in the detection of hepatic fibrosis and steatosis. Br Med J (Clin Res Ed) 1986;292:13–5. Crossref

24. Wieckowska A, McCullough AJ, Feldstein A E. Noninvasive Diagnosis and Monitoring of Nonalcoholic Steatohepatitis: Present and Future. Hepatology 2007;46:582-9. Crossref

25. Castera L, Foucher J, Bernard PH, et al. Pitfalls of liver stiffness measurement: a 5 years prospective study of 13,369 examinations. Hepatology 2010;51:828-35. Crossref

26. Saadeh S, Younossi ZM, Remer EM et al. The utility of radiological imaging in nonalcoholic fatty liver disease. Gastroenterology 2002;123:745-50. Crossref

27. Roldan-Valadex E, Favila R, Martinex-Lopez M, Uribe M, Medex-Sanchez N. Imaging techniques for assessing hepatic fat content in nonalcoholic fatty liver disease. Ann Hepatol 2008;7:212-20.

28. Dowman JK, Tomlinson JW, Newsome PN. Systematic review: the diagnosis ans tagging of non-alcoholic fatty liver disease and non-alcoholic steatohepatitis. Alimentary Pharmacology and Therapeutics,2011;33:525-40. Crossref

29. Obika M, Noguchi H. Diagnosis and evaluation of Nonalcoholic fatty liver disease. Experimnetal diabetes research Volume 2012, Article ID 145754, 12 pages. Crossref

30. Yilmaz Y. Cytokeratins in hepatitis. Clin Chim Acta 2011;412:2031-6. Crossref

31. Dixon JB, Bhathal PS, O'Brien PE. Nonalcoholic fatty liver disease: predictors of nonalcoholic steatohepatitis and liver fibrosis in the severely obese. Gastroenterology 2001;121:91-100. Crossref

32. Palekar NA, Naus R, Larson SP, Ward J, Harrison SA. Clinical model for distinguishing nonalcoholic steatohepatitis from simple steatosis in patients with nonalcoholic fatty liver disease. Liver Int 2006;26:151-6. Crossref

33. Poynard T, Ratziu V, Charlotte F, et al. Diagnostic value of biochemical markers (NashTest) for the prediction of non alcoholo steato hepatitis in patients with non-alcoholic fatty liver disease. BMC Gastroenterol 2006;6:34. Crossref

34. Gholam PM, Flancbaum L, Machan JT, Charney DA, Kotler DP. Nonalcoholic fatty liver disease in severely obese subjects. Am J Gastroenterol 2007;102:399-408. Crossref

35. Campos GM, Bambha K, Vittinghoff E, et al. A clinical scoring system for predicting nonalcoholic steatohepatitis in morbidly obese patients. Hepatology 2008;47:1916-23. Crossref

36. Sumida Y, Yoneda M, Hyogo H et al. A simple clinical scoring system using ferritin, fasting insulin, and type IV collagen 7S for predicting steatohepatitis in nonalcoholic fatty liver disease. J Gastroenterol 2011;46:257-68. Crossref

37. Brunt EM, Janney CG, Di Bisceglie AM, Neuschwander- Tetri BA, Bacon BR. Nonalcoholic steatohepatitis: a proposal for grading and staging the histological lesions. Am J Gastroenterol 1999;94:2467-74. Crossref

38. Kleiner DE, Brunt EM, Van Natta M, Behling C, Contos MJ, Cummings OW, Ferrell LD, Liu YC, Torbenson MS, Unalp-Arida A, Yeh M, McCullough AJ, Sanyal AJ. Design and validation of a histological scoring system for nonalcoholic fatty liver disease. Hepatology 2005;41:1313-21. Crossref

39. Angulo P, Hui JM, Marchesini G, et al. The NAFLD fibrosis score: a noninvasive system that identifies liver fibrosis in patients with NAFLD. Hepatology 2007;45:846-54. Crossref

40. Harrison SA, Oliver D, Arnold HL, Gogia S, Neuschwander-Tetri BA. Development and validation of a simple NAFLD clinical scoring system for identifying patients without advanced disease. Gut 2008;57:1441-7. Crossref

41. Shah AG, Lydecker A, Murray K, Tetri BN, Contos MJ, Sanyal AJ. Comparison of noninvasive markers of fibrosis in patients with nonalcoholic fatty liver disease. Clin Gastroenterol Hepatol 2009;7:1104-12. Crossref

42. McPherson S, Anstee QM, Henderson E, Day CP, Burt AD. Are simple noninvasive scoring systems for fibrosis reliable in patients with NAFLD and normal ALT levels? Eur J Gastroenterol Hepatol 2013;25:652-8. Crossref

43. Sumida Y, Ohno T, Sakai K, Kanemasa K, Imai S. Usefulness of combination of platelet count and AST/ALT ratio (PAAR index) for excluding advanced fibrosis in nonalcoholic fatty liver disease. Kanzo 2011; 52: 383-386. Available from: Crossref