Zagazig University Medical Journal
www.zumj.journals.ekb.eg

ASSESSMENT OF LIVER STIFFNESS IN NONALCOHOLIC FATTY LIVER DISEASE

Mahmoud M A Amer [a], Emad A Emam [a], Ayman F Ahmed [b] , Emad F Hamed [a]

Internal Medicine Department, Faculty of Medicine, Zagazig University, Zagazig, Egypt [a]
Radiology Department, Faculty of Medicine, Zagazig University, Zagazig, Egypt [b]

Corresponding author:
Mahmoud Mohamed Abdel Hamid Amer.
Email:aliamer006@yahoo.com

ABSTRACT

Introduction and aim: Several liver fibrosis markers which have been previously evaluated for the patients with viral hepatitis particularly HCV-infected patients. The combination of liver stiffness measurement by transient elastography (TE) and fibrosis scores like APRI, FIB -4 and NAFLD fibrosis score (NFS) are able to accurately diagnose or exclude the presence of severe liver fibrosis and they also reducing the number of needed diagnostic liver biopsies. The purpose of the present study was to assess liver stiffness severity in NAFLD patients by TE, and also to test diagnostic accuracy of FIB-4, NFS and APRI as simple noninvasive markers of liver stiffness in those patients. **Method**: This study included, 153 individuals who were divided into three groups according to fibrosis grade measured by TE, group I consisted of 62 subjects 32 were males (51.6%) and 30 females (48.4%), their mean age ± SD (44.29 ± 9.65 y), Whereas the group II consisted of 57 subjects, 22 males (38.6%) and 35 females (61.4%), their mean age ± SD (44.70 ± 9.12 y), As regard group III, it consists of 34 subjects, 13 males (38.2%) and 21 females (61.8%), their mean age ± SD (45.82 ± 9.65 y). Full history taking, thorough clinical examination and routine laboratory investigations together with different noninvasive fibrosis score and LSM was analyzed in three groups. **Results**: There was statistically significant difference between the three groups as regard FIB-4 and NF scores, while there was no statistically significant difference between the three groups as regard APRI. By post hoc analysis the most significant difference was between group I and III as well as group II and III. There was statistically significant positive correlation between fibrosis stage by TE and FIB4 score, glycated hemoglobin (HBA1c) and NF score. **Conclusion**; LSM by TE is easy, accurate way to anticipate advanced fibrosis together with other simple noninvasive measures like NFS and FIB-4 may lower the threshold for liver biopsies in NAFLD patients.

Keywords: Liver Stiffness, nonalcoholic fatty liver disease, transient elastography, non invasive scoring systems

1- INTRODUCTION

Nonalcoholic fatty liver disease (NAFLD) is one of the most common causes of chronic liver disease and its prevalence is increasing worldwide. Nonalcoholic steatohepatitis (NASH), is the progressive form of NAFLD, which leads to liver cirrhosis and hepatocellular carcinoma. [(1).]

In current practice, assessment of liver fibrosis and grading in NAFLD depends on liver biopsy. However, many patients may not consent to do it as it has many drawbacks such as sampling error, biopsy size and intra and inter-observer variability and a risk of complication. Furthermore, it's not realistic to perform liver biopsies on all NAFLD patients. [(2)]

Given the patient preference to avoid liver biopsy, noninvasive alternatives to assess liver fibrosis are in high demand. The most accurate noninvasive methods are based on liver elastography that included vibration controlled transient elastography, magnetic resonance elastography, shear-wave elastography and acoustic radiation force impulse. [(3)]

So, the aim of this study was to assess liver stiffness severity in NAFLD patients by TE, and also to test diagnostic accuracy of

FIB-4, NFS and APRI as simple noninvasive markers of liver stiffness in these patients.

2- SUBJECTS AND METHODS

2.1- Study design and classification:

A comparative cross-sectional study was carried out in Gastroenterology and Hepatology unit, Internal Medicine Department, in collaboration with Radiology Department, Faculty of Medicine, Zagazig University Hospitals between April 2017 and April 2018.

Based on fibrosis stage measured by TE, our subjects (n=153) were classified into three groups**:**[(4)]

Group I: Individuals with F 0/F 1: (LSM by TE was ≤ 7 Kpa.). Sixty-two subjects 32 of them were males (51.6%) and 30 of them were females (48.4%), their mean age ± SD (44.29 ± 9.65 y). **Group II: Individuals with F 2:** (LSM by TE was > 7 and < 10 Kpa.). Fifty-seven subjects 22 of them were males (38.6%) and 35 of them were females (61.4%), their mean age ± SD (44.70 ± 9.12 y). **Group III: Individuals with F 3:** (LSM by TE was ≥ 10 Kpa.). Thirty-four subjects 13 of them were males (38.2%) and 21 of them were females (61.8%), their mean age ± SD (45.82 ± 9.65 y).

2.2- Inclusion and Exclusion Criteria:

Individuals of both sexes, their age > 18 years old. Ultrasound criteria for NAFLD diagnosis: Bright hepatic echoes, increased hepatic to renal echogenicity and vascular blurring of the portal or hepatic vein have been classified as unique sonographic features of NAFLD [(5)].

Exclusion criteria included patients with liver disease other than fatty liver such as chronic viral hepatitis, autoimmune hepatitis, other metabolic liver diseases, hepatocellular carcinoma, chronic alcohol consumption and infiltrative liver diseases.

2.3- Ethical Clearance:

Written Informed consent was taken from the patients' relatives to participate in the study. Approval for performing the study was obtained from Institutional Review Board (IRB) approval.

2.4- Study tools:

All members of the study were subjected to:

Full history and thorough clinical examination as well as drug prescriptions. General examination and Local examination of different systems with **t**horough gastrointestinal examination. Routine investigations were done according to protocol of clinical pathology and laboratories of Zagazig University Hospital: 10 ml of blood was collected for Complete blood count (CBC), liver function tests, kidney function tests, coagulation profile. Fasting blood glucose, HbA1c and lipid profile.

Special investigations: meas**u**rement of liver stiffness was performed by transient elastography using Fibroscan (Echosens, Paris, France) after overnight fasting [(6)]. Briefly, the tip of an ultrasound probe was placed in an intercostals space on the right lobe of the liver with the patient lying in dorsal decubitus position and the right arm in maximal abduction. Vibrations with mild amplitude and low-frequency were transmitted to the liver tissue. The velocity of the induced shear wave is directly related to liver stiffness. A minimum of 10 valid readings, with at least a 60% success rate and an interquartile range of ≤30% of the median value, are taken with the results expressed in kilopascals (kPa).

Underlying liver fibrosis was estimated using:

- **APRI=** (AST in IU/L) / (AST Upper Limit of Normal in IU/L) / (platelets in 10^9/L)
- **FIB-4** = (Age x AST) / (platelets x (sqr (ALT))
- **NFS** = -1.675 + (0.037*age [years]) + (0.094*BMI [kg/m2]) + (1.13*IFG/diabetes [yes = 1, no = 0]) + (0.99*AST/ALT ratio) – (0.013*platelet count [×109/L]) – (0.66*albumin [g/dl])

2.5- Statistical Analysis:

All data were analyzed using SPSS 20.0 for windows (SPSS Inc., Chicago, Illinois, USA), MedCalc Statistical Software version 15.8 (MedCalc Software bvba, Ostend, Belgium; https://www. medcalc. org; 2015).

3- RESULTS

There were statistically significant differences between the three groups regarding ALT and glycosylated hemoglobin. Also, there were statistically significant differences between the three groups as regard FIB-4 and NF scores, while there is no statistically significant difference between the three groups as regard APRI.

By the application of post hoc analysis the most significant differences were between group I and III as well as group II and III.
There was statistically significant positive correlation between fibrosis stage by fibroscan and FIB-4 score. As well as significant positive correlation between fibrosis stage by fibroscan and BG, NF score.
Cut-off value of FIB-4 > 1.94 with sensitivity of 50% and specificity of 90.8%. AUC = 0.67. Cut-off value of NFS-4 > 0.11 with sensitivity of 47.1% and specificity of 96.6%.AUC = 0.64.

Table 1. Demographic data of the studied population:

	The studied Population (n=153)	
	No	**%**
Age (Years)		
Median (Range)	45 (29 – 60)	
Sex		
Male	67	43.8%
Female	86	56.2%
Smoking Status		
Non Smoker	113	73.9%
Smoker	40	26.1%
BMI (Kg/m^2)		
Mean± SD	34.87 ± 3.40	
Diabetes		
No	65	42.5%
Yes	88	57.5%
Hypertension		
No	63	41.2%
Yes	90	58.8%

Table 2. Comparison of glycated hemoglobin and lipid profile between studied groups (n=153):

	Group I (n=62)	**Group II (n=57)**	**Group III (n=34)**	**Test**	**P**
ALT(U/L)					
Median (Range**)**	55 (13-92)	59 (14-95)	43 (13-90)	**7.78**	**0.02**
HbA1c (%)					
Median (Range**)**	4 (3 – 6.2)	5.3 (3.9 – 7)	7.4 (5.3- 9)	77.46	**<0.001**
Cholesterol (mg/dL)				**F**	0.64
Mean± SD	191.52 ± 42.29	198.88 ± 44.63	197.94 ± 48	0.46	(NS)
LDL (mg/dL)				**F**	0.29
Mean± SD	123.50 ± 28.69	129.33 ± 23.67	121.15 ± 24.54	1.27	(NS)
HDL (mg/dL)				**F**	0.73
Mean± SD	39.73 ± 5.40	40.02 ± 4.79	39.15 ± 4.97	0.31	(NS)
TG (mg/dL)				**F**	0.29
Mean± SD	161.74 ± 62.64	168.58 ± 64.41	182.65 ± 57.61	1.24	(NS)

KW = Kruskal Wallis test F= One-way ANOVA.
p value <0.05 was considered statistically significant(S).

Table 3. Correlation coefficient between elastography score (Kpa.) and study parameters:

	Total population (n=153)		
	r	P	
HbA1c (%)	**0.68**	**<0.001**	**(S)**
ALT (U/L)	**-0.18**	**0.03**	**(S)**
AST (U/L)	0.11	0.18	(NS)
ALB (g/dL)	0.04	0.66	(NS)
INR	-0.04	0.60	(NS)
Cholesterol (mg/dL) *	0.15	0.07	(NS)
Fib4 score	**0.24**	**0.003**	**(S)**
APRI index	0.15	0.07	(NS)
NFS	**0.22**	**0.008**	**(S)**

r = Spearman's rank correlation coefficient
*= Pearson correlation coefficient

Table 4. Correlation coefficient between elastography score (Kpa.) and fibrosis score:

	Total population (n=153)		
	r	P	
Fib4 score	**0.24**	**0.003**	**(S)**
APRI index	0.15	0.07	(NS)
NFS	**0.22**	**0.008**	**(S)**

r = Spearman's rank correlation coefficient
*= Pearson correlation coefficient

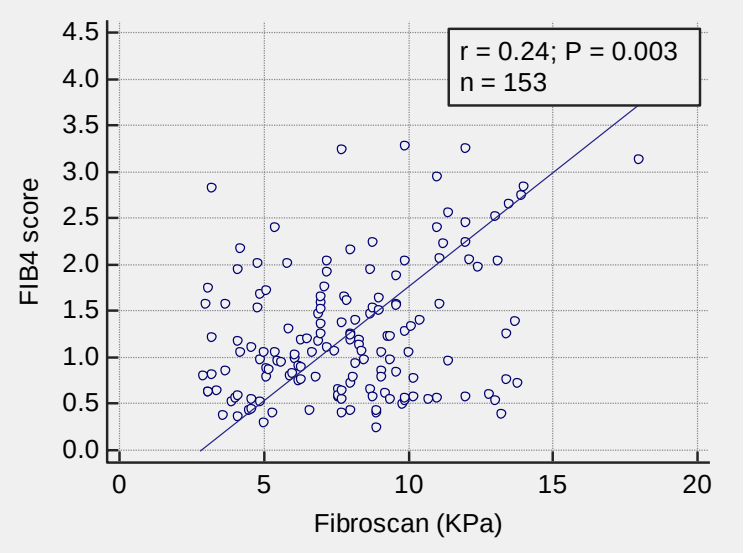

Figure 1. linear regression line between FIB-4 score and TE by fibroscan examination (n=153).

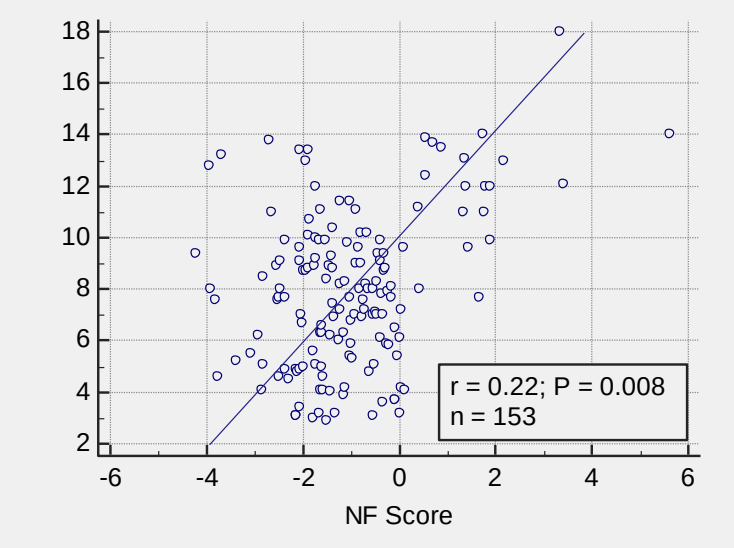

Figure (2): Linear regression line between NF score and TE by fibroscan examination (n=153).

Table 5. Comparison of fibrosis scores between studied groups:

	Group I (n=62)	Group II (n=57)	Group III (n=34)	Test	P
FIB4 Median (Range)	0.97 (0.29 – 4.16)	1.10 (0.24 – 3.28)	1.77 (0.39 – 3.25)	**KW** 9.75	**0.008 (S)**
APRI Mean± SD	0.45 ± 0.20	0.48 ± 0.23	0.55 ± 0.26	**F** 2.21	0.11 (NS)
NFS Mean± SD	-1.37 ± 0.93	-1.17 ± 1.22	-0.14 ± 2.17	**F** 8.95	**< 0.001 (S)**

KW = Kruskal Wallis test
F= One-Way ANOVA

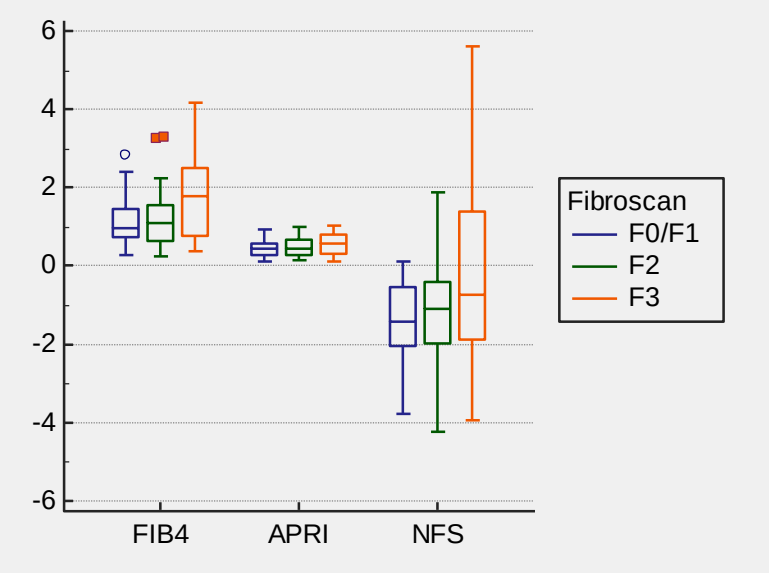

Figure (3): Boxplot chart show different fibrosis scores in study groups.

Table 6. LSD Post Hoc analysis of mean ± SD of FIB-4 and NFS:

	Group II	Group III
FIB-4		
Group I	0.458 (NS)	**<0.001 (S)**
Group II	-------	**<0.001 (S)**
NFS		
Group I	0.451 (NS)	**<0.001 (S)**
Group II	-------	**0.001 (S)**

4- DISCUSSION

Nonalcoholic fatty liver disease (NAFLD) is the most common cause of liver disease worldwide with prevalence estimates ranging from 25% to 45% in most studies, increasing in parallel with that of obesity and diabetes. NAFLD defined by the presence of hepatic steatosis in the absence of excess alcohol and is considered to represent the hepatic component of the metabolic syndrome. [(7)]

Fibrosis assessment in patients with chronic liver disease is useful for prognostication, identification of those patients who require more intensive monitoring or treatment as well as for those who may benefit from screening and surveillance strategies. In NAFLD patients, significant fibrosis increases the risk of liver related morbidity and mortality. So, we can categorize a set of patients who may benefit from, monitoring and more intensive management of their metabolic risk factors. [(8)]

Several liver fibrosis markers which have been previously evaluated for the patients with viral hepatitis particularly HCV-infected patients. The combination of liver stiffness measurement by Fibroscan and NAFLD fibrosis score like APRI and FIB-4, NFS may be able for diagnosis or exclusion of severe liver fibrosis, also reducing the number of needed diagnostic liver biopsies. [(9)].

Our study demonstrated that a large portion of included people were considered as having obesity as indicated by their BMI being more than 30 kg/m^2, however it is not always the case, a study by Younossi et al., NAFLD may occur actually to lean people in a mechanism that is not clear. This is in line with other study by Wattacheril and Sanyal, they stated that lean individuals with NAFLD are not rare but represent one significant end of the phenotypic spectrum of NAFLD. This is, of course, important because there is multinational investigation reveals an increased mortality in lean individuals with nonalcoholic steatohepatitis (NASH). Many aspects of lean NAFLD need further exploration including epidemiology, clinical risk assessment, histologic changes unique to lean NAFLD, genetic and pathophysiologic mechanisms predisposing at risk individuals, natural history, and treatment strategies in this underrecognized population. [(10) (11)]

The relationship between rising body mass index (BMI) and prospective risk of NAFLD/NASH is virtually universal, also in our study patients with higher BMI have more fibrosis by TE compared to their peers. Our study showed that patient with significant fibrosis have more BMI than other groups median 36.3 kg/m^2 and ranging from 32.3 to 38.4 kg/m^2. In a study by Loomis et al., they found that the prospective risk for being recorded as having a diagnosis of NAFLD/NASH increased linearly with increasing BMI such that risk of NAFLD/NASH diagnosis was approximately 5 - to 9-fold higher at BMI of 30–32.5 kg/m^2 rising to around 10 - to 14-fold higher at BMIs of 37.5–40 kg/m^2 compared with patients with BMI 20–22.5 kg/m^2. [(12)]

Another study by Yen et al., reported that elevated BMI is independently associated with possible liver cirrhosis and clinically relevant fibrosis in patients with different etiologies of CLD. Hence, weight loss could be beneficial for these patients. In study by Amiri Dash Atan et al., reported that obesity is one of the most important factors involved in NAFLD and high BMI in type 2 diabetes mellitus patients is associated with NAFLD. [(13) (14)]

As regard sex, our study showed slight female predominate. This was consistent with Fernandes et al., who found metabolic

differences between male and female adolescents with NAFLD. However, this is against two studies by Lu et al., and Caballería et al**.,** who reported male predominance in NAFLD. [15, 16,17]

As regard prevalence of diabetes in NAFLD patients, there was a sided relationship between both diabetes and NAFLD, they follow each other and they were considered as a part of metabolic spectrum that affect a wide variety of individuals [18] [19]. There was, therefore, no doubt that these two common conditions co-exist and that there was significant amount of unrecognized advanced NAFLD within asymptomatic diabetic patients. In our study the prevalence of diabetes in NAFLD was high (57.5%), being more statistically significant in the group with advanced fibrosis (>50%). This was consistent with Wong et al., who reported that NAFLD was independently associated with T2DM. [20]

In our study there was statistically significant positive correlation between HbA1c and LSM by TE. This was in agreement with Amiri Dash Atan et al., who found that that there was a relationship between HbA1c and NAFLD. [14]

As regarding our study, TE by fibroscan using M probe was unreliable in about 15% of NAFLD patients due to obesity, this result being in line with a range of 5 – 10% reported in the literature on adult patients with NAFLD when using the standard M probe [21]

In the present study individuals with F 0/F 1(TE was ≤ 7 Kpa.) were 62/153 subjects (40.5%), Individuals with F 2 (TE was > 7 and < 10 Kpa.) were 57/153 subject (37.3%), while Individuals with F 3 (LSM by TE was ≥ 10 Kpa.) were 34/153 subject (22.2%).

Our results were comparable to Imajo et al., who found that F 2, the LSM cut-off values range from 6.2 to 11 kPa, with 62%-90% sensitivity and 74%-100% specificity and F 3, the LSM cut-off values range from 8 to 12 kPa, with 84%-100% sensitivity and 83%-97% specificity. This also was in agreement with Pathik et al., who settled that F 2, the LSM cut-off values range from 5.9 to 9.1 kPa, and for F 3, the LSM cut-off values range from 8 to 11.4 kPa. [22,23]

In a study by Petta and colleagues [21], LSM values of 7.25 kPa and 8.75 kPa were the best cut-offs for discriminating significant fibrosis (F 2-F4) and severe fibrosis (F3-F4), respectively. These stiffness cut-off values were quite comparable to those identified in other studies by Gaia et al., and Lupsor et al., [24,25]

Non-alcoholic fatty liver disease is the most common cause of elevated liver enzymes [27]. In this regard the current study showed that there was statistically significant difference between the three groups as regard ALT being higher in group III by post hoc analysis. This is logical and is consistent with previous studies. [26,27,28]

Another study found a relationship between presence of steatohepatitis and increased level of ALT, and even ALT had been considered as a part of some steatohepatitis diagnostic panels, like the BAAT score, FIB4 index, FibroTest, FibroMeter, NashTest and NFS [29].

The FIB-4 scoring system uses a combination of patient age, platelet count, AST and ALT which is available to the primary care physician. The scoring system creates a score - <1.45 has a negative predictive value of over 90% for advanced liver fibrosis of multiple aetiologies. A score of >3.25 has a positive predictive value of 65% for advanced fibrosis with a specificity of 97% [30]. Interestingly, when the FIB4 index was compared to other noninvasive markers of fibrosis—including the AST/ALT ratio and the NFS—it had the highest AUROC for predicting advanced fibrosis (0.80–0.86) [31].

The present study showed that the baseline FIB-4 score for the whole population had mean of 1.27 ± 0.74. There was statistically significant difference between the three groups as regard FIB4. By post hoc analysis the most significant difference was between group I and III as well as group II and III. There was statistically significant positive correlation between fibrosis stage(fibroscan) and FIB4 score.

According to Bonder and Afdhal, study that established cut off values of liver fibrosis using fibroscan, where F 0/F 1 have mean FIB-4 score of 0.97, F2 have mean FIB-4 of 1.1

while F 4 have mean FIB-4 score of 1.77. FIB-4 was assessed in a study by Sumida et al., found that FIB-4 index was superior to other tested noninvasive markers of fibrosis in Japanese patients with NAFLD, with a high negative predictive value for excluding advanced fibrosis with a cut off value of 1.45 that predict significant fibrosis. Another study by Shah et al., reported that FIB-4 with a cut off value of 1.98 could predict significant fibrosis ≥ F 3. [4,32,33]

The NFS was developed by Angulo and colleagues in a large cohort of patients with NAFLD that was confirmed on the biopsy. The NFS has been validated in multiple studies, and meta-analysis. The NAFLD guidelines acknowledged that the NFS was a clinically useful tool for identifying advanced fibrosis in patients with NAFLD [34,31].

As regard NFS, in our study mean NFS was -1.02 ± 1.47, this was consistent with findings by Bugianesi et al., who reported a cut off value to define significant fibrosis in individuals with NAFLD is > 0.675. Also, in our study individuals in group III (**≥ F3**) have NFS of -0.14 ± 2.17. In a study by McPherson et al., it was reported that NF score and other non-invasive fibrosis scoring systems can reliably exclude advanced fibrosis in patients with non-alcoholic fatty liver disease, the baseline NF score was 1.47 ± 1.73 also mean NF score that can predict significant fibrosis F 3/F 4 was 1.52 ± 1.75. [35,30]

In this work there was statistically significant positive correlation between fibrosis stage by fibroscan with FIB4 score and NFS. This was in agreement with a study by Fallatah et al., who found that there was a significant positive correlation between the Fibroscan results and the AST/ALT ratios, the APRI scores, and the FIB-4 results. Also, this was supported by findings by Kumar et al., who reported a correlation between liver stiffness measured by TE and different study parameters and other fibrosis markers including NFS and FIB-4. This may be logical as either score include parameters e.g. liver enzymes any increase with affect liver stiffness measurement. [36,37]

ROC curve analysis was used in our study to detect the optimal cut off values of fibroscan using categories of fibrosis according to noninvasive scores that could detect significant fibrosis by FIB-4 and NFS. Using NFS to define F3-F4 categories the cut-off value of TE > 9.4 KPa with sensitivity of 93.75% and specificity of 78.8% with AUC = 0.91. Compared to a histology proven NAFLD study by Wong et al., the accuracy of fibroscan to detect significant fibrosis ≥ F 3 with a cut off value of 9.6 KPa with sensitivity of 75% and specificity of 91.6% with AUC = 0.93. [38]

Boursier et al., and Petta et al., found that the accuracy of fibroscan to detect significant fibrosis ≥ F 3 with the cut off value of 8.7 and 10.1 respectively with sensitivity of 88% and 78% and specificity of 63 and 78% respectively. [39,9]

In conclusion; LSM by TE is easy, accurate way to anticipate advanced fibrosis together with other simple noninvasive measures like NFS and FIB-4 and this will lower the threshold for liver biopsies in NAFLD patients.

5- REFERENCES

1. **Yuta Aida, Hiroshi Abe, Yoichi Tomita, et al.:** Serum cytokeratin 18 fragment level as noninvasive biomarker for nonalcoholic fatty liver disease; (2014)7: 4191–4198.
2. **Strassburg MM and Manns MP:** Approach to liver biopsy techniques –revisited. Semin Liver Dis; (2006)26: 318-27.
3. **Tapper, E. B., and Loomba, R.** Noninvasive imaging biomarker assessment of liver fibrosis by elastography in NAFLD. Nature Reviews Gastroenterology & Hepatology (2018)15, pages 274–282.
4. **Bonder, A., and Afdhal, N.** Utilization of Fibroscan in clinical practice. Current gastroenterology reports, (2014)16 (2), 372.
5. **Khov, N., Sharma, A., and Riley, T. R.** Bedside ultrasound in the diagnosis of nonalcoholic fatty liver disease. World Journal of Gastroenterology; (2014) 20(22), 6821–6825.
6. **Sandrin L, Fourquet B, Hasquenoph JM, et al.** Transient elastography: a new noninvasive method for assessment of hepatic fibrosis. Ultrasound Med Biol (2003) ;29(12):1705–1713.
7. **Chalasani N, Younossi Z, Lavine JE, et al;** American Gastroenterology Association; American Association for the Study of Liver Diseases; American College of Gastroenterology. The diagnosis and management of non-alcoholic fatty liver disease: practice guideline by the American Gastroenterological Association, American Association for the Study of Liver Diseases, and American College of Gastroenterology. Gastroenterology; (2012) 142 (7): 1592-1609.

8. **Adams, L. A., George, J., Bugianesi, E., et al.** Complex non-invasive fibrosis models are more accurate than simple models in non-alcoholic fatty liver disease. Journal of gastroenterology and hepatology, (2011) 26(10), 1536- 1543.
9. **Petta S, Vanni E, Marco V, et al.:** The combination of liver stiffness measurement and NAFLD fibrosis score improves the noninvasive diagnostic accuracy for severe liver fibrosis in patients with nonalcoholic fatty liver disease. Liver international; (2015) 35: 1566-1573.
10. **Younossi, Z. M., Stepanova, M., Negro, F., et al.** Nonalcoholic fatty liver disease in lean individuals in the United States. Medicine, (2012) 91(6), 319-327.
11. **Wattacheril, J., and Sanyal, A. J.** Lean NAFLD: an underrecognized outlier. Current hepatology reports, (2016) 15(2), 134-139.
12. **Loomis, A. K., Kabadi, S., Preiss, D., et al.** Body Mass Index and Risk of Nonalcoholic Fatty Liver Disease: Two Electronic Health Record Prospective Studies. The Journal of Clinical Endocrinology and Metabolism, (2016)101(3), 945–952.
13. **Yen, Y. H., Chang, K. C., Tsai, M. C., et al.** Elevated body mass index is a risk factor associated with possible liver cirrhosis across different etiologies of chronic liver disease. Journal of the Formosan Medical Association, (2018)117(4), 268-275.
14. **Amiri Dash Atan, N., Koushki, M., Motedayen, M., et al.** Type 2 diabetes mellitus and non-alcoholic fatty liver disease: a systematic review and meta-analysis. Gastroenterology and Hepatology from Bed to Bench, (2017) 10 (Suppl1), S1–S7.
15. **Fernandes, M. T. B., Ferraro, A. A., De Azevedo, R. A., et al.** Metabolic differences between male and female adolescents with non-alcoholic fatty liver disease, as detected by ultrasound. Acta Paediatrica, (2010)99(8), 1218-1223.
16. **Lu, H., Zeng, L., Liang, B., et al.** High prevalence of coronary heart disease in type 2 diabetic patients with non-alcoholic fatty liver disease. Archives of Medical Research, (2009) 40(7), 571-575.
17. **Caballería, L., Pera, G., Auladell, M. A., et al.** Prevalence and factors associated with the presence of nonalcoholic fatty liver disease in an adult population in Spain. European journal of gastro-enterology & hepatology, (2010)22(1), 24-32.
18. **Mantovani, A., Byrne, C. D., Bonora, E., et al.** Nonalcoholic fatty liver disease and risk of incident type 2 diabetes: a meta-analysis. Diabetes care, (2018). 41(2), 372-382.
19. **Shen, X., Cai, J., Gao, J., et al.** Non-Alcoholic Fatty Liver Disease and Risk of Diabetes: A Prospective Study In CHINA. Endocrine Practice. (2018).
20. **Wong, C. A., Araneta, M. R. G., Barrett-Connor, E., et al.** Probable NAFLD, by ALT levels, and Diabetes among Filipino-American Women. Diabetes Research and Clinical Practice, (2008). 79 (1), 133–140.
21. **Petta, S., Di Marco, V., Cammà, C., et al.** Reliability of liver stiffness measurement in non-alcoholic fatty liver disease: the effects of body mass index. Alimentary pharmacology & therapeutics, (2011)33(12), 1350-1360.
22. **Imajo K, Kessoku T, Honda Y, et al.** Magnetic Resonance Imaging More Accurately Classifies Steatosis and Fibrosis in Patients with Nonalcoholic Fatty Liver Disease Than Transient Elasto-graphy. Gastroenterology.; (2016) 150:626–637.e7.
23. **Pathik P, Ravindra S, Ajay C, et al.** Fibroscan versus simple noninvasive screening tools in predicting fibrosis in high-risk nonalcoholic fatty liver disease patients from Western India. Ann Gastroenterol.; (2015)28:281–286.
24. **Gaia S, Carenzi S, Barilli AL, et al.** Reliability of transient elastography for the detection of fibrosis in non-alcoholic fatty liver disease and chronic viral hepatitis. J Hepatol; (2011)54: 64–71.
25. **Lupsor M, Badea R, Stefanescu H, et al**. Performance of unidimensional transient elastography in staging non-alcoholic steatohepatitis. J Gastrointestin Liver Dis; (2010) 19: 53–60.
26. **Vernon, G., Baranova, A., and Younossi, Z. M.** Systematic review: the epidemiology and natural history of non-alcoholic fatty liver disease and non-alcoholic steatohepatitis in adults. Alimentary pharmacology & therapeutics, (2011) 34(3), 274-285.
27. **Díez-Vallejo J., and Comas-Fuentes A.** Asymptomatic hypertrans-aminasemia in patients in primary care. Rev Esp Enferm Dig., (2011)103:530–535.
28. **Pourshams A, Malekzadeh R, Monavvari A, et al.** Prevalence and etiology of persistently elevated alanine aminotransferase levels in healthy Iranian blood donors. J Gastroenterol Hepatol.; (2005). 20:229–233.
29. **Hadizadeh, F., Faghihimani, E., and Adibi, P.** Nonalcoholic fatty liver disease: Diagnostic biomarkers. World Journal of Gastrointestinal Pathophysiology, (2017)8(2), 11–26.
30. **McPherson, S., Stewart, S. F., Henderson, E., et al.** Simple non-invasive fibrosis scoring systems can reliably exclude advanced fibrosis in patients with non-alcoholic fatty liver disease. Gut, (2010) 59(9), 1265-1269.
31. **Alkhouri, N., and McCullough, A. J.** Noninvasive diagnosis of NASH and liver fibrosis within the spectrum of NAFLD. Gastroenterology & hepatology, (2012)8(10), 661.
32. **Sumida, Y., Yoneda, M., Hyogo, H., et al.** Validation of the FIB4 index in a Japanese nonalcoholic fatty liver disease population. BMC Gastroenterol; (2012)12: 383-386.
33. **Shah, A. G., Lydecker, A., Murray, K., et al.** Comparison of noninvasive markers of fibrosis in patients with nonalcoholic fatty liver disease. Clinical Gastroenterology and Hepatology, (2009). 7(10), 1104-1112.
34. **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; 45:846–854. [PubMed: 17393509]

35. **Bugianesi, E., Rosso, C., and Cortez Pinto, H.** How to diagnose NAFLD in 2016. Journal of hepatology, (2016) 65(3), 643-4.
36. **Fallatah, H. I., Akbar, H. O., and Fallatah, A. M.** Fibroscan Compared to FIB-4, APRI, and AST/ALT Ratio for Assessment of Liver Fibrosis in Saudi Patients with Nonalcoholic Fatty Liver Disease. Hepatitis Monthly, (2016)16(7), e38346.
37. **Kumar R, Rastogi A, Sharma MK, et al.** Liver stiffness measurements in patients with different stages of nonalcoholic fatty liver disease: diagnostic performance and clinicopathological correlation Dig Dis Sci. (2013);58(1):265–74.
38. **Wong, V. W. S., Vergniol, J., Wong, G. L. H., et al.** Diagnosis of fibrosis and cirrhosis using liver stiffness measurement in nonalcoholic fatty liver disease. Hepatology; **(2010).** 51(2), 454-462.
39. **Boursier J, Vergniol J, Sawadogo A, et al** The combination of a blood test and FibroScan improves the non-invasive diagnosis of liver fibrosis. LiverInt; (2009). 29(10): 1507–1515.