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Original Research Article.

Association of Liver Histology with Sonological Grading and Liver Stiffness Measured by Fibroscan in Patients with NAFLD

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ABSTRACT

Background: Non-alcoholic fatty liver disease (NAFLD) is the term for a range of situations caused by a build-up of fat in the liver. It's usually seen in people who are obese.

Objective: In this study our main goal is to evaluate the association of liver histology with sonological grading and liver stiffness measured by Fibroscan in patients with NAFLD.

Methods: This cross-sectional study was done at Department of Hepatology, Bangabandhu Sheikh Mujib Medical University from July 2007 to June 2009. Total 45 patients of fatty liver disease were studied. All collected data was recorded in a structured questionnaire and was analyzed by SPSS version 20. Values were expressed either as mean ± SD or in frequency or in percentages.

Results: During the study majority of the patients were 30-50 years old (73.33 %), which indicates that the patients were somewhat younger and out of 45 of study population 21 (46.7%) patients was grade I fatty liver, 23(51.1%) were grade II fatty liver and only one (2.2%) was grade III fatty liver. This study also showed that correlation between fibroscan of liver and grading of fibrosis in liver histology reveals significant association.

Conclusion: Our results show a significant correlation between liver histology with sonological grading and liver stiffness measured by fibroscan and fibrosis stage in NAFLD patients. Further large-scale cohort study is recommended to validate these results.

Keywords: Nonalcoholic Fatty Liver Disease (NAFLD), Fibroscan, Fibrosis Stage.

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INTRODUCTION

Nonalcoholic fatty liver disease (NAFLD) is the common liver disease observed in the clinical practice of Hepatology, with a prevalence rate of 20-30 % in the Western population.The prevalence in general population of Bangladesh has not been reported. Ludwig and his colleagues first described this condition in 1980 in a series of patients whose liver histology mimicked alcoholic hepatitis and yet had no history of heavy alcohol use.

Nonalcoholic fatty liver disease represents a spectrum of liver diseases characterized mainly macro vesicular steatosis that occurs in the absence of alcohol consumption in amounts considered injurious to the liver. The hepatic histology can vary from isolated hepatic steatosis alone to steatohepatitis and are referred to as non-alcoholic fatty liver (NAFL) and non-alcoholic steatohepatitis (NASH) respectively. In addition to predominantly macro vesicular steatosis, the diagnosis of steatohepatitis also requires the additional presence of varying combinations of findings including cytological ballooning, Mallory's hyaline, scattered inflammation and pericellular. Although the exact etiology is not clear, it could possibly be a part of a metabolic syndrome associated with insulin resistance, diabetes, obesity and hypertension. Patients typically present with asymptomatic serum aminotransferase elevation of 2-3 times the normal. Symptoms may include fatigue and abdominal pain. Physical examination may show hepatomegaly. Liver biopsy is useful and effective as a prognostic indicator, but it is an invasive and costly tool to diagnose fatty liver. The imaging modalities most often used to identify hepatic steatosis is ultrasonography. Hyperechogenic liver indicates steatosis by ultrasonography.[1,2]

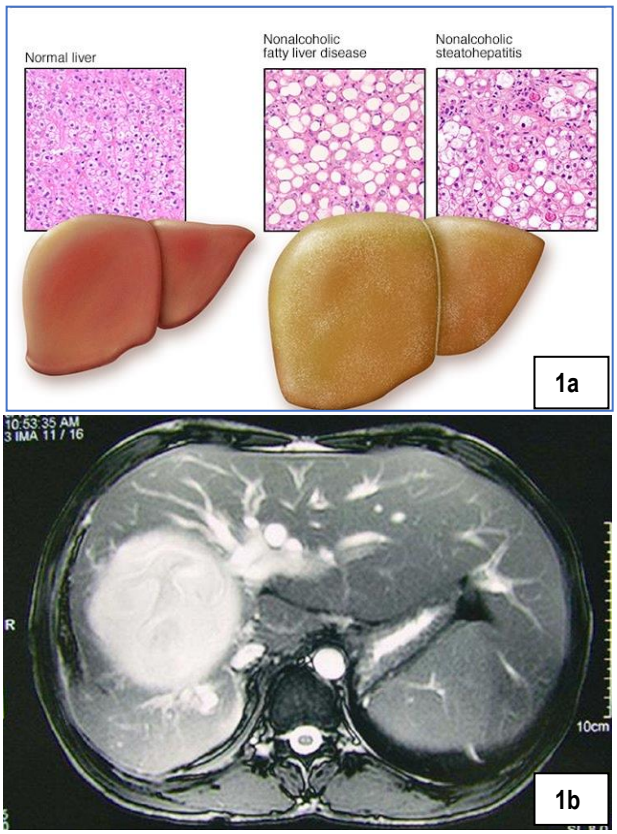

Figure 1a & 1b: Shows normal liver vs Nonalcoholic fatty liver disease and x-ray of Nonalcoholic fatty liver[3]

NAFLD has been recognized as a disease of affluent countries and considered basically a Western disease. However reports on NAFLD have now come from Asia-Pacific region as well. Prevalence rate of fatty liver of more than 30% have been reported from two studies from Taiwan and Japan. And a study from Shanghai, China recorded a rate of 20% in patients above the age of 50 years. There has indeed been great concern about the impact of this fast- growing disease in our part of the world, especially with the recognition that it is not necessarily a benign condition with the evolution in a proportion of cases to liver cirrhosis and cancer.[4]

The prevalence of overweight and obesity is increasing in Bangladesh especially in females due to a change in dietary habits and sedentary life style. In clinical practice, we frequently encounter bright echogenic liver on ultrasonography in Bangladesh. Many of those patients have elevated liver enzymes especially alanine aminotransferase (ALT). In this study our main goal is toevaluate the association of liver histology with sonological grading and liver stiffness measured by Fibroscan in patients with NAFLD.

OBJECTIVES

General Objective

- To evaluate the relation of liver histology with sonological grading and liver stiffness measured by Fibroscan in patients with NAFLD.

Specific Objective

- To measure sonological grading in patients with NAFLD.
- To evaluate the liver stiffness by fibroscan in patients with NAFLD.
- To identify degree of steatosis and fibrosis in patients with NAFLD by liver histology.
- To assess the relation between sonological grading and Fibroscan with histological changes in NAFLD.

METHODOLOGY

Study Type, Place and Period

- This study was a Cross sectional study; conducted at Department of Hepatology, Bangabandhu Sheikh Mujib Medical University from July 2007 to June 2009 where 45Patients of Fatty Liver Disease were studied.

Inclusion Criteria

- Patients with ultrasonographic evidence of fatty liver.

Method

- A patient presents with the features of Metabolic Syndrome or sonographic evidence of fatty liver was asked history and examined for evidence
- If the patient has features of NAFLD, Ultrasonography was done if not previous done.
- If fatty liver disease is present, the patient was preliminarily included in the study.
- Then, after explanation about the necessity of the investigations and about the study, his/her blood sample was drawn and sent to laboratory.
- Then ultrasonologal grading and liver elasticity measured by Fibroscan.
- After baseline assessment and informed written consent, liver biopsy of the patient was done by Tru-cut biopsy needle. The liver biopsy tissue sample will be sent for histopathological examination including NAS score by H&E stain and Mansson-Trichrom stain.
- All data was collected from structured questionnaire and will be analyzed by SPSS (version 10). P values <0.05 will be considered significant.
- Lastly, data was analyzed by Pearson correlation analysis. Data was expressed as mean ± standard deviation. Comparative analysis will be done by ANOVA test and Chai Square test.

RESULTS

In figure-2 shows Age distribution of patients where majority of our patients were 30-50 years old (73.33 %), which indicates that our patients were somewhat younger. Mean = (39.59± 9.14) years; range = 20-60 years.

In figure-3 shows gender distributions of the patients where out of 45 cases selected for study, 20(45%) were males and 25(55%) females giving a male- female ratio of 4:5.

In figure-4 shows BMI of patients (n=45) where Based on BMI of Asian population, the obesity status of the patients was defined. Nearly half (46.7 %) of the patients were obese stage I, 28.9 % was obese stage II, 17.8 % was overweight and only 6.7% was with normal BMI. The mean BMI was 27.79± 3.33 kg/ m^2. And the lowest and height BMI were 18.75 and 34.15.

In figure-5 shows Dyslipidaemic status of NAFLD patients (n=45) where Out of 45 patients, 38 (84.44%) had dyslipidaemia, 34 (75.55 %) exhibited triglyceride > 150mg/dl, 25 (55.55 %) had cholesterol > 200 mg/dl, and both cholesterol and triglyceride were high in 23 (51.11 %) patients.

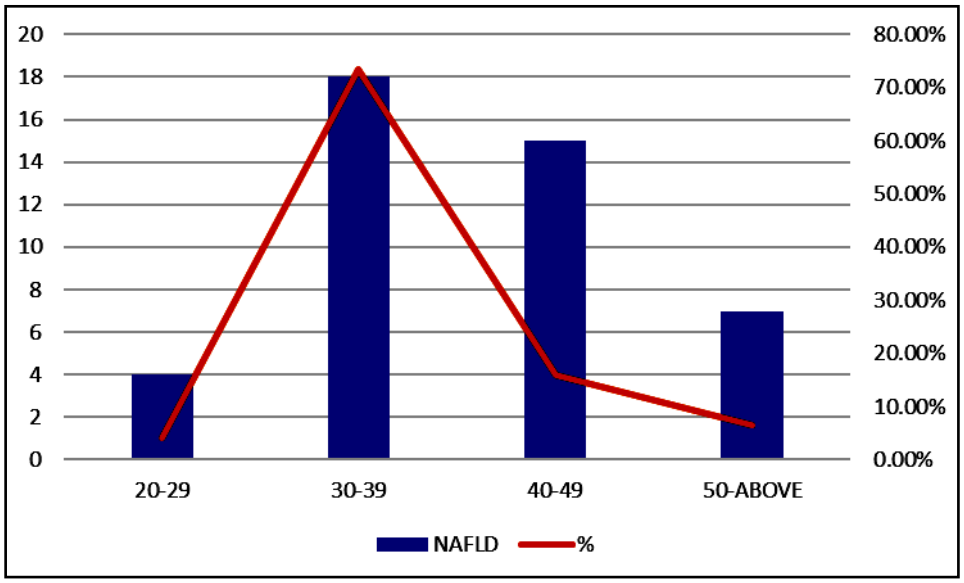

Figure 2: Age distribution of patients.

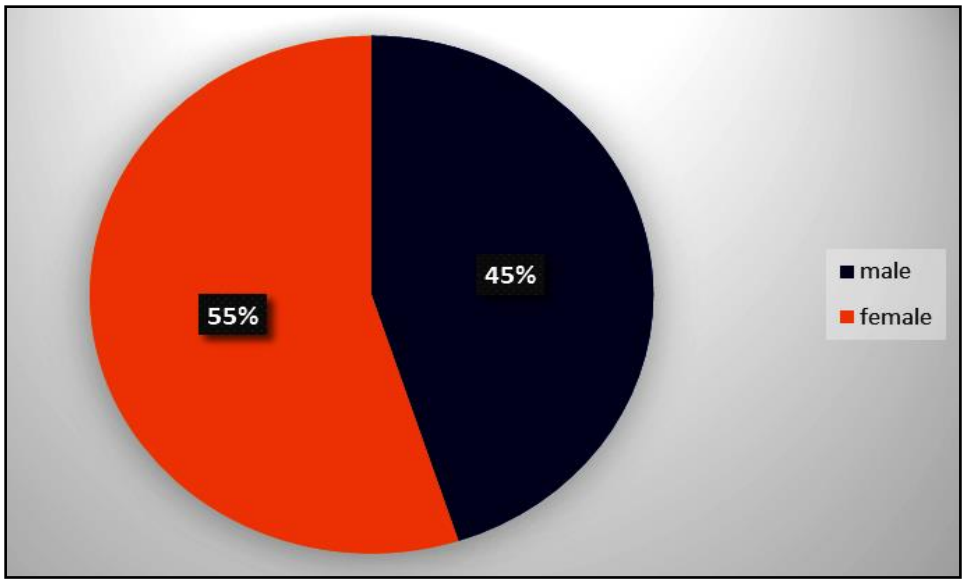

Figure 3: Gender distributions of the patients

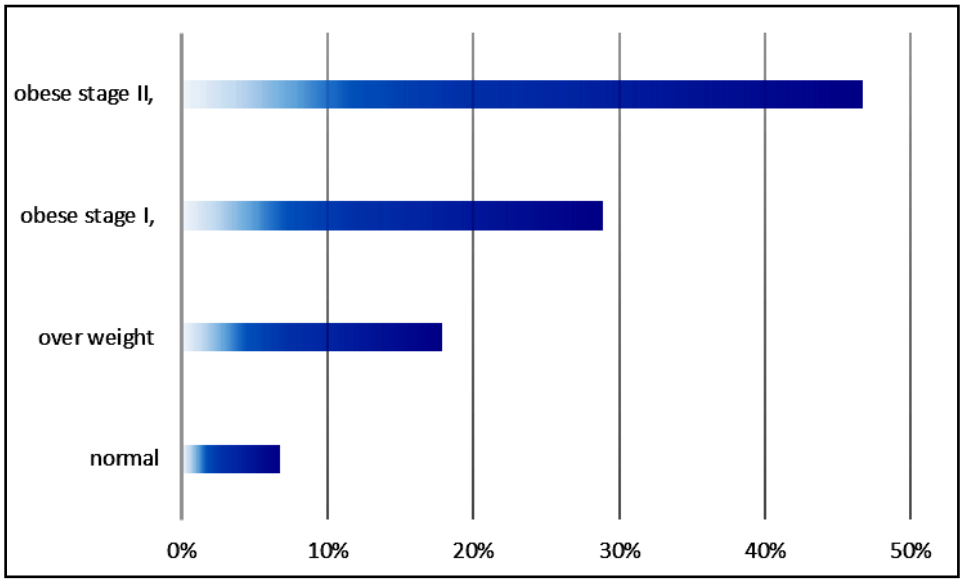

Figure 4: BMI of patients (n=45)

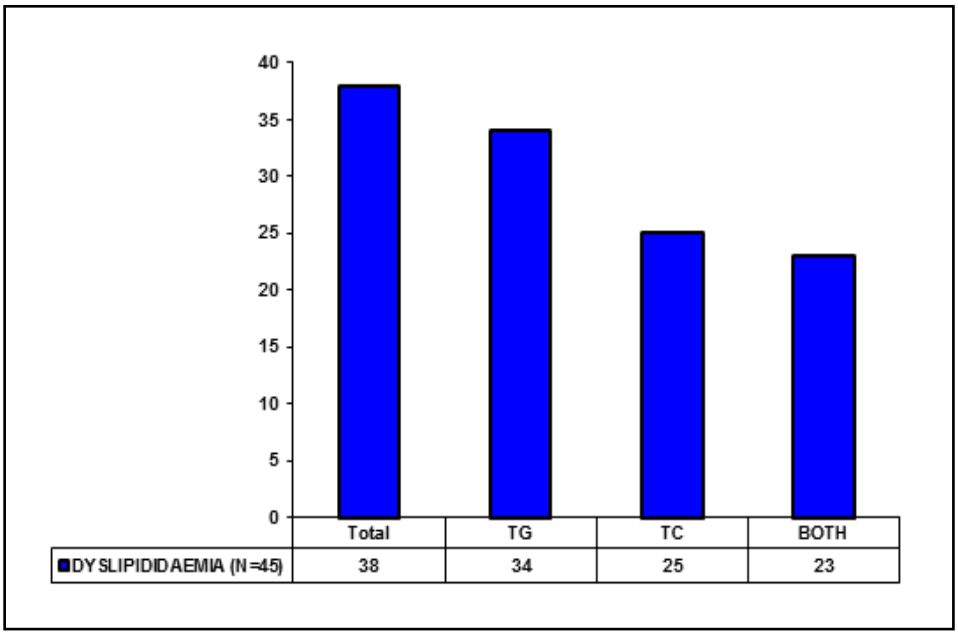

Figure 5: Dyslipidemia status of NAFLD patients (n=45)

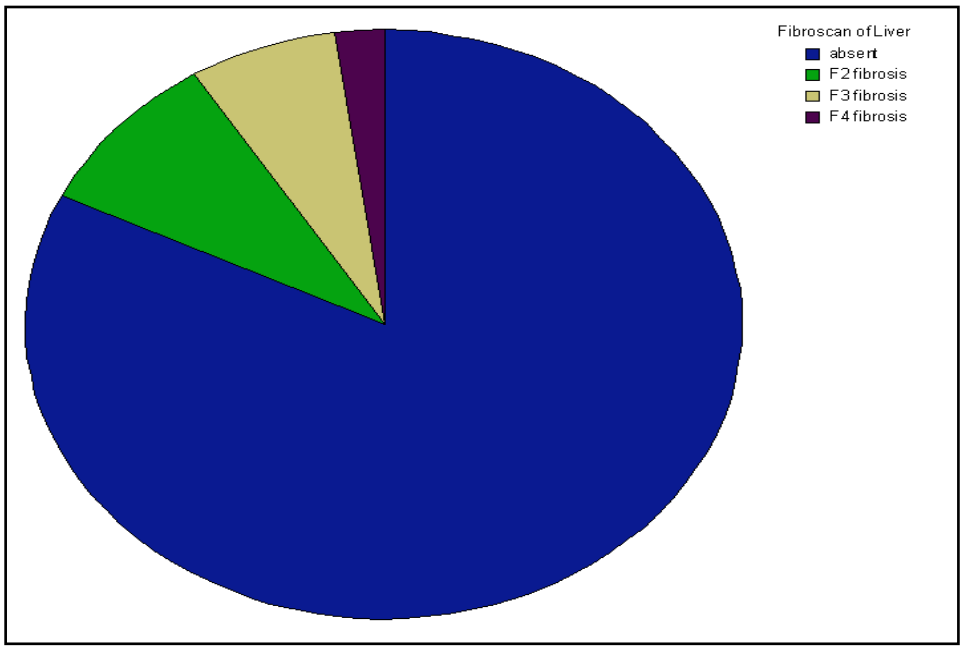

Figure-6: Liver elastography measured by Fibroscan of NAFLD patients (n=45).

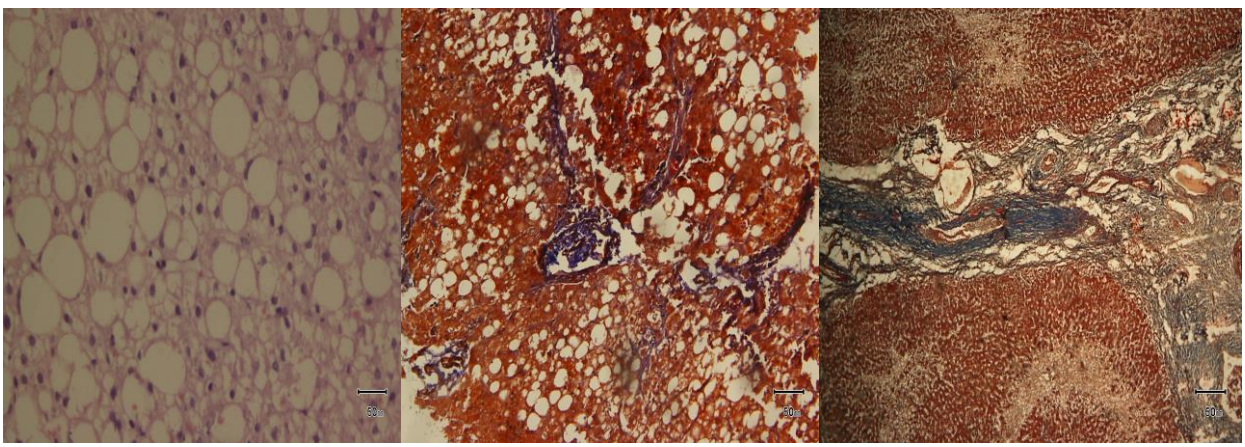

Figure 7a, 7b, 7c: Macrovesicular fatty change in NAFL (case no 13), Masson-Trichrom Stain show F2 fibrosis (case no 20), Masson-Trichrom Stain show F3 fibrosis (case no 34)

Table 1: Ultrasonological grading of NAFLD patients

Variable	n	%
Grade I fatty liver	21	46.7%
Grade II fatty liver	23	51.1%
Grade III fatty liver	1	2.2%

Table 2: Histological evaluations of NAFLD patients (n=45)

Parameter	Number	Percentage
Grade of steatosis (0-3)		
Grade 0	00	00
Grade I	13	28.9
Grade II	21	46.7
Grade III	11	24.4
Stage of fibrosis (0-4)		
Stage o	15	33.3
Stage 1	15	33.3
Stage 2	6	13.3
Stage 3	7	15.6
Stage 4	2	4.4
Ballooning degeneration		
Absent	3	6.7
Present	42	93.3
Lobular inflammation		
Absent	7	15.6
Mild	27	60.0
Moderate	10	22.2
Severe	1	2.2
Diagnosis		
Definite NASH	14	31.11
Probable NASH	28	62.22
Not NASH	03	6.7

Table 3: Correlation between histologic grading steatosis with baseline variable of NAFLD patients

	Variable	r value	p value
Grading of steatosis	**Age**	-0.108	0.481
	Sex	-0.177	0.244
	Weight	0.079	0.606
	Height	0.313	0.036#
	BMI	-0.147	0.337
	Waist circumference	-0.236	0.119
	DM	0.259	0.086
	HTN	0.033	0.832
	ALT	0.185	0.225
	AST	-0.076	0.695
	Alk. Phosphatase	0.115	0.451
	S. Bilirubin	-0.047	0.761
	S.cholesterol	-0.026	0.865
	Serum TG	-0.012	0.938

Table 4: Correlation between histological grading of fibrosis with baseline variable of NAFLD patients

	Variable	r value	p value
Grading of fibrosis	**Age**	0.321	0.032
	Sex	-0.004	0.978
	Weight	0.162	0.288
	Height	0.108	0.479
	BMI	0.100	0.513
	Waist circumference	0.007	0.965
	DM	0.502	0.000##
	HTN	0.293	0.051
	ALT	0.026	0.867
	AST	-0.078	0.613
	Alk. Phosphatase	0.212	0.161
	S. Bilirubin	0.095	0.537
	S.cholesterol	-0.028	0.854
	Serum TG	0.137	0.370

Table 5: Summaries of liver elasticity with different grade of fibrosis

Group (i)	Group (j)	Mean difference (I –j)	P value
F0	F2	.00	1.000
	F3	-1.57	.000
	F4	-3.00	.000
F2	F0	.00	1.000
	F3	-1.57	.005
	F4	-3.00	.000
F3	F0	1.57	.000
	F2	1.57	.005
	F4	-1.43	.197

Data were analyzed using ANOVA

In table-1 shows Ultrasonological grading of NAFLD patients where Out of 45 of study population 21 (46.7%) patients was grade I fatty liver, 23 (51.1%) were grade II fatty liver and only one (2.2%) was grade III fatty liver.

In figure-6 shows Liver elastography measured by Fibroscan of NAFLD patients where the available Fibroscan machine of our country cannot detect F1 fibrosis. Based on cut of value of this study 8.9 % were F2 fibrosis, 6.7% were F3 fibrosis, 2.2% were F4 fibrosis and 82.2% had no fibrosis.

Histological evaluation: Steatosis and associated inflammation where Histological study reveals that 28.9% (13) patients had mild (< 33%) steatosis and 46.7 % (21) patient had moderate (33-66%) steatosis and rest 24.4% (11) patients had severe (>66%) steatosis. All the 45 patients exhibited fatty change with macrovesicular type being predominant. The ballooning degeneration was observed in 93.3% (42) of cases. Mild degree of lobular inflammation was found in 60% (27) cases, moderate and severe degree of lobular inflammation was observed 22.2% and 2.2% cases respectively. Only 7 (15.6%) cases are free from lobular inflammation.

In table-2 shows Histological evaluation where Histological evaluation based on Kleiner et al. 2005, demonstrates that 28.9% (13) patients had mild steatosis and 46.7 % (21) patient had moderate (33-66%) steatosis and rest 24.4 (11) patients had severe (>66%) steatosis. In terms of staging of fibrosis in liver, 33.3 % (15) patients had stage 1 fibrosis, 13.3 % (6) patients had stage 2 fibrosis, 15.6 % (7) patients had stage 3 fibrosis, 4.4%(2) patients had stage 4 fibrosis, rest 33.3 % (15) patients did not have any fibrosis. In figure-7a, 7b, 7c shows Macrovesicular fatty change in NAFL, Masson-Trichrom Stain show F2 fibrosis, Masson-Trichrom Stain show F3 fibrosis of the patients.

In table-3 shows Correlation between histologic grading of steatosis with baseline variable of NAFLD patients (n = 45) where none of the variables except height could correlate with grade of steatosis in the study population.

In table-4 shows Correlation between histological grading of fibrosis with baseline variable of NAFLD patients (n = 45) where none of the variables except DM could correlate with grade of fibrosis in the study population.

In table-5 shows Summaries of liver elasticity with different grade of fibrosis where One-way ANOVA was done for liver elasticity with different grade of fibrosis and significant for p =0.05. There was strong correlation of liver elasticity in cases of F0 with F3 and F4 fibrosis (p = < 0.05), F2 with F3 and F4 (p =< 0.05), F3 with F0 and F2 (p =< 0.05).

In figure-8 shows Correlation between Fibroscan of liver and grading of fibrosis in liver histology where Correlation between Fibroscan of liver and grading of fibrosis in liver histology reveals significant association between these two variables (Pearson correlation .581, P = .000).

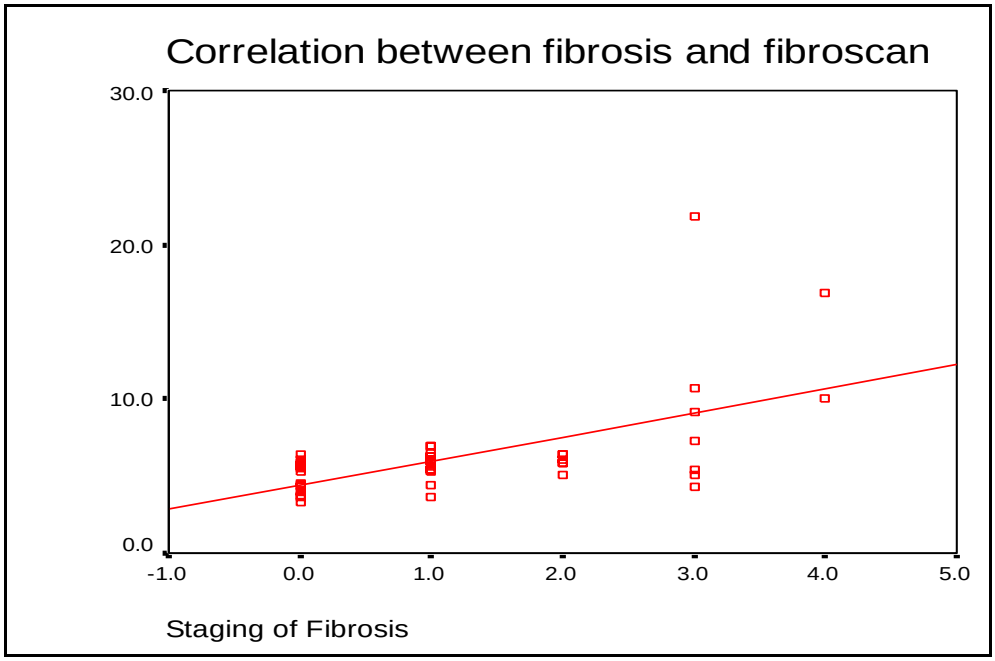

Figure 8: Correlation between Fibroscan of liver and grading of fibrosis in liver histology

DISCUSSION

Nonalcoholic fatty liver disease (NAFLD) is an increasingly recognized medical condition that was thought to be a benign disease. However, studies on the natural history of NAFLD have shown that it may evolve into severe hepatic fibrosis and subsequently liver cancer.[5]The clinical implications of NAFLD are derived mostly from its common occurrence in general population, especially in obese individuals, and its potential to progress to cirrhosis and liver failure. In Asia-Pacific region, a prevalence of NAFLD ranging from 20-30% has been reported.[6]

In outpatient clinics of Bangladesh, we encounter many patients having fatty liver on ultrasonography and/ or elevated liver enzymes, especially who are diabetic and dyslipidemia. If hepatitis B and hepatitis C infection and history of alcohol consumption are excluded, these patients are presumed to have NAFLD. As other forms of chronic liver disease especially autoimmune hepatitis and Hemochromatosis are rare in our country and socioeconomic status of the population is low, we did not go for investigations to exclude these diseases.

As histopathological examination reveals the confirmed diagnosis of NAFLD, we performed liver biopsy of all our patients. Nearly thirty two percent of our patients had definite NASH and sixty two percent had probable NASH this is remarkable finding and is probably due to precise patient selection in our study. Moreover, most of our patients were obese (75.6 %), dyslipidemia (84.44%), diabetic (20%), hypertensive (22.2%), which are establish risk factors for NAFLD.

Majority of our patients were 30-50 years old (73.33 %), which indicates that our patients were somewhat younger than those in previous studies.[7] Our study also shows female (55.6%) predominance, which corresponds with study conductedby others.[5] Consistent with the published literatures, we found that almost all of our patients (93.33 %) were either overweight (17.8%) or, obese (75.6 %) with a mean BMI of 27.79.

Out of 45 patients, 38 had dyslipidemia, 34 (75.55 %) exhibited triglyceride > 150mg/dl, 25 (55.55 %) had cholesterol > 200 mg/dl, and both cholesterol and triglyceride were high in 23 (51.11 %) patients. These higher percentages of hyper triglyceridemic are consistent with study conducted by other articles.[8]

Out of 45 of study population 21 (46.7%) patients was grade I fatty liver, 23(51.1%) were grade II fatty liver and only one (2.2%) was grade III fatty liver, that was similar to study conducted by other.[8]

The proposed cut-off value by one report thatof liver stiffness to determination of liver fibrosis in NAFLD are F1 = 5.90, F2 = 6.65, F3 = 9.80, and F4= 17.5.[9] The available Fibro scan machine of our country cannot detect F1 fibrosis, as there is no cut-off value for F1 fibrosis, the value for F2= 6.90, F3 = 10, F4 = 18.83, which is more or less similar to other study. [9]Based on cut of value of this study 8.9 % were F2 fibrosis, 6.7% were F3 fibrosis, 2.2% were F4 fibrosis and 82.2% had no fibrosis.

The fatty changes of NAFLD are both macro vesicular and micro vesicular in type. All the 45 patients exhibited fatty change with macro vesicular type being predominant. The ballooning degeneration was observed in 93.3% (42) of cases. Mild degree of lobular inflammation was found in 60% (27) cases, moderate and severe degree of lobular inflammation was observed 22.2% and 2.2% cases respectively. Only 7 (15.6%) cases are free from lobular inflammation.

Histological evaluation based on one report demonstrates that 28.9% (13) patients had mild steatosis and 46.7 % (21) patient had moderate (33-66%) steatosis and rest 24.4% (11) patients had severe (>66%) steatosis.[10]

In terms of staging of fibrosis in liver, 33.3 % (15) patients had stage 1 fibrosis, 13.3 % (6) patients had stage 2 fibrosis, 15.6 % (7) patients had stage 3 fibrosis, 4.4% (2) patients had stage 4 fibrosis, rest 33.3 % (15) patients did not have any fibrosis. Regarding diagnosis 84.4 % of study population are NASH only 15.6% are NAFL that were not consistent with western study where only 10-20% were NASH. These are alarming for our general population, as 3-5% of NASH may complicate with cirrhosis in 20 years. But this high percentage of NASH might be due to sampling error.

Correlation between Ultrasonological grading and grading of steatosis in Histology where Out of 45 of study population 21 (46.7%) patients was grade I fatty liver, 23(51.1%) were grade II fatty liver and only one (2.2%) was grade III fatty liver. Liver histology demonstrates that 28.9% (13) patients had mild steatosis and 46.7 % (21) patient had moderate (33-66%) steatosis and rest 24.4% (11) patients had severe (>66%) steatosis. Correlation between Ultrasonological grading and grading of steatosis in liver histology reveals strong association between these two variables (Pearson correlation .402, p = .006). Correlation between ultrasonological grading and grading of steatosis in liver histology reveals strong association between these two variables (r = .402, p = .006).

One-way ANOVA was done for liver elasticity with different grade of fibrosis and significant for p =0.001. There was strong correlation of liver elasticity in cases of F0 with F3 and F4 fibrosis (p = < 0.05), F2 with F3 and F4 (p =< 0.05), F3 with F0 and F2 (p =< 0.05). A study conducted by one report shows the median value (95% CI) of the liver stiffness in relation to the severity of liver fibrosis as assessed in liver biopsy specimens in patients with NAFLD.[9] The results of the analysis revealed a stepwise increase of liver stiffness with increasing histological severity of liver fibrosis (p < 0.0001 by Kruskal-Wallis test). Our result also shown the correlation between liver elasticity measured by Fibroscan and grading of fibrosis in liver histology reveals significant association between these two variables (Pearson correlation .581, p = .000) with a stepwise increase of liver stiffness with increasing histological severity of liver fibrosis.

CONCLUSION

Our results show a significant correlation between liver histology with sonological grading and liver stiffness measured by Fibroscan and fibrosis stage in NAFLD patients, as confirmed by the results of liver biopsy, the gold standard for evaluation of the severity of liver pathology in patients with NAFLD. Thus, measurement of the liver stiffness is a non- invasive, clinically useful method for predicting the severity of liver fibrosis in patients with NAFLD. However, further large-scale prospective study is required to find more precise correlation among different noninvasive diagnostic tools for finding different stage of NAFLD.

REFERENCES

1. Shira Zelber-Sagi, DoritNitzan- Kaluski, Zamir Halpern, Ran Oren. Prevalence of primary non-alcoholic fatty liver disease in a population based study. Liver International 2006; 26: 856-863.

2. Ratziu V, Poynard T. NASH: a hidden and silent fibroser finally revealed? J. Hepatol. 2005; 42: 12–14.

3.https://www.google.com/search?q=Nonalcoholic+fatty+liver+disease+(NAFLD&rlz=1C1GGRV_enBD810BD810&source=lnms&tbm=isch&sa=X&ved=0ahUKEwicuN-F_NPfAhUJr48KHb-OBmgQ_AUIDygC&biw=1366&bih=662#imgrc=_

4. Stefano Bellentani, Mariano Marino. Epidemiology and natural history of non-alcoholic fatty liver disease (NAFLD). Annals of Hepatology 2009; 8: S4-S8

5. Powell EE, Cookesly EGE et al. The natural history of steatohepatitis: a follow- up study of forty- two patients for upto 21 years. Hepatology 1990; 11: 74-80.

6. Laurent Castera, Xavier Forns, Alfredo Alberti. Noninvasive evaluation of liver fibrosis using transint elastography. Journal of Hepatology 2008; 48: 835-847.

7. Langman G, Hall PM, Todd G. Role of non-alcoholic steatohepatitis in methotrexate-induced liver injury. J. Gastroenterol. Hepatol. 2001; 16: 1395–1401.

8. P. Angulo, J.M. Hui and G. Marchesini et al., The NAFLD fibrosis score: a noninvasive system that identifies liver fibrosis in patients with NAFLD, Hepatology 2007; 45: 846–854.

9. Yoneda M, Fujita K et al. Transient elastography in patients with non-alcoholic fatty liver disease (NAFLD).Gut 2007; 56: 1330–31.

10. Kleiner et al. Design and validation of a histological scoring system for nonalcoholic fatty liver disease. Hepatology. 2005;41:1313-21.

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