

A Light Microscopic Study of Steatosis in Non Alcoholic Fatty Liver Disease and its Correlation with Fibrosis

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ABSTRACT

Objective: To assess the steatosis by microscopic study of liver biopsies in patients labeled as fatty liver on ultrasound and study its association with fibrosis.

Study Design: Case series study.

Place and Duration of Study: This study was conducted at the Gastroenterology unit of Hayatabad Medical Complex and Histology laboratory of Khyber Medical College and Histopathology Lab of CMH, Peshawar from June 2016 to December 2016.

Materials and Methods: Ten cases diagnosed as fatty liver on ultrasound were selected for liver biopsy followed by histological study. They were divided in to two groups. Group 1: between the ages of 45-65 years, obese having diabetes and hyperlipidemia. Group 2: 25-44 years, overweight, diabetic and hyperlipidemic.

Results: Histological examination clearly indicates that 60% of total cases developed grade 1 steatosis, while 30% and 10% of cases revealed grade 2 and 3 respectively. The results revealed highly significant association between the two groups and that of steatosis grade after application of Chi square. Azonal/panacinar steatosis was found in 60% of total cases while 10% and 30% of cases were reported with zone 1 and 3 steatosis. Only single case did not develop fibrosis although, the rest of patients revealed various stages of fibrosis. Stage 4 (cirrhosis) could not be recorded in any of the case.

Conclusion: Mix pattern of steatosis is present, with higher incidence of steatosis in old and obese patients having diabetes and hyperlipidemia. Furthermore, there exists no substantial correlation between steatosis grade and fibrosis stage.

Key Words: Steatosis, Fibrosis, Fatty liver.

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INTRODUCTION

Non-alcoholic fatty liver disease NAFLD is one of the most widespread condition, affecting men, women and children^{1,2}. NAFLD is now recognized as a clinic-pathological entity that involves a wide spectrum of pathological conditions extending from accumulation of fat in hepatocytes to various degree of inflammation and fibrosis^{3,4,5}. The fibrosis ultimately leads to cirrhosis and hepatocellular carcinoma^{6,7}. The most common risk factors for development of NAFLD are obesity, diabetes and hyperlipidemia^{8,9}.

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In Pakistan the incidence of NAFLD is higher in type-2 diabetic patients and is found in both sexes with higher incidences in females¹⁰.

The possible mechanism for the pathogenesis of fatty liver is a net retention of lipid in liver cells, commonly in form of triglycerides. The primary metabolic abnormalities resulting in lipid deposition are not clear, but the reason could be alteration in the various pathways of hepatic lipid metabolism resulting from insulin resistance¹¹. The type of steatosis that predominates in adults and in children is macrovesicular steatosis having a single fat droplet occupying the entire cytoplasm resulting in nuclear eccentricity¹². It may be found as several smaller and well defined droplets in the cell cytoplasm. It is not uncommon for there to be a mixture of large single fat globule and multiple small droplets. Clusters of hepatocytes with delicate intracytoplasmic septation due to microvesicular steatosis may also be present¹³.

Among the diagnostic tools commonly applies for diagnosis of fatty liver (steatosis) are ultrasonography and liver biopsy^{14,15}. Examination of liver biopsies by skilled pathologist is vital diagnostic complement. Moreover, liver biopsy helps in providing essential

prognostic information. In the view of above mentioned facts, we carried out a study to assess the morphometric and microscopic features of steatosis along with study of other histological features specifically fibrosis, which is useful in determining the risk of progression to more advanced condition.

MATERIALS AND METHODS

The study was conducted on ten patients labeled as fatty liver on ultrasound. Percutaneous liver biopsies were performed on outdoor patient in Gastroenterology unit of Hayatabad Medical Complex Peshawar. Tissues processing, morphometry and histological analysis were performed at Histology laboratory of Khyber Medical College and Histopathology lab of CMH, Peshawar June 2016 to December 2016.

Patients selected for study were between 25 to 65 years, having serum ALT level more than 45u/l and BMI more than 25. They had hemoglobin, TLC, platelet count, clotting factors and serum bilirubin within normal limit. The patients with history of alcohol intake, advance liver disease and systemic disorders were excluded from the study. The patients were categorized into two groups on the basis of age, BMI, certain hematological and biochemical findings.

Group 1: Comprised of 07 cases between the ages of 45 to 65 years. These patients were obese, diabetic and having high levels of cholesterol & triglycerides.

Group 2: Comprised of 03 cases between the ages of 25 to 44 years. They were overweight, with no history of diabetes, hypercholesterolemia and hypertriglyceridemia.

Patients were briefed to avoid aspirin, NSAID to reduce the risk of bleeding after biopsy. Fresh blood investigations were sent to rule out any bleeding disorders. Patients were kept empty stomach for at least 12 hours prior to the procedure. All the patients were informed about the complication of procedure. Every case was given injection diazepam intravenously to keep them relaxed during the procedure. Percutaneous liver biopsy was performed under local anesthesia. The size of specimen was between 1 and 1.5cm in length and 1 mm in diameter. For assessment of various hepatic diseases, the appropriate size of specimen should be 1.5 cm in length¹⁶. The specimens were fixed in formalin followed by tissue processing. Two tissue slides of every case were prepared in serial order and following staining methods were applied.

- Hematoxylin and Eosin for routine microscopy and demonstration of fat globules.
- Masson's Trichrome for study of fibrosis.

Stained sections were studied under light microscope. The morphometric measurements were taken by means of occlusometer. Histological staging and grading was done according to Histological Scoring System for NAFLD¹⁷.

Statistical analysis was done using SPSS version 16. The statistical results were presented as mean and standard deviation. The two-tailed Student's-t test and Chi-Square test were carried out. The 95% confidence interval was taken for the study and p value < 0.05 was considered as statistically significant.

RESULTS

Morphometric Parameters: The morphometry of fat globules was done under 40 × objective. Both macrovesicular and microvesicular steatosis were observed in group 1 and group 2. Detail of results is given in table No: 1.

Table No.1: Comparison of mean morphometric parameters of Group 1 and Group 2.

parameters	Group 1 (n=7)		Group 2(n=3)		P value
	Mean (µm)	SD	Mean (µm)	SD	
Macrosteatotic fat globule size	23.24	4.59	22.33	5.55	<0.793
Microsteatotic fat globule size	8.31	4.60	5.98	0.49	<0.422

Scoring of Steatosis and Fibrosis: Scoring and grading of steatosis and fibrosis was done under 4× and 10× objectives. Details of results are depicted in table 2 and 3.

Table No.2: Showing grade and location of steatosis of group 1 and 2.

Group	Steatosis Grade			Steatosis Location		
	1 5%-33%	2 >33%-66%	3 >66%	Zone 3	Zone 1	Azonal/panacinar
1	30%	20%	10%	20%	10%	50%
2	30%	10.0%	0.0%	10%	0.0%	10%
Chi-Sq	4.27			1.43		
p-value	0.001			0.481		

There is substantial association (< p 0.05) between two groups in that of steatosis grade, whereas there exists no significant correlation in that of steatosis location.

Table No.3: To show Correlation of steatosis grading and stage of fibrosis.

Case No:	Steatosis Grade	Fibrosis Stage
1	1(5%-33%)	1C (Portal/Periportal)
2	1 (5%-33%)	1C (Portal/Periportal)
3	3 (>66%)	1(Perisinusoidal/periportal)
4	1 (5%-33%)	1C (Portal/Periportal)
5	1 (5%-33%)	1(Perisinusoidal/periportal)
6	2 (33%-66%)	3 (Bridging fibrosis)
7	2 (33%-66%)	0 (None)
8	1 (5%-33%)	1(Perisinusoidal/periportal)
9	2 (33%-66%)	1C (Portal/Periportal)
10	1 (5%-33%)	1C (Portal/Periportal)

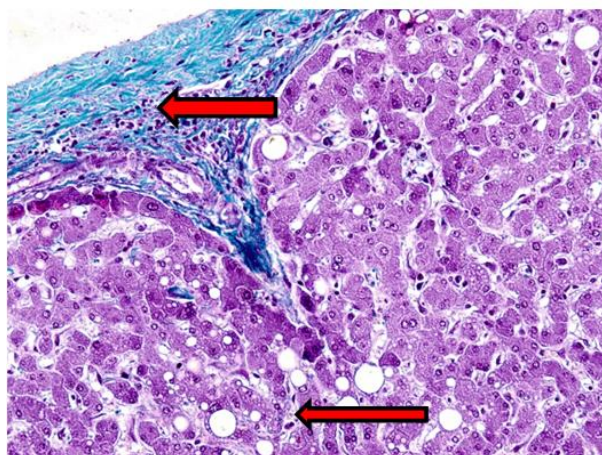


Figure No.1: Photomicrograph of Masson's Trichrome stained 5 μ thick section of liver showing fibrosis and steatosis with macrosteatotic, microsteatotic cells (40 \times).

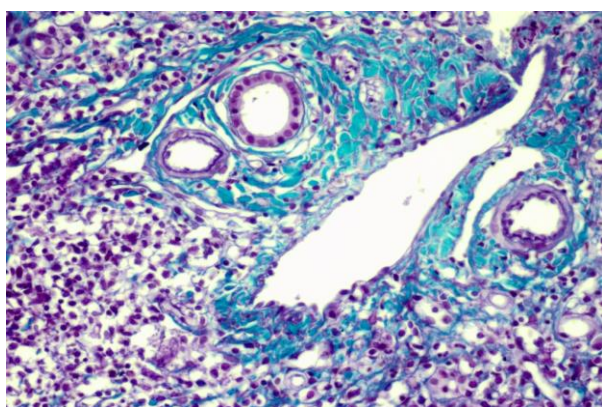


Figure No.2: Photomicrograph of Masson's Trichrome stained 5 μ thick section of liver showing portal fibrosis (40 \times).

DISCUSSION

The incidence of NAFLD is growing rapidly worldwide in association with the overall increase in type 2 diabetes mellitus and obesity^{18,19}. The spectrum of NAFLD is extending from simple steatosis to NASH and finally fibrosis²⁰. Present study was designed to evaluate the steatosis by microscopic study of liver biopsies in patients labeled as fatty liver on ultrasound and to study its correlation with fibrosis. Furthermore, the relationship of steatosis with age, BMI, diabetes and hyperlipidemia were also considered. Histological grading of steatosis and fibrosis were performed to preclude hazards of steatosis by timely diagnosis. In the present study, results of the morphometric analysis (size of macrosteatotic and microsteatotic fat droplets) match with findings documented in one study on chronic hepatitis C²¹. Regarding steatosis grade, it was found that majority of cases revealed grade 1 and 2 steatosis as compared to grade 3, which is in accordance with the study of Brunt et al. (1999)²². The

patients of group 1 were noted with high grades of steatosis whereas low grade was noticed in group 2, indicating higher occurrence of steatosis in old and obese patients with history of diabetes and hyperlipidemia. Panacinar steatosis was one of a prominent finding, which is in line with the observation of Zubair A and Jamal S (2009), who carried out their study on morphometry of fatty changes in patients with hepatitis C infection. Location of steatosis showed no significant relation between group 1 and 2. This indicates that age, BMI, serum lipid profile and blood sugar level has no effect on steatosis location.

Both macrosteatosis and microsteatosis (mixed pattern of steatosis) was appreciated in majority of the cases. It was noted that macrosteatosis was fairly prominent feature of steatosis. These findings were in accordance with the observations of Brunt et al²² but did not correlate with the study of Zubair A and Jamal S, who reported a high incidence of microsteatosis in chronic hepatitis C infection²¹. This indicates that the pathogenesis of steatosis in hepatitis C infection and NAFLD might be different.

Varying stages of fibrosis was observed in 90% of patients. Stage 1 fibrosis was noted in most of the members, while bridging fibrosis was found only in single case. Furthermore, cirrhosis was not observed in any of the cases. No remarkable association was observed between stage of fibrosis and grades of steatosis. Only one case revealed bridging fibrosis (slightly advanced phase of fibrosis) was having low score of steatosis. These findings were in accordance with the observation of Brunt et al (2007), who conducted their study on pathology of fatty liver disease²³.

CONCLUSION

Our study demonstrates that cases diagnosed as fatty liver on ultrasound do have some degree of steatosis on microscopic examination of biopsy specimen. Mix pattern of steatosis is observed with higher incidences of steatosis in older, obese patients having diabetes and hyperlipidemia. Macrosteatosis is predominant feature in majority of the cases. Furthermore, there exists no significant correlation between the stage of fibrosis and grades of steatosis.

Author's Contribution:

Concept & Design of Study:	Hamid Ali Khan
Drafting:	Shabnum Aamir & Munila Shabnum Khattak
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Final Approval of version:	Shabnum Aamir & Hamir Ali Khan

Conflict of Interest: The study has no conflict of interest to declare by any author.

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