Document heading doi: 10.21276/apjhs.2019.6.2.9 **Research Article**

Impact of the size of liver biopsies on the evaluation of chronic liver disease –a study by artificial sampling

Naheena Bashir[1] ,Zubaida Rasool[2] ,Duri Mateen[1]

[1]MD Pathology,Senior Resident,Department of Pathology,SKIMS,Soura,Srinagar
[2]MD Pathology,Additional Professor, Department of Pathology,SKIMS,Soura,Srinagar
Received: 28-01-2019 / Revised: 27-03-2019 / Accepted: 30-04-2019

Abstract

Chronic liver disease is a disease process of the liver that involves progressive destruction and regeneration of the liver parenchyma leading to fibrosis and cirrhosis. We first evaluated the 70 percutaneous liver biopsies from patients with a clinically established diagnosis of chronic hepatitis and chronic liver diseases of various etiologies. All biopsies were obtained through percutaneous approach using 16 F biopsy gun (manufactured by Bard) in adults and 18 F in children. . The mean biopsy size was 1.8cm ± 0.20 and a median size was 2cm. Out of the 70 liver biopsies, a total of 24 (34.2%) liver biopsies were less than 1.5cm in size, and 46(65.7%) were ≥ 1.5cm.

Keywords:biopsy,chronic,liver,disease

Introduction

Chronic liver disease is a disease process of the liver that involves progressive destruction and regeneration of the liver parenchyma leading to fibrosis and cirrhosis. Chronic liver disease (CLD) is the major cause of morbidity and mortality worldwide. More recently, the increasing prevalence of obesity and the metabolic syndrome has resulted in increasing incidence of cirrhosis secondary to nonalcoholic fatty liver disease (NAFLD), especially in developed countries[1]. Viral hepatitis is the most common cause of acute and chronic liver disease in the world with over half the world's population exposed to the different hepatotropic viruses[2].Approximately 18% of patients with auto-immune liver disease present with features characteristic of a second auto-immune hepatobilliary disease, usually Primary Biliary Cirrhosis (PBC) or Primary Sclerosing Cholangitis (PSC)[3]. Inspite of recent advances in non-invasivetechniques, liver biopsy continues to be the gold standard method in evaluating chronic hepatitis and fibrosis[4].

Correspondence
Dr. Naheena Bashir
Senior Resident,Department of Pathology,
SKIMS,Soura,Srinagar
E Mail: naheena786@gmail.com

Materials & methods

This was a prospective study carried out over a period of 2 years extending from June 2013 to June 2015.
We first evaluated the 70 percutaneous liver biopsies from patients with a clinically established diagnosis of chronic hepatitis and chronic liver diseases of various etiologies. All biopsies were obtained through percutaneous approach using 16 F biopsy gun (manufactured by Bard) in adults and 18 F in children. All samples ≥ 1.5cm were reviewed blindly at two different sessions, changing the length of the sample with the aid of opaque paper tape [Artificial Sampling, (AS)]so that only specific specimen lengths (5 mm, 10mm) were visible. All the parameters including the diagnosis, scoring and staging of the various diseases were repeated by reducing the length of the specimen from ≥1.5 cm to 1 cm (AS1) and further to 0.5cm (AS2). The Ishak's scoring system[5] was used for grading and staging of chronic hepatitis. For NASH, Kleiner's score was used and staging in primary biliary cirrhosis was done by Ludwig's method[6,7].

Statistical analysis

For assessing the impact of length on the scoring and fibrosis, agreement between the 5-mm, 10-mm, with the 15-mm or greater length was estimated by using weighted κ statistics. The statistical evaluation was based on the prerequisite that the biopsy length of 1.5cm or greater was the gold standard, and that smaller biopsy specimens were compared with that

gold standard. The interpretation of the magnitude of the weighted κ score is that greater than 0.75 signifies excellent agreement, the closer to 1.0 the greater the agreement; and less than 0.40 indicates poor agreement[8].

Results

. The mean biopsy size was 1.8cm ± 0.20 and a median size was 2cm. Out of the 70 liver biopsies, a total of 24 (34.2%) liver biopsies were less than 1.5cm in size, and 46(65.7%) were ≥ 1.5cm.

Histopathological Diagnosis

Out of the 70 liver biopsies,27(38.6%) were reported as chronic hepatitis which was the most common histopathological diagnosis made, followed by PBC which was seen in 6(8.5%) biopsies. The other histopathological diagnoses made included cirrhosis in 6(8.5%), cholestatic hepatitis in 5(7.2%), non-alcoholic steatohepatitis (NASH) in 6(8.5%), glycogen storage disease in 5(7.2%),AIH-PBC overlap in 4(5.7%), AIH in 3(4.28%),Wilson's disease in 2(2.9%) biopsies. 1(1.4%) case reported was uncertain for NASH. Because of inadequate size, diagnosis was not possible in 5(7.2%) liver biopsies.12 out of 13 biopsies of AIH/PBC were from female patients. The other diseases however showed no particular gender distribution.

Impact of length of liver biopsy on the number of complete portal tracts

Artificial sampling at 1cm could be done in 46 liver biopsies (which were ≥ 1.5cm in size). The number of complete portal tracts in these biopsies ranged from of a minimum of 1 to a maximum of 6 and overall a decrease in the number of CPTs was observed in 41(93.1%) biopsies.In 5(10.8%) biopsies, the number of complete portal tracts remained the same.The mean number of CPTs reduced from 7.07 ± 1.73 to 4.46 ± 1.20.(**Chart 1**). **Table 1** shows comparison of complete portal tracts from liver biopsies 1.5cm or longer with 1cm and 0.5cm.Artificial sampling at 0.5 cm could be done in a total of 46 cases (liver biopsies with a size greater than 1.5cm). With the exception of 1(1.6%) liver biopsy, all other biopsies (45/97.8%) showed a decrease in the number of complete portal tracts with the mean number of CPTs decreasing from 7.07 ± 1.73 to 1.30 ± .91. The number of CPT's ranged from a minimum of zero to a maximum of 3. Interface hepatitis could be seen in 6(40%) out of 15 cases and portal inflammation could be seen in 12(48%) out of 25 cases in which it was originally seen.Focal necrosis could be seen only in 2(13.3%) out of 15 cases.(**Chart 2**)

Impact of length of liver biopsy on histopathological diagnosis

As the diagnosis was reviewed after a decrease in the biopsy size to 1cm (by artificial sampling), 5 out of these 46 biopsies (10.8%) showed a change in diagnosis. The diagnosis however remained same in 41 (89.2%) biopsies. On decreasing the size of the biopsy further by 0.5cm , the original diagnosis could be maintained in only 26(56.6%) biopsies.These included the cases which were initially reported as cirrhosis, glycogen storage disease, wilsons disease, few cases of cholestatic hepatitis and chronic hepatitis. five (10.8%) cases showed a change in diagnosis, all of which were that of NASH. In the remaining 15 (32.6%) cases no diagnosis was possible at the size of 0.5cm.(**Chart 3**)

Impact of length of liver biopsy on ishak's score

In addition, artificial sampling at 1cm also resulted in a change in the scoring of various diagnoses made.Out of the 27 cases of chronic hepatitis which were artificially sampled, 11(40.7%) cases showed a decrease in Ishak's score.One-score difference was seen in 10(37%) cases and 2-score difference was seen in 1 case(3.7%) .The score however remained same in 16(59.3%) cases. Artificial sampling was done in 5 out of 6 cases of NASH ,as one case had an initial biopsy size of <1.5cm and artificial sampling could not be done in it.All these 5 cases showed a decrease in Kleiner score and hence a change in diagnosisOut of 27 cases of chronic hepatitis, the Ishak's score decreased in 17(63%) cases.13(48.2%) out of these 17 cases showed a 2-score difference and 4(14.8%) cases showed a 1-score difference .Scoring was not possible in 6(22.3%)cases owing to the complete loss of complete portal tracts after artificial sampling. Five (83.3 %) out of six cases of NASH showed a decrease in Kleiner score and hence a change in diagnosis.The scoring was not possible in the remaining 1(16.6%) case of NASH (**Chart 4**)

Impact of length of liver biopsy on fibrosis/staging

The fibrosis/staging done in a total of 39 cases which included chronic hepatitis, primary biliary cirrhosis and NASH showed a decrease in stage in 18(46.2 %) cases.A 1-stage difference was seen in 14(35.9%) cases and a 2-stage difference was seen in 4(10.3%) cases whereas the stage remained same in 21(53.8 %) cases. The fibrosis/staging was attempted in 39 cases which included cases of chronic hepatitis,primary biliary cirrhosis and NASH. Out of 39 cases,the stage remained same only in 1(2.6%) case and decreased in 26(66.7%) cases.3(7.7%) out of 29 cases showed a 1 stage difference and 23(59%) showed a 2-stage difference.Thus there were more cases with a milder

stage. However no staging was possible in 12(30.7%) cases because of inadequate CPTs and markedly reduced liver parenchyma.Only one case had the same stage as at 1.5cm(**Chart 5**)

Discussion

This two-year study was conducted in the department of Pathology Sher-i-Kashmir Institute of Medical Sciences, Soura, Srinagar. The most common clinical indication for liver biopsy was chronic hepatitis which has also been reported by Sporea et al[9] in their study of 250 liver biopsies. Most common histopathological diagnosis made in our study was chronic hepatitis (in 38.6% cases) followed by primary biliary cirrhosis, NASH and cirrhosis in 8.5% each. Other histopathological diagnoses made included cholestatic hepatitis and glycogen storage disease in 7.2% each, AIH-PBC overlap in 5.7%, AIH in 4.3% and Wilson's disease in 2.9%. One (1.4%) case reported was uncertain for NASH. Because of inadequate size, diagnosis was not possible in 7.2% of liver biopsies. Khokhar N[10] and Samaila et al[11] have also reported chronic hepatitis to be the most common histopathological diagnosis in their respective studies (68.3% and 40.5% cases respectively). In this study the clinical diagnosis was confirmed on histopathological examination in 78.5% cases while the histopathological diagnosis did not match the clinical diagnosis in 14.3% cases. In the study by (Spycher et al)[12], the clinical diagnosis was confirmed histopathologically in 84.4% of cases and changed in 6.8%. In the study by Gilmore et al the diagnosis made clinically was confirmed histopathologically in 63% of patients[13].The results of this study provide the evidence of a significant relationship between biopsy size and histopathological diagnosis along with grading and staging of chronic viral hepatitis and various other chronic liver diseases(in which grading or staging is done).The specimens were examined 3 times (at a size of ≥1.5cm, at 1cm and finally at 0.5cm).After decreasing the size of the biopsy to 1cm, 10.8% cases showed a change in diagnosis. All these cases were those of non-alcoholic steatohepatitis. The diagnosis however remained same in 89.2% cases.On further decreasing the size of biopsies to 0.5cm the original diagnosis could be maintained in only 56.6% of the biopsies. These included the cases which were initially reported as cirrhosis, glycogen storage disease, Wilson's disease, and a few cases of cholestatic hepatitis and chronic hepatitis. This was because in glycogen storage disease and Wilson's disease, the clinical details provided and positive staining for orcein and rhodanine (in Wilson's) and PAS with diastase digestion (in glycogen storage disease) were sufficient to make the diagnosis.These parameters were least affected by the reduction in size. Also the cases of cirrhosis that we diagnosed were all micronodular and showed complete replacement of the liver parenchyma by regenerative nodules which were visible even after reducing the size to 0.5cm. 10.8% cases showed a change in diagnosis, all of which had the initial diagnosis of NASH. As the score decreased, the cases were diagnosed as 'uncertain for NASH' or 'not NASH' after the decrease in size. In the remaining 32.6% cases no diagnosis was possible at the size of 0.5cm because of either the complete absence of CPTs or CPTs showing normal morphology only. Also in cases of cholestatic hepatitis and primary biliary cirrhosis because of the patchy nature of the biliary injury, the diagnosis was impossible when the size and hence the complete portal tracts markedly reduced. The cases of cholestatic hepatitis which were diagnosed at 0.5cm had marked cholestasis and showed severe ductular destruction in the portal tracts which were visible.Our data demonstrates that shorter liver specimens result in a significant underestimation of both the grade and the stage of the liver disease: In assessing the overall grade of necroinflammatory activity, the weighted κ score at 1cm and 0.5cm was 0.447 and 0 respectively indicating poor agreement for both. Reducing the length to 1 cm lead to a decrease in score of 46.2% of cases with a 1-score difference in 35.9% cases and a 2-score difference in 10.2% of cases. However no change in score was seen in 53.8%of cases. Further reduction of size to 0.5cm lead to a decrease in score of 63% of cases with a 1-score difference in 10.8% and 2-score difference in 48.2% probably because at 0.5cm most of the biopsies showed a complete absence of CPTs or the CPTs showed normal morphology only. This indicated that as we decrease the size of the biopsy, the score of the disease becomes milder and hence results in the underestimation of the grade of the disease. Cases with severe grades decreased markedly as the specimen size was reduced. Colloredo et al in their study also reported an almost absence of severe grades in the shorter specimens in their study[14].The cases in which the fibrosis/staging was done included cases of chronic hepatitis, primary biliary cirrhosis and NASH. When the fibrosis/staging was done at AS1 there was a decrease in stage in 46.2% of the cases. A 1-stage difference was seen in 35.9% cases and a 2-stage difference was seen in 10.2% cases whereas the stage remained same in 53.8% of cases. In the study by Schiano et al in a 1-stage difference was seen in 22% cases and 6% cases showed a 2-stage difference[15].At AS2, staging remained same only in 1(2.6%) case. It decreased in 66.7 % cases, with 59% cases showing a 2-stage difference and 7.7% cases showing a 1-stage

difference. However no staging was possible in 30.7% of cases because of inadequate CPTs and markedly reduced liver parenchyma. In the study by Schiano et al, 31% cases showed a 1-stage difference and 13% cases showed a 2-stage difference.This indicated a milder degree of fibrosis with the decrease in size of the biopsy and hence an underestimation of the stage of the disease which could hence alter the management of the patient.In this study when the specimens size of the biopsies originally diagnosed as NASH was reduced, there was a significant change in the score, which subsequently changed the diagnosis of NASH. The fibrosis in these cases was also markedly reduced. This was because of the unavailability of parenchyma showing steatosis, ballooning and lobular inflammation (parameters required for scoring) and decrease in the number of portal tracts, which showed inflammation and fibrosis. Similar findings were observed when the specimen length was reduced in the cases of primary biliary cirrhosis. The biopsies showed a marked reduction in fibrosis on decreasing the specimen length. In assessing the stage of fibrosis, the weighted κ score at 1cm and 0.5cm was 0.671 and 0.124 respectively indicating poor agreement for assessing fibrosis at 0. 5 cm. Since closer is the value to 1 better is the agreement[5], the agreement for fibrosis was better(but not excellent which is for 0.75) with 1cm specimens(**Table 2**) .These studies could comment on the larger biopsy specimens which we could not because of limited setup. The most important factor influencing the diagnostic inadequacy of the smaller biopsy sizes is probably the significant decrease in the number of complete portal tracts in the smaller specimens. Colloredo et al[14], Rocken et al[16] and Petz et al[17]reported in their studies that the number of complete portal tracts correlated with the size of the biopsy. Rocken et al[16], and Vargas-Tank et al suggested in their studies that the lower number of complete portal tracts may be the reason for the lower diagnostic accuracy obtained with smaller samples[18].It is widely believed that an adequate liver biopsy specimen should contain a minimum number of portal tracts. Most pathologists are satisfied with specimens containing 6–8 portal tracts [19]**,** although some studies now suggest that a greater number of portal tracts may be required for adequacy[14]. Colloredo et al[14]**,** Zarski et al[20]and Guido et al[21]also suggested in their study that a specimen size of 2 cm and a minimum of 11 complete portal tracts are considered adequate for diagnosis of cirrhosis and chronic active hepatitis. Although decreasing the size of the biopsy specimen to 1cm did not alter the diagnosis, there was a definite change in scoring. There was also a decrease in the stage of the disease but the agreement was still better as compared to 0.5cm size. Further decrease in the size of the biopsy samples to 0.5cm altered all the three parameters i.e. diagnosis, scoring and staging. Infact a considerable number of cases (32.6%) the diagnosis was not possible after decreasing the sample size to 0.5cm.This was because the number of CPTs decreased with decrease in size of the biopsy. Our study indicated that at least 7-8 complete portal tracts should be available to assess the different parameters accurately.

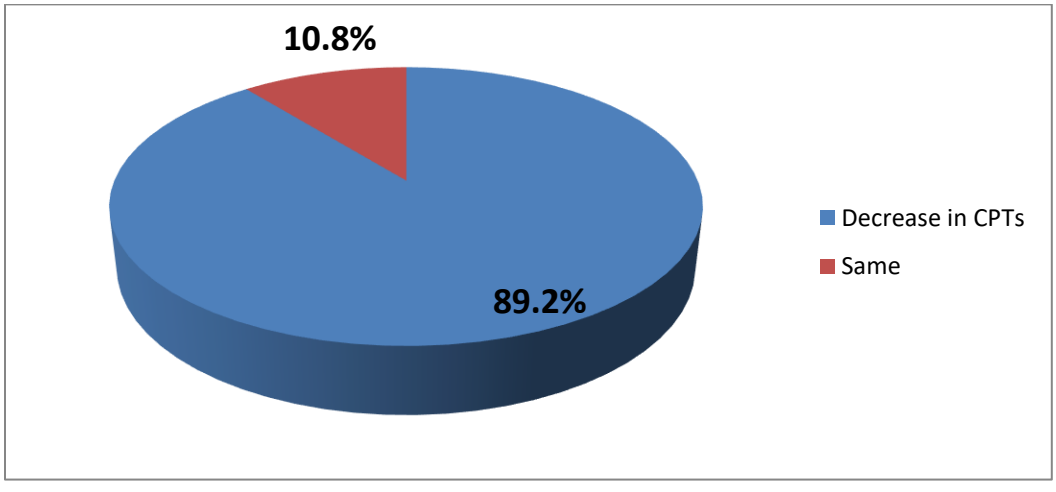

Chart 1: Complete portal tracts at AS1(1cm)

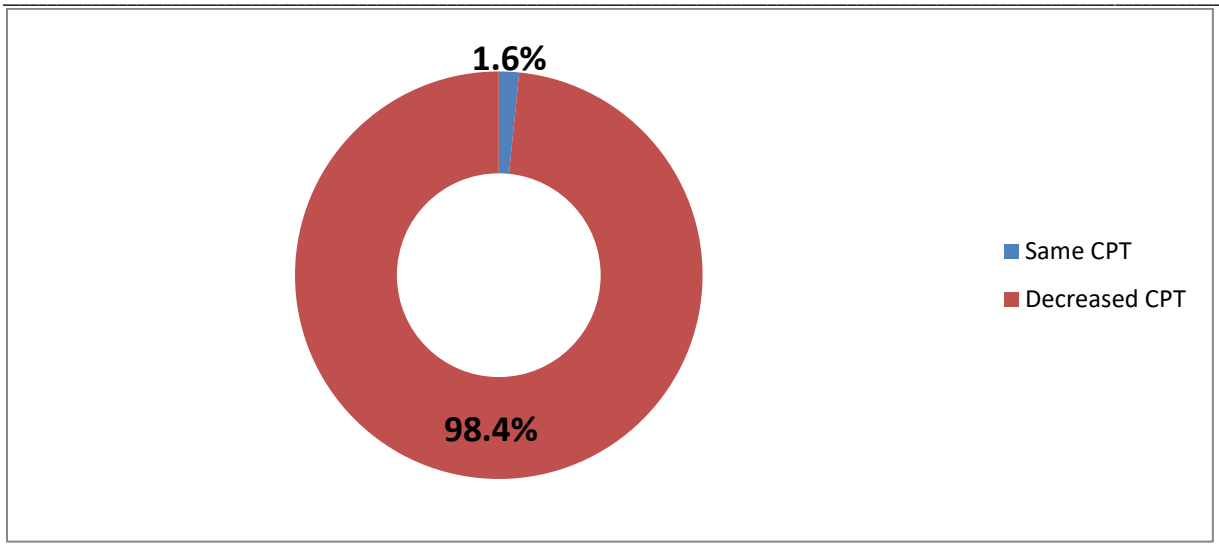

Chart 2 : Number of complete portal tracts at AS2(0.5cm)

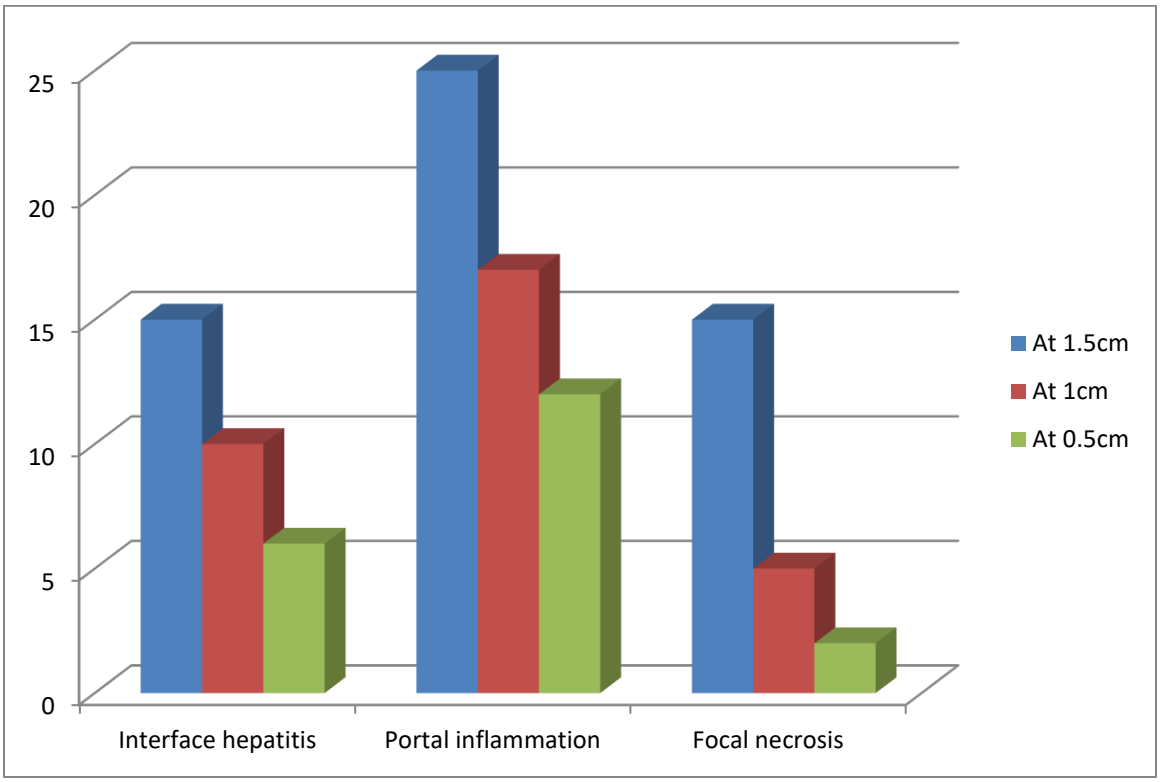

Chart 3: Comparison of histological lesions diagnosed at AS1(1cm) and AS2(0.5cm)

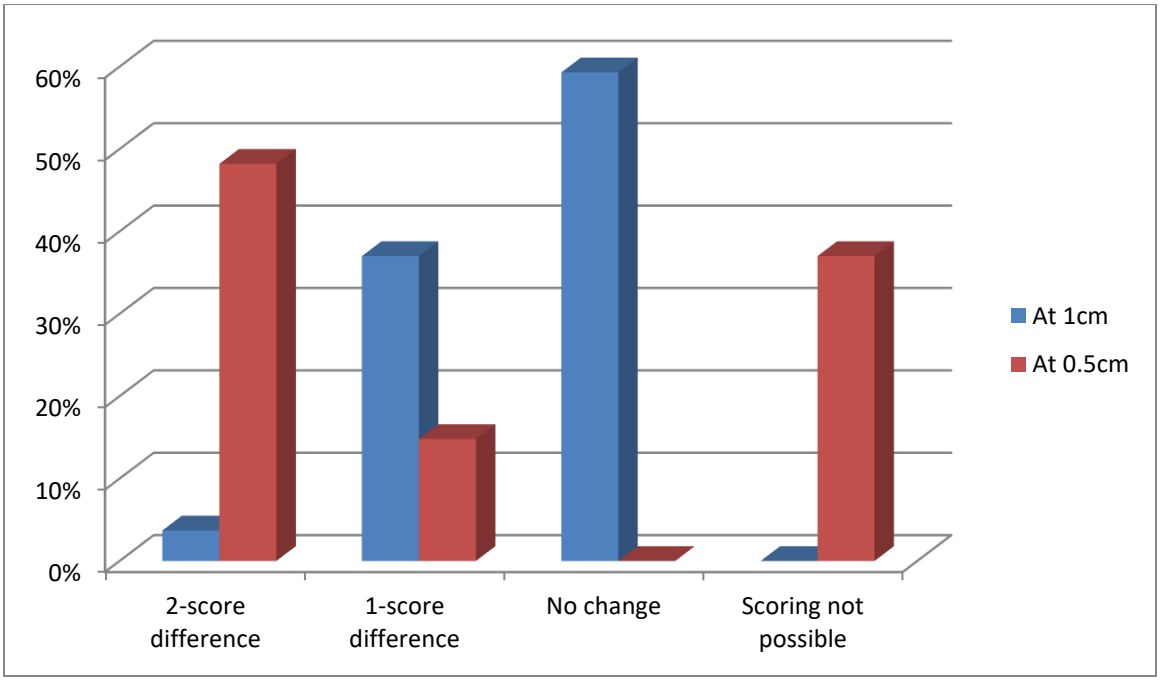

Chart 4: Comparison of scoring in chronic hepatitis

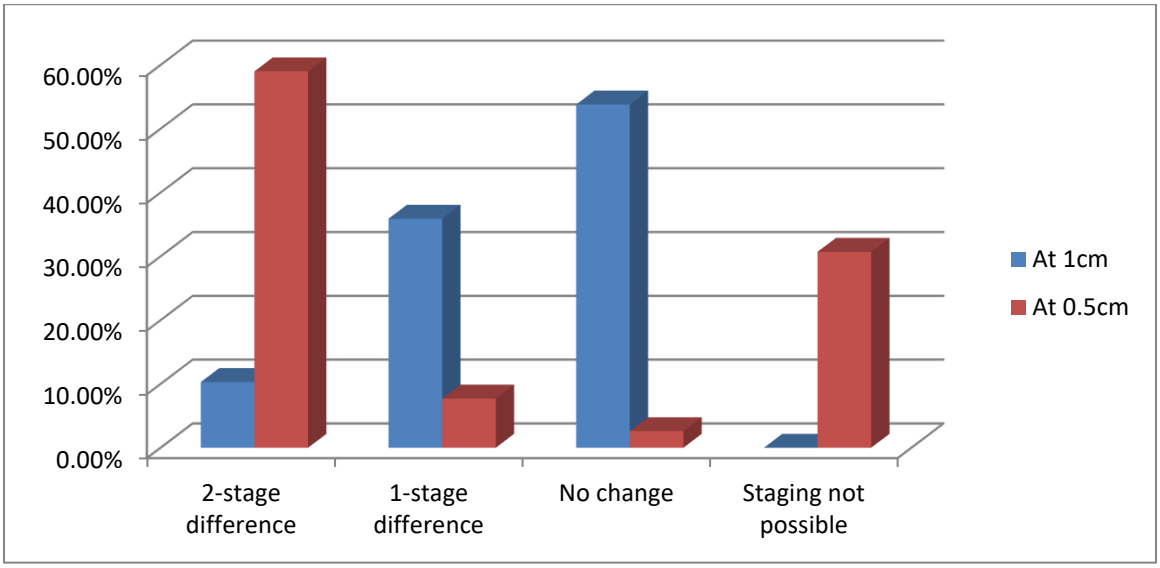

Chart 5: Comparison of staging

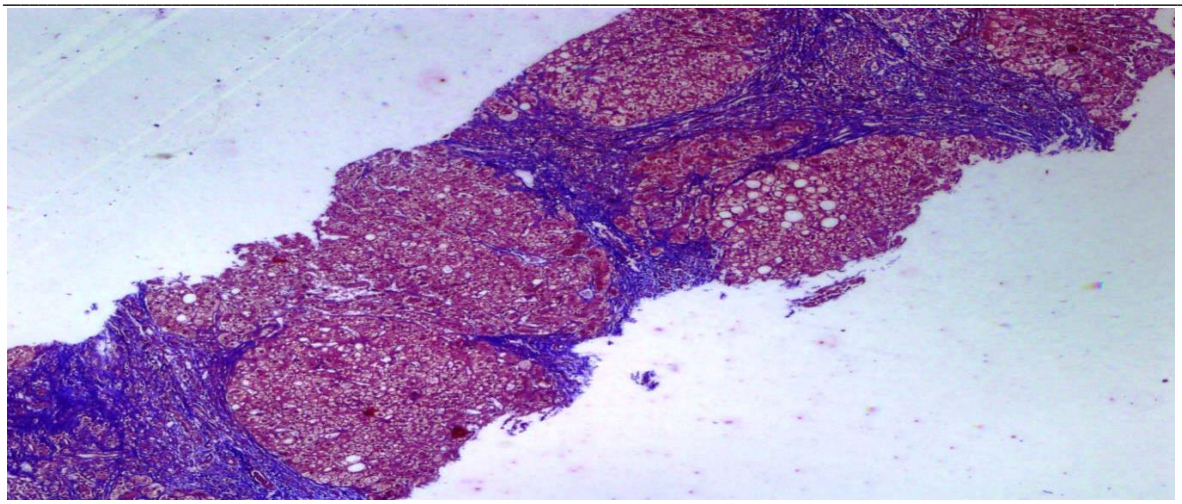

Fig 1 : Photomicrograph of liver biopsy showing altered hepatic architecture with formation of cirrhotic nodules.(MTC 10X)

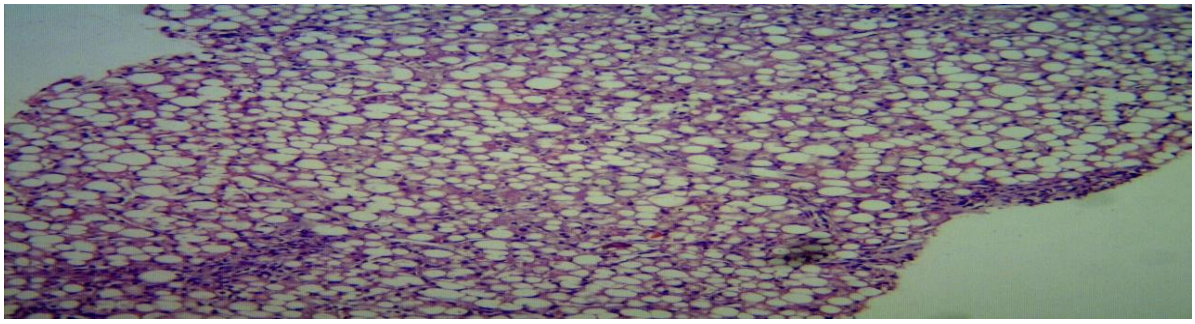

Fig 2: Non alcoholic steatohepatitis.Photomicrograph of liver biopsy showing steatosis,lobular inflammation and fibrotic tendrils.(H&E 10X)

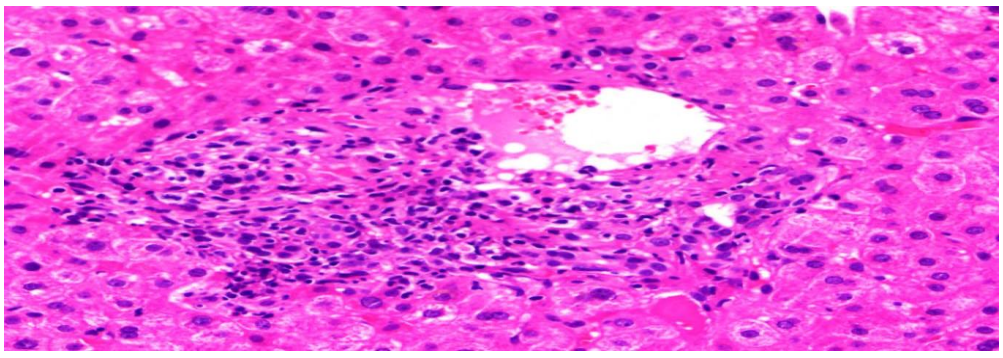

Fig 3: Chronic hepatitis.Liver biopsy showing portal inflammation comprising predominantly of lymphocytes.(H&E 40X)

Conclusion

The study concluded with the results that a biopsy size of less than 1.5cm can be considered inadequate as there was definite improvement in the diagnostic accuracy at the biopsy size ≥1.5cm when compared to 1cm and 0.5cm. Also, at least 7-8 complete portal tracts should be visualized for accurately diagnosing, scoring and staging chronic liver disease.

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Conflict of Interest: None
Source of Support: Nil