Fibrosis stage of benign and malignant lesions in the liver based on Metavir with reticulin and trichrome massons staining

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Background : Liver fibrosis is a healing process in progressive chronic liver disease. For years, fibrosis was considered irreversible. Over the past three decades, liver cirrhosis has been accepted as a reversible condition. Histologic levels of fibrosis is the main thing in evaluating liver damage, it is also useful for predicting responses to antiviral treatment and also in predicting the endpoint of fibrosis to be able to continue into cirrhosis and end-stage liver complications.
Materials and Methods : Paraffin blocks from 22 patients with benign and malignant liver disease were given reticuline and trichrome massons staining and then assessed for fibrosis stage using the Metavir scoring system.
Results : Each staining gave the same results which were dominated by F3 fibrosis stage which was 9 cases (40.91%). Stadium F0 was not found in this study. Stage F1 in 1 case (4.54%), stage F2 in 7 cases (31.82%) and stage F4 in 6 cases (27.27%). Benign lesions in the liver in this study were dominated by F4 in 3 cases (42.86%) of a total of 7 cases and 9 cases of primar malignant lesions in the liver were dominated by F3 in 4 cases (44.44%). Metastasis lesion was found in 6 cases (27.27%) and in this study were dominated by F3 in 3 cases (50%).

keywords : fibrosis, liver, reticulin, trichrome massons

I. Introduction

Liver fibrosis is a central pathological healing process in progressive chronic liver disease. For many years, fibrosis was thought to be irreversible. The first notion on the regression of liver fibrosis appeared in the medical literature in 1979, when Perez-Tamayo, analyzing the activity of liver collagenase, presented data supporting that cirrhosis could be reversible. During the last three decades, fibrosis has been widely accepted as a dynamic process with a strong potential for significant resolution. Substantial evidence originated from data showing that successful treatment of the underlying liver disorders, could reverse fibrosis and probably even cirrhosis.[1]

Liver fibrosis is a significant health problem, which can ultimately lead to end stage cirrhosis and hepatocellular carcinoma. A wide spectrum of chronic liver injuries, including viral hepatitis, cholestatic liver diseases, alcohol abuse, non-alcoholic steatohepatitis, and nonalcoholic fatty liver disease, can cause chronic hepatic inflammation and deregulated wound healing process in the liver, which give rise to fibrosis. Liver fibrosis is characterized by excessive extracellular matrix (ECM) deposition and fibrous scar formation. The destruction of the normal liver architecture by fibrous scar and the loss of hepatocytes can prevent the liver from its physiological functions and in the end, result in liver failure.[2] In Indonesia, chronic liver disease caused by hepatitis viruses (B, C and D). Eighty percent of patients with liver disease, especially those caused by the hepatitis C virus, develop into chronic from mild symptoms that can progress to liver cirrhosis by 20%, and 4% towards long-term liver cell carcinoma.[3] World Health Organization (WHO) in 2014 reported that liver disease ranks the eighth most cause of death in Indonesia with a mortality rate of 3.49%, which is around 48,858 people.[4]

II. Material and Methods

A. Patients and samples

This is cross sectional study was conducted on biopsy and surgical specimens which diagnosed benign and malignant lesions in liver were included. The specimens were collected and stored at the laboratory of anatomical pathology of universitas sumatera utara and unit of anatomical pathology in Haji Adam Malik General Hospital in Medan, North sumatera, Indonesia.

B. Reticuline and trichrome massons

Reticuline and trichrome massons staining was performed in 22 paraffin blocks using formalin-fixed and paraffin-embedded tissue sections. Reticulin staining is a special coloring used to show reticular fibers. Reticulin will stain type III collagen The silver metal is able to settle on reticular fibers, and this can be visualized using silver salt. Initially salt will be oxidized by potassium permanganate. Next, metallic silver will be formed from ammonia silver nitrate solution, and will settle as a brownish deposit on the target structure. Formaldehyde, is a strong reducing agent, which will accelerate this process.

Furthermore, when the toning process with gold chloride, silver metal is transferred to a more stable gold compound, which leads to more intensive results. Furthermore, nonspecific silver will be bonded with sodium thiosulfate. In trichrome massons staining method, three dyes are used selectively for staining collagen fibres, muscles, erythrocytes and fibrin. The principle of

trichrome stain is that the smallest molecular size dye stains the less permeable tissues. However, if a large dye molecule is capable in penetrating the tissue, staining will take place in the rate of the smallest molecule. The tissue is initially stained with Biebrich Scarlet (acid dye), which binds to the acidophilic components. Collagen fibres will be stained blue, nuclei stained black and cytoplasm, muscle and erythrocytes stained red. The tissue sections were independently examined by three researchers, including two pathologist.

C. Metavir scoring system

In assessing fibrosis the Metavir system is relatively simple, categorizing the fibrosis stage in 5 scale levels, namely the scale of 0 to 4. The Metavir system categorizes the fibrosis stage in 5 scale levels namely scale F0 to F4. Fo if no fibrosis was found, F1 if fibrosis was limited to the portal, F2 if portal fibrosis was found with little septic fibrosis, F3 if fibrosis was found at a clear septa, F4 if fibrosis was found at septa and extends to the center.[5]

III. RESULTS

The majority of samples were retrieved from male in 14 cases (63.64%) and 8 (36.36%) from female. The mean (SD) age at diagnosis was 44 ± 13,09 years. Most of the 34 patients were primer malignant lesion in liver in 9 cases (40.91%). (Table 1).

Table 1. Clinicopathological characteristic

characteristic	(n)	Persentage (%)
age		
0 - 10 years	2	9,09
11-20	1	4,54
21-30	2	9,09
31-40	2	9,09
41-50	5	22,73
51-60	7	31,82
61-70	2	9,09
71-80	1	4,54
sex		
male	14	63,64
female	8	36,36
lesions		
benign	7	31,82
primer malignant	9	40,91
Metastasis	6	27,27

This study was approved by National Ethics Committee Medical faculty of Universitas Sumatera Utara.

In this study we was found that fibrosis stage based on metavir using reticulin and trichrome massons staining gave the same results. Each staining was dominated by F3 fibrosis stage in 9 cases (40.91%). Stadium F0 was not found in this study. F1 stage was found in 1 case (4.54%), stage F2 in 7 cases (31.82%) and stage F4 in this study were found in 6 cases (27.27%) (table 2).

Table 2. distribution of fibrosis stage based on metavir with reticulin and trichrome massons staining

Fibrosis stage	retikulin [n /(%)]	trichrome massons [n /(%)]
F0	0	0
F1	1 (4,54%)	1 (4,54%)
F2	6 (27,27%)	6 (27,27%)
F3	9 (40,91%)	9 (40,91%)
F4	6 (27,27%)	6 (27,27%)
total	22 (100%)	22 (100%)

Distribution of fibrosis stages in this study showed that in male there were 14 cases (63.64%). In both stains found the most fibrosis stage in male were F2 and F3, each of which was 5 cases (35.71%). In female we were found eight cases (36.36%) and the most were in 4 cases (50%) (table 3).

Table 3. distribution of fibrosis stage based on metavir by sex

sex	retikulin					trichrome massons				
	F1	F2	F3	F4	total	F1	F2	F3	F4	total
male	0	5	5	4	14	0	5	5	4	14
female	1	1	4	2	8	1	1	4	2	8
total	1	6	9	6	22	1	6	9	6	22

Based on the type of lesions in this study it was divided into 3 types, there were benign liver lesions , primary malignant lesions of the liver and liver metastatic lesions. The results of this study showed that the fibrosis stage in benign lesions in the liver was mostly found in F4, namely as many as 3 cases (42.86%) of a total of 7 cases encountered. This is found to be the same in both reticular staining and trichrome masson. Primary malignant lesions in the liver in 9 cases (40.91%) of the total cases were found. The most fibrosis stage found in stage F3 in 4 cases (44.44%) and the group of liver metastatic lesions found in 6 cases (27.27%) in this study. The most fibrosis stage was stage F3 in 3 cases (50%). The least encountered fibrosis stage is stage F4 in 1 case (16.67%). And in this study no F1 stage was found in malignant lesions that metastasized to the liver. (table 4.)

Table. 4 distribution of fibrosis stage based on lesions type

lesions	retikulin					trichrome massons				
	F1	F2	F3	F4	Jumlah	F1	F2	F3	F4	total
benign	1	1	2	3	7	1	1	2	3	7
Primer malignant	0	3	4	2	9	0	3	4	2	9
Metastasis	0	2	3	1	6	0	2	3	1	6
Total	1	6	9	6	22	1	6	9	6	22

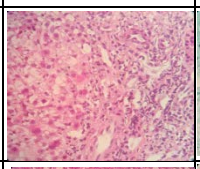
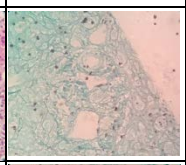
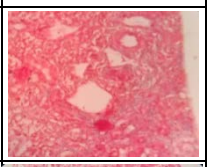
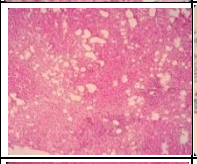
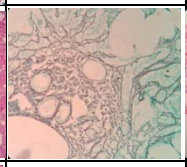
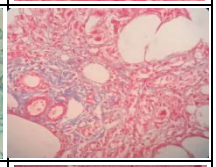
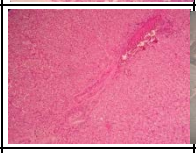
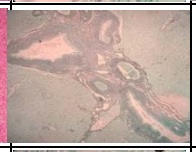
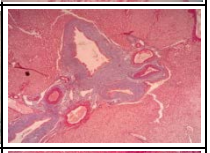
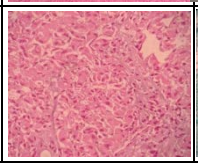
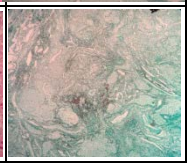
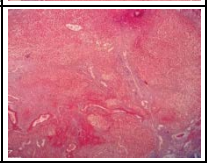
stage fibrosis	H&E	RETIKULIN	TRICHROM MASSON
F1			
F2			
F3			
F4			

Figure 1. Fibrosis staga F1,F2,F3,F4 in HE, retikulin,and trichrome massons

IV. DISCUSSIONS

Fibrosis is a characteristic feature of accumulation extracellular matrix (ECM) which leads to scar tissue formation and will eventually cause organ dysfunction.[6] In Indonesia, especially chronic liver disease caused by hepatitis viruses (B, C and D). Eighty percent of patients with liver disease, especially those caused by the hepatitis C virus, develop into chronic symptoms ranging from mild to can progress towards liver cirrhosis by 20%, and 4% towards long-term liver cell carcinoma.[7] According to Nur Aisyah's research at RSU Dr. Pirngadi Medan in 2002-2006 there were 669 patients with liver cirrhosis. From 251 patients there were 56.6% of patients who had a history of hepatitis.[8] From the recent research, it was found that the most common cause of fibrosis was nonalcoholic fatty liver disease (NAFLD), which is around 77%. This is much higher than fibrosis caused by hepatitis C which is only about 8% and liver disease which is only caused by alcohol by 8%. Poynard et. al., in the study found that liver fibrosis was generally caused by alcoholic and non-alcoholic fatty liver disease (66%), NAFLD 13%, 9% alcohol, 6% HCV, and 6% other.[9] In this study the diagnosis of hepatitis was only found in two cases (9.1%) of a total of 22 cases examined for fibrosis stage.

Malignancy cases were the highest in the study, which amounted to 15 cases (68.18%) which were dominated by cases of hepatocellular carcinoma in 5 cases (22.73 %). The average age of patients with liver disease in this study is 44 years with standard deviation 13.09. which was dominated by male in 14 cases (63.64%). This is not much different from the study of Maria L et. al., who conducted the Prevalence of Liver Fibrosis and Its Association with Non-invasive Fibrosis and Metabolic Markers in Patients with Unhealthy Obesity with Vitamin D Deficiency of 46 cases of liver biopsy found that the average age was 42 years with a standard deviation of 13 years.[10] Fung J et. al., who conducted a study of 1315 patients found that 319 patients (34%) had severe fibrosis. He also found that the overall prevalence of severe fibrosis in patients with hepatitis B in the older age group was seen to increase by 34%. Severe fibrosis in patients aged <25 years is seen as 20% and increases to 81% in patients with patients aged> 65 years.[11] Poynard et. al., analyzed 1,312 patients with positive hepatitis C and confirmed by PCR examination from 3 health centers (Paris n 537, Marseille n 601, Bordeaux n 174) found that the average age is 48 years and 58% are male.[12]

Male and female ratio in this study were found to be 7: 4 with a percentage of male in 63.64% cases. This is slightly different from the study of Fung J et al., Found that the ratio of male and female was 39: 42%.[11] Research what Maria L et. al., is dominated by women which is as much as 80% of the total cases.[10] This may be caused by factors of habits and lifestyle of the people in this research area and also because of the different number of samples.

In this study both staining reticuline and trichrome massons found that F3 had 9 cases (40.91%) of 22 cases which was encountered in the age range of 51-60 years,in 4 cases out of a total of 7 cases of F3. Stage F4 only found in 6 cases (27.27%) of 22 cases in this study. Most stages F4 are found in the age range of 41-50 years in 2 cases. Stadium F2 in this study found as in 6 cases (27.27%) and the most in the age range 51-60 years in 2 cases. This is slightly different from previous with Maria L et. al., found that of the total cases 30% showed significant fibrosis (≥F 2) with 9% representing advanced fibrosis (F3) and 4% representing cirrhosis (F4).[10]

Benign lesions in the liver in this study found in 7 cases (31.82%) both in reticulin and trichrome Massons staining found F1, F2, F3 and F4 fibrosis respectively 1 (14.28%), 1 (14, 28%), 2 (28.57%) and 3 (42.86%) cases. Poynard et. al., who observed the distribution of natural fibrosis stages in patients with chronic hepatitis C who had liver biopsies of 2235 cases. The prevalence of fibrosis was F0 10%, F1 36%, F2 21%, F3 15%, and F4 18%. While patients who were reconfirmed with the Polymerase Chain Reaction (PCR) examination of 1312 cases he found the prevalence of fibrosis stage was 11% for F0 (n 141), 40% for F1 (n520), 22% for F2 (n 295), 16% for F3 (n 208), and 11% for F4 (n 148).[12] This is probably due to the fact that the number of samples in this study was only 22 cases, and it can also be because early detection of fibrosis in liver disease using biopsy is still minimal in the institutions where this research was conducted.

In this study 15 cases (68.18%) of malignancy were found. Six cases (40%) of them were metastatic cases from other places to the liver. Fibrosis stage in malignancy cases in both reticulin staining and Trichrome Massons staining was dominated by F3, in 7 cases out of 15 malignancy cases (46.66%). F2 in 5 cases (33.33%) and F4 remaining 3 cases (20%). In this study, most fibrosis stage in benign lesions was found in stage F4 and most malignant lesions was stage F3. This shows that in this study fibrosis trips in benign lesions continued and did not receive good treatment so that the fibrosis stage to be able to continue into cirrhosis and end-stage liver complications. Cirrhosis. And in this study most of malignant lesions was stage F3 this

indicates that malignancy should not to be preceded by cirrhosis (F4) malignancy can take place when the liver does not have fibrosis or at the time of fibrosis at a low stage Radisky et. all., states that increasing the expression of MMP matrix metalloproteinase can stimulate fibrosis, tumorigenesis, and also the development of tumors by inducing extracellular matrix especially in transdifferentiate epithelial cells and causing myofibroblast to be activated. This connection provides a new perspective on the development of micro-fibrosis and tumor environments.[12] Fibrosis is strongly associated with cancer which affects tumorigenesis, metastasis and inhibits drug delivery to the tumor. Early detection of fibrosis provides a great opportunity for the management of fibrotic disease.[13]

V. CONCLUSION

In this study, the use of reticulin staining and staining of trichrome massons showed no difference in assessing fibrosis stage using the metavir scoring system. The most common fibrosis stage in this study is stage F3. Most cases are in the sixth decade of life and more men are found with a ratio of 3.5: 2. In this study, fibrosis stage in benign lesions was found in stage F4 and in most malignant lesions found was stage F3. Fibrosis is also associated with cancer which affects tumorigenesis, metastasis and inhibits drug delivery to the tumor.

COMPETING INTERESTS

The authors have no relevant financial interest in the products or companies described in this article.

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