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Presence of perivenular elastic fibers in nonalcoholic steatohepatitis Fibrosis Stage III

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Summary. Elastic fibers appear in extensive old fibrotic foci in general. We examined an association between hepatic fibrosis stage and the presence of perivenular elastic fibers in nonalcoholic steatohepatitis (NASH). A total of 48 liver needle biopsy specimens were used, taken from 48 cases with NASH. Fibrosis Stage (Brunt E, et al. Am. J. Gastroenterol. 1999) of the cases was as follows; six Fibrosis Stage I, twenty-two Fibrosis Stage II, and twenty Fibrosis Stage III. We examined Orcein stain sections in all of the liver needle biopsy specimens. In all twenty Fibrosis Stage III cases, perivenular elastic fiber bundles were observed. In contrast, perivenular elastic fibers were detected only in one of the six Fibrosis Stage I and two of the twenty-two Fibrosis Stage II cases. In liver needle biopsy specimens of NASH, detection of perivenular elastic fibers is useful in deciding Fibrosis Stage III.

Key words: Elastic fiber, Steatohepatitis, Nonalcoholic steatohepatitis, Central vein, Perivenular fibrosis

Introduction

Nonalcoholic steatohepatitis (NASH) was first described as a clinical entity (Ludwig et al., 1980). NASH is a progressive form of nonalcoholic fatty liver disease (NAFLD) that can lead to hepatic fibrosis, cirrhosis, and hepatocellular carcinoma (Sanyal, 2002; McCullough, 2002). Recently, histological grading and staging score system of NASH was proposed (Brunt et al., 1999; Kleiner et al., 2005). Fibrosis stage is closely associated with patient prognosis.

In NASH, fibrosis is restricted to Rappaport zone 3 in Stage I and restricted to Rappaport zone 3 and periportal areas in Stage II (Brunt et al., 1999). Perivenular fibrosis appears in early fibrosis stage of NASH. Elastic fibers appear in extensive old fibrotic foci in general (Roten et al., 1981). It is valuable to examine a distribution of elastic fibers in liver biopsy specimens with NASH, in order to recognize the fibrosis stage and patient prognosis. However, the presence of elastic fibers are not included in the scoring system.

In the present study, we examined the presence of perivenular elastic fibers in liver biopsy specimens of NASH, and compared the results with their Fibrosis Stage.

Materials and methods

A total of 48 liver needle biopsy specimens with NASH (Stage I, Stage II, and Stage III) from the Surgical Pathology File of Clinical Laboratory Department, Saiseikai Kure Hospital, from April 2002 to February 2007, were used. In addition to hematoxylin and eosin stains, both Masson's trichrome-stained sections and silver stain sections were reviewed, in order to evaluate the extent of fibrosis in all of the biopsy specimens. We decided the fibrosis stage of each biopsy specimen according to the criteria of Brunt et al. (1999); Stage I (only perivenular/perisinusoidal fibrosis); six cases, Stage II (perivenular/perisinusoidal fibrosis with portal fibrosis) twenty-two cases, Stage III (perivenular/perisinusoidal fibrosis portal fibrosis plus bridging fibrosis); twenty cases.

Then, Orcein-stained sections were examined to evaluate the presence of elastic fibers, especially

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focusing on perivenular areas, in the present study.

Statistical analysis was carried out using Fisher's exact probability test and p values <0.005 were considered to be significant.

Results

The results are summarized in Table 1.

In all twenty Stage III cases (Figs. 1, 2), elastic fibers were detected in perivenular areas; central venules were surrounded by thick elastic fiber bundles (Fig. 3).

In contrast, elastic fiber bundles were detected in perivenular areas of only one of the six NASH Stage I and two of the twenty-two NASH Stage II cases.

The presence of perivenular elastic fiber bundles was significantly higher in cases with Stage III than those with Stage I and Stage II (p=0.0000000001).

Discussion

Histopathological, immunohistochemical and molecular morphological studies regarding steatohepatitis are few in number (Washington et al., 2000; Lefkowitch et al., 2002). Kupffer cells are aggregated in perivenular areas in steatohepatitis (NASH and alcoholic steatohepatitis (ASH)) (Lefkowitch et al., 2002). Alpha smooth muscle actin positive activated hepatic stellate cells are distributed mainly in zone 3 of NASH (Washington et al., 2000).

Elastic fibers appeared in perivenular areas in all of the NASH Fibrosis Stage III cases, but not in nearly 90% of the Stage I and Stage II cases in the present study. There is a possibility that the production of elastic

Table 1. Association between fibrosis stage and presence of perivenular elastic fiber bundles in nonalcoholic staetohepatitis (NASH).

Fibrosis stage	Number	Presence of elastic fibers
Stage I	6	1*
Stage II	22	2*
Stage III	20	20*

*p=0.0000000001

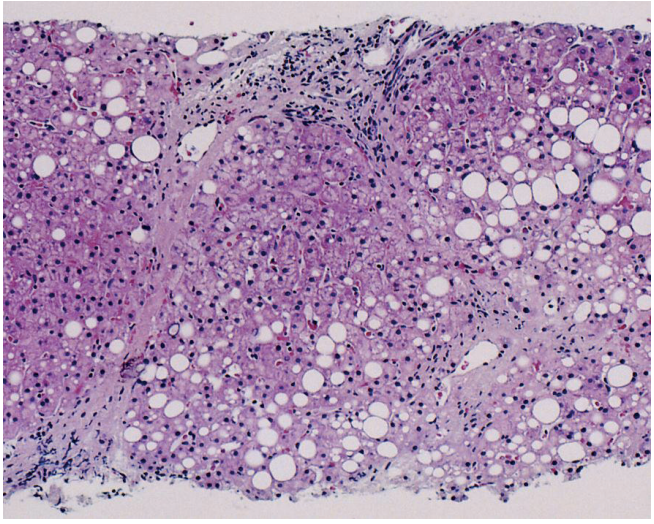

Fig. 1. Representative NASH Fibrosis Stage III case (note: Figs. 2 and 3 are the same site as Fig. 1). At the upper left and lower right sides, central veins are observed (Hematoxylin eosin stain). x 150

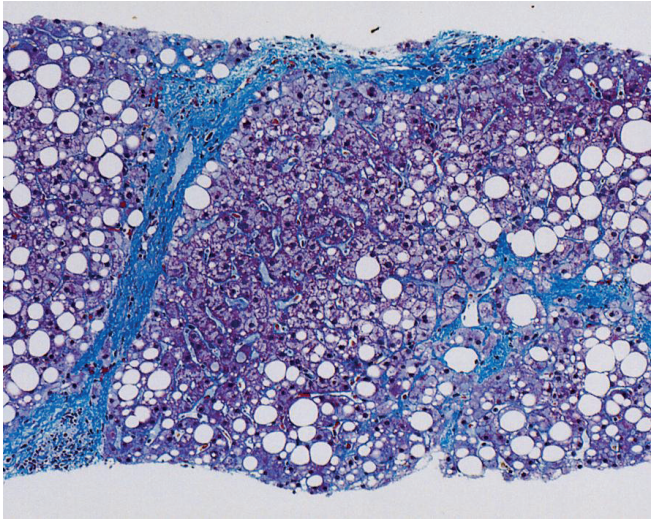

Fig. 2. Representative NASH Fibrosis Stage III case. At the upper left and lower right sides, perivenular fibrosis, bridging fibrosis with nodular architecture is detected (Masson-trichrome stain). x 150

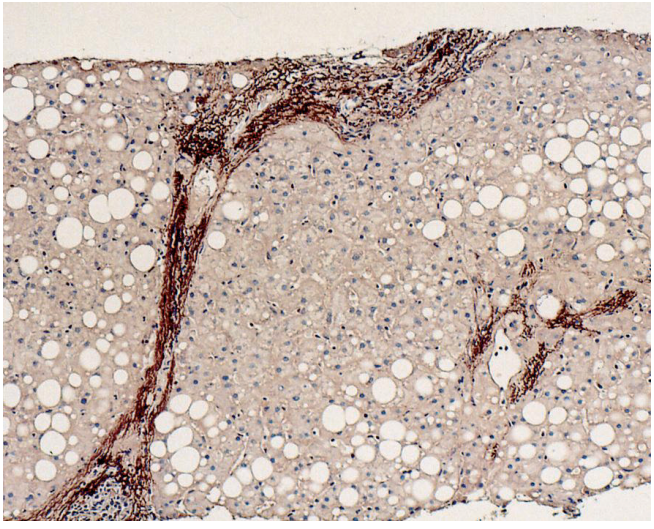

Fig. 3. Orcein stain section of representative NASH Fibrosis Stage III case. At the upper left and lower right sides, central veins are observed; perivenular thick elastic fibers are detected. x 150

fibers is associated with alpha smooth muscle actin positive activated hepatic stellate cells; Kupffer cells and other inflammatory infiltrates may play a supportive role in the production of elastic fibers by alpha smooth muscle actin positive activated hepatic stellate cells. In NASH Stage III, bridging fibrosis exists between portal tract and perivenular areas; there is a possibility that portal fibroblasts migrate from portal tract to perivenular areas. The roles of portal transformed fibroblasts, namely portal myofibroblasts, in perivenular elastosis should also be considered. A recent study elucidated that myofibroblasts which originated from peribiliary fibroblasts deposit elastin, whereas myofibroblasts derived from hepatic stellate cells, namely activated hepatic stellate cells, do not deposit elastin (Ramadori and Saile, 2004). Actually, elastic fibers are observed in portal areas of normal liver. Elastic fibers are detected adjacent to old fibrous bridge in chronic viral hepatitis and steatohepatitis (Scheuer and Lefkowitch, 2005). On the other hand, portal myofibroblasts express transforming growth factor receptor (TGF) beta 2 and, unlike activated hepatic stellate cells, express all three TGF beta receptors and are inhibited by TGF beta 1 and TGF beta 2 (Wells et al., 2004), suggesting that activated hepatic stellate cells eclipse portal myofibroblasts (Crawford, 2007). Central veins/terminal hepatic veins have a very thin wall lined by endothelial cells and reticulin fibers, but no advetitial fibroblasts. The possibility of elastin production in adventitial fibroblasts of small hepatic efferent veins should be considered. To elucidate the mechanism of perivenular elastosis, further molecular and biological investigations are needed.

One of the six NASH Stage I and two of the twenty-two NASH Stage II cases have perivenular elastic fibers in the present study. In general, liver fibrosis is reversible when patients received effective treatments for underlying diseases (Dufour et al., 1997, 1998). Especially, fibrosis in NASH spontaneously regresses nearly 30 % after a mean interval of 3.2 years (Adams et al., 2005). So there is a possibility that NASH fibrosis stage III with perivenular elastic fibers reversed to NASH fibrosis stage I and stage II; bridging fibrosis regressed and perivenular elastic fibers persisted.

In conclusion, the presence of perivenular elastic fiber bundles is associated with NASH Stage III. Detection of perivenular elastic fiber bundles is useful in deciding fibrosis stage in NASH.

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