Fibronectin as predictor of cirrhosis in men who abuse alcohol

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Abstract

In a study of 142 male alcohol abusers without evidence of cirrhosis the presence of intralobular fibronectin in the liver was investigated in relation to the subsequent development of the disease. All 142 initial biopsy samples showed preserved architecture. During a follow up period of 10-13 years 23 patients (16%) developed cirrhosis. Twelve of 110 patients with normal or slightly increased amounts of parenchymal fibronectin in the initial biopsy specimen developed cirrhosis, whereas eight out of 27 patients with moderately increased amounts and three out of five with significantly increased amounts later developed the disease ($p < 0.005$).

Semiquantitative assessment of the amount of parenchymal fibronectin at an early stage of alcoholic liver disease is of definite predictive value for the development of cirrhosis.

Introduction

Among the various hepatic lesions caused by chronic alcohol ingestion, cirrhosis is regarded as the end stage and is thought to be a result of progressive fibrosis.^{1,2} Prediction of cirrhosis early in alcoholic liver disease would be of biological as well as clinical importance. In a recent prospective study of male alcohol abusers drinking habits and biochemical liver measurements were of little predictive value; liver biopsy, however, provided valid information, the presence of alcoholic hepatitis and degree of steatosis being significantly correlated with the later development of cirrhosis.³

Investigations on animal models⁴ and human liver tissue⁵ show that fibrosis may occur independently of alcoholic hepatitis, thus indicating the need for other morphological markers of active fibrogenesis. Fibronectin may be such a marker as it is present in early granulation tissue⁶ and in increased amounts in the liver in early liver fibrosis.⁷ This study aimed at assessing the predictive value of fibronectin for the development of cirrhosis in male alcohol abusers.

Patients and methods

The study group comprised 328 patients who during 1968 to 1971 were admitted to the second department of internal medicine at Copenhagen City Hospital with a history of drinking more than 50 g alcohol daily for more than one year. All were offered a liver biopsy, and of the 330 biopsy samples obtained 72 were excluded for the following reasons: unsuitable initial sample (one case); double entry into initial study (two); patients were women (nine); patients emigrated after initial biopsy (two); initial biopsy showed cirrhosis, chronic aggressive hepatitis, or acute viral hepatitis (58). The remaining 258 samples, from 258 different patients, showed no signs of cirrhosis and, these patients were therefore eligible for the follow up study.

Morphological evaluation—The 258 initial biopsy samples were fixed, embedded, cut, and stained by routine procedures.⁹ Alcoholic hepatitis, morphologically defined as liver cells with Mallory bodies surrounded by neutrophils, was recorded. Steatosis was assessed semiquantitatively as 0 (absent), + (slight), ++ (moderate), and +++ (severe). Sections were stained with antibodies against fibronectin (Dakopatts, Denmark) by the indirect immunoperoxidase technique, as described.¹⁰ As fibronectin normally occurs around vessels and bile ducts in the portal tracts¹¹ this positive reaction was used to control for suitability of the staining reaction. When an inappropriate staining reaction for fibronectin was recorded in the portal tracts the specimen was excluded. (Lack of reactivity may have been caused by antigenicity being decreased or eliminated as a result of high temperatures that may have occurred with the technique used to embed the initial biopsy specimens in paraffin.) The amount of fibronectin in acinar zone 3 was assessed without knowledge of the clinical data as either normal or slightly increased (+), moderately increased (++), or significantly increased (+++). We also recorded whether increased amounts of fibronectin occurred focally or diffusely in zone 3 or in the hepatocytes.

Follow up of the 258 eligible patients was performed 10-13 years after the initial biopsy.³ For the 139 who had died we examined death certificates, discharge records, and necropsy records. Of the 119 patients still alive, 86 were examined clinically at the hospital and 16 reported on by their general practitioners. A second liver biopsy sample was available in 31 patients. Results of follow up were recorded as: (a) cirrhosis verified morphologically ($n=16$); (b) clinical and biochemical evidence of cirrhosis but no morphological verification ($n=22$); (c) no clinical evidence of cirrhosis and no morphological examination ($n=133$); and (d) cirrhosis excluded morphologically ($n=87$).³ In the analyses categories (a) and (b) were considered as cirrhosis.

Statistical analysis—95% Confidence intervals of proportions were based on the binomial distribution. Bivariate relations, with or without stratification by a single variable, were analysed by the Goodmann-Kruskal gamma test for trends in contingency tables based on ordinal scales.¹² Gamma (γ) is a measure of the degree of association and may extend from -1 to $+1$, 0 indicating no association. Groups with small numbers were combined with neighbouring groups.

Results

Of the 258 liver biopsy samples examined for fibronectin, 142 showed a staining reaction suitable for semiquantitative assessment. The remaining 116 were excluded. Suitability for assessment of fibronectin decreased with increasing degree of steatosis (table I; $\gamma = -0.24$, $p < 0.01$).

Fibronectin was detected in normal or slightly increased amounts in the acinar zone 3 of 110 biopsy samples as a fine linear outline of the perisinusoidal spaces. In 27 cases fibronectin was present in moderately increased amounts (figure) and in five cases significant amounts. In all these 32 samples fibronectin was detected diffusely along the sinusoids in zone 3 and encircling single or small groups of liver cells (figure). Fibronectin was not observed intracellularly in any case, and there was no topographical relation to liver cells with steatosis or alcoholic hepatitis. The amount of fibronectin was positively correlated with the degree of steatosis (table I; $\gamma = 0.34$, $p < 0.02$). As only three biopsy samples showed alcoholic hepatitis, for analysis this group was combined with the group showing severe steatosis without alcoholic hepatitis.

Of the 142 patients, 23 (16%; confidence interval 11% to 24%) were found to have developed cirrhosis at follow up. A similar rate of cirrhosis was found among the 116 men with biopsy samples unsuitable for assessment of fibronectin (15 cases (13%; confidence interval 7% to 21%); $\gamma = 0.13$, $p > 0.2$). A similar result was obtained when stratifying by the degree of steatosis ($\gamma = 0.26$; $p > 0.09$). Thus suitability of the biopsy sample for assessment of fibronectin was not significantly related to the follow up results.

Table II shows that the amount of fibronectin was related to the later development of cirrhosis ($\gamma = 0.62$; $p < 0.005$). Of the 110 men whose liver biopsy samples contained normal or slightly increased amounts of fibronectin in acinar zone 3, the follow up examination showed cirrhosis in 12 (11%; confidence interval 6% to 18%). Of the patients with moderately increased

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TABLE 1—Amounts of fibronectin in liver biopsy samples in relation to degree of steatosis and presence or absence of alcoholic hepatitis. Figures are numbers (percentages) of samples

	Fibronectin normal or slightly increased (+)	Fibronectin moderately increased (++)	Fibronectin significantly increased (+++)	Total examined	Not examined	Total samples
<i>No alcoholic hepatitis</i>						
No steatosis	30 (27)	4 (15)	0	34 (24)	20 (17)	54 (21)
Slight steatosis	47 (43)	11 (41)	3 (60)	61 (43)	41 (35)	102 (40)
Moderate steatosis	29 (26)	7 (26)	1 (20)	37 (26)	37 (32)	74 (28)
Severe steatosis	3 (3)	3 (11)	1 (20)	7 (5)	11 (10)	18 (7)
<i>Presence of alcoholic hepatitis</i>						
Moderate/severe steatosis	1 (1)	2 (7)	0	3 (2)	7 (6)	10 (4)
Total	110 (100)	27 (100)	5 (100)	142 (100)	116 (100)	258 (100)

Acinar zone 3 showing moderately increased amounts of fibronectin (++) in both perisinusoidal and pericellular patterns. Normal amount of fibronectin present at periphery of sample.

TABLE 2—Amounts of fibronectin in initial biopsy samples and subsequent occurrence of cirrhosis

	Fibronectin normal or slightly increased	Fibronectin moderately increased	Fibronectin significantly increased	Total
No of men	110	27	5	142
No (%) who developed cirrhosis	12 (11)	8 (30)	3 (60)	23 (16)

Increased amount of fibronectin initially and risk of subsequent cirrhosis: $p < 0.005$.

amounts of fibronectin, cirrhosis developed in eight (30%; confidence interval 14% to 50%), and of the patients with significantly increased fibronectin, the disease occurred in three (60%; confidence interval 15% to 95%).

When stratified by the degree of steatosis fibronectin remained significantly related to the development of cirrhosis ($\gamma = 0.58$; $p < 0.02$). The previously reported relation between degree of steatosis and development of cirrhosis³ remained when the amount of fibronectin was taken into account in the analysis ($\gamma = 0.41$; $p < 0.03$).

Discussion

Fibronectin is a non-collagenous glycoprotein found in an insoluble form in most tissues as part of the extracellular matrix.¹³ In normal liver parenchyma only small amounts of fibronectin are present in the perisinusoidal spaces along the sinusoids¹¹ whereas increased amounts have been found in conditions with progressive fibrosis of different aetiology.¹⁴

The relevance of fibronectin in the development of fibrosis is not fully understood but has been attributed to its role as a primary

matrix for the new deposition of collagen.⁶ Large amounts of fibronectin occur especially in early granulation tissue, and during maturation of the tissue fibronectin gradually disappears.⁶ Thus the amount of fibronectin does not simply reflect the actual amount of collagen present but rather the fibrogenetic activity. Assessment of fibronectin may therefore be expected to be valuable for predicting the development of progressive fibrosis and later cirrhosis.

We found a highly significant correlation between the amount of fibronectin in initial biopsy samples and the subsequent development of cirrhosis. Of five patients whose initial biopsy samples contained abundant fibronectin (+++), three developed cirrhosis (table II). Interestingly, with an increasing degree of steatosis it became more difficult to assess the amount of fibronectin, but when assessment was possible the amount appeared to increase. Thus there seems to be a strong correlation between the amount of fibronectin and the degree of steatosis. When stratification was performed according to the degree of steatosis, however, fibronectin remained strongly related to the development of cirrhosis.

A previous study of the same series of patients showed a significant correlation between the occurrence of steatosis and steatosis combined with alcoholic hepatitis and the subsequent development of cirrhosis.³ This correlation remained when the amount of fibronectin was taken into account. Thus the results of our analysis of fibronectin do not merely reflect the previous results. Furthermore, there was no topographical relation between the occurrence of fibronectin and steatosis and steatosis combined with alcoholic hepatitis. An increased amount of parenchymal fibronectin may therefore be a new and important marker of the later development of alcoholic cirrhosis.

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