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EFFECT OF SPLENECTOMY ON LIVER FIBROGENICITY IN PORTAL HYPERTENSION PATIENTS

By
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This study was designed to assess the role of the spleen on the presence and localization of liver fibronectin, to measure the amount of hepatic collagen and serum level of fibronectin and procollagen III in cases of portal hypertension.

The study was performed on 40 patients divided into two groups: Portal hypertension group (20 patients) included one pure schistosomal hepatic fibrosis, three mixed schistsomal and virus C cirrhosis, ten virus C hepatic cirrhosis, two virus B hepatic cirrhosis and four mixed B&C infections. Control group (20 patients) presented with different splenic insults without hepatic affection. In the first group, through clinical examinations and sonographic findings revealed hepatosplenomegaly and grade III & IV varices with history of injection sclerotherapy in 65% of cases.

Preoperatively, marked hepatic fibronectin deposition in portal tracts and around blood vessels raised the stage of fibrosis. This was accompanied by decrease in plasma level of fibronectin and increase of serum level of procollagen III in relation to the control group.

Six months post-operatively, tissue fibronectin, collagen content and serum procollagen III were insignificantly decreased. However, plasma level of fibronectin was increased significantly.

These results denoted that fibronectin is one of the predominant early fibrogenic components in chronic liver diseases, stimulating further collagen deposition. It is concluded that the role of the spleen in the development of hepatic fibrosis seems ancillary. Multifactorial influences, including etiology, timing of splenectomy, hepatic vascular adjustment, and factors associated with the biology of extracellular matrix of the liver, probably play a more significant role. Further studies were recommended to assess the role of splenectomy on liver fibrogensis at different stages of fibrosis. Intervention with splenectomy could improve or reverse fibrogenic activity at early stages.

Key words: *Splenectomy, fibrogenicity, fibronectin, procollagen III, liver fibrosis, portal hypertension*

INTRODUCTION

Liver cirrhosis results from the majority of chronic liver insults and is associated with a significant morbidity and mortality. At present, the only curative treatment for end stage cirrhosis is transplantation, but even in the developed world, the number of donor organs available and the clinical condition of the potential recipient limit the applicability of this technique. Thus, there is a considerable imperative to develop antifibrotic strategies that are applicable to liver cirrhosis. In clinical circumstances where an effective treatment for the underlying insult is available, remodeling of the scar tissue can occur and a return towards architectural normality has been documented even in advanced cirrhosis. This has been most clearly documented in autoimmune disease, but is paralleled by observations of haemochromatotic patients after

venesection and patients with hepatitis B and C after successful interferon therapy. [1]

The role of the spleen on the pathogenesis of schistosomal peri-portal fibrosis of the liver was evaluated. It was found that peri-portal fibrosis appeared less frequently in splenectomized bilharzial mice.[2] It was suggested that the spleen should be an immunomodulatic organ in playing an exacerbation role of hepatic cirrhosis, and the effect of splenectomy played a preventive role against the induced rat liver cirrhosis, at least, at certain stage.[3]

Markers of fibrogenesis represent modern noninvasive tests for fibrotic liver process detection in different disease. The most often used markers of hepatic fibrogenesis are the following: procollagen III peptide, procollagen IV, hyaluronic acid, fibronectin, tenascine and unduline. Biochemical markers of fibrogenesis are useful in regular monitoring of disease development and treatment effectiveness and should be an inseparable part of progression assessment in all-chronic hepatopathies.[4]

AIM OF WORK:

Evaluation of the fibrogenic activity in normal and diseased liver before and after splenectomy by estimation of serum procollagen III and plasma fibronectin, and their correlation with tissue fibronectin and collagen content of the liver in both groups.

PATIENTS AND METHODS

This study was conducted in Theodor Bilharz Research Institute and Kasr El-Aini University Hospital from January 2000 to June 2001 on 40 patients classified into two groups:

1-Portal hypertension group:

Include 20 patients diagnosed as portal hypertension by past history of bilharzial infestation or treatment of bilharziasis, hepatomegaly or splenomegaly or both by clinical examination, dilated portal vein>10mm and/or cirrhotic pattern of the liver by sonography, esophageal varices by gastroscopy and/or liver biopsy. This group underwent elective splenectomy and devascularization.

2- Control group :

Include 20 patients with no evidence of either portal hypertension or chronic liver disease admitted to the Surgical Department for splenectomy (13 urgent splenectomy because of traumatic ruptured spleen, 5 with splenic hydatid disease and 2 with splenic abscess).

Both groups were subjected to proper history taking, full clinical examination, routine blood investigation as well as abdominal sonography& gastroscopy . Wedge liver biopsies were taken during splenectomy, while needle liver biopsies were taken 6 months post-operatively for the portal hypertension group.

Fresh frozen biopsies and formalin-fixed, paraffine embedded sections were used in this study. Routine haematoxyline and eosin stain was performed for proper diagnosis, Masson trichrome stain was used for estimation of fibrosis score (mild, moderate and marked).

Indirect immunofluorescent technique was applied on frozen liver sections to identify tissue fibronectin (intensity and distribution) [5]

Hepatic total collagen content was estimated by measurements of hydroxyproline in hepatic tissue according to the equation [6]:

-Collagen content in the liver = concentration of hydroxyproline in the liver X 10 ug/gm liver X 2 .

-Concentration of hydroxyproline in liver tissue = concentration from the curve X 31.25 ug/gm liver.[7]

Determination of serum procollagen III and plasma fibronectin was done preoperatively as well as two weeks, three months and six months post- operatively.

Serum procollagen III was measured by radioimmunoassay technique using Farmos diagnostic kits, oulusalo, Finland .[8] While plasma fibronectin was determined by single radial immunodiffusion plates from Bohring Institute, Germany.[9]

RESULTS

1-Portal hypertension group:

Include 20 patients, their ages ranged between 17-55 years old. They were 6 females and 14 males. Patients usually presented with dragging left hypochondrial pain (85%), easy fatigability (60%), history of haematemsis (55%), history of melena without haematemsis (30%) and jaundice (10%).

Sonography revealed hepatosplenomegaly in 65% and shrunken liver in 35%.

Upper endoscopy showed grade I varices (10%), grade II (25%), grade III & IV with history of injection sclerotherapy in (65%) of cases.

Routine laboratory investigations revealed anaemia, leucopenia, thrombocytopenia, hypoprothrombinaemia as well as hypoalbuminaemia with mild elevation of liver function tests in the portal hypertension group when compared with the control group (table 1).

Pre-operatively, plasma fibronectin showed highly significant decrease (P<0.001); while serum procollagen III was significantly increased when compared with the control group.Following splenectomy; Plasma fibronectin level was significantly increased (P<0.05) three & six months post-operatively. However, the changes in procollagen level was insignificant (table 2).

Histopathologically, only one patient was diagnosed as pure bilharzial fibrosis(5%), two patients (10%) had post virus B hepatitic cirrhosis ,three patients (15%) had mixed liver cirrhosis (bilharzial peri-portal fibrosis as well as post-hepatitic C cirrhosis), four (20%) showed mixed B&C hepatitic cirrhosis (fig.1), and the last ten patients(50%) were found to be of post virus-C hepatitic cirrhosis (fig.2).

Development of liver fibrosis ,regardless of aetiology, entails major alterations in the both quantity and quality of hepatic extracellular matrix and there is overwhelming evidence that activated hepatic stellate cells (HSC, fat storing cell, or lipocyte) are the major producers of the fibrotic neomatrix.

Marked hepatic fibronectin deposition was found in portal tracts as well as perivascular spaces in advanced stages of fibrosis (Fig.3&4).

There was significant increase of total hepatic collagen content in the portal hypertension group when compared with the control group (P<0.001). Insignificant decrease seen in liver pathology after splenectomy as regards the hepatic total collagen & intensity of tissue fibronectin (table 3).

2- Control group:

Include 20 patients, their age ranged between 25-60 years old. They were 9 females and 11 males. The clinical presentation varied from cardiovascular instability, upper left hypochondrial brusies as well as haemoperitonium in 13 patients to dyspepsia , heartburn and left hypochondrial pain and/or constitutional symptoms in 7 patients respectively.Thorough clinical examination and investigations(routine lab tests, upper endoscopy, and sonography or computed tomography if needed) as well as surgical exploration revealed traumatic rupture of the spleen (13 patients), hydatid cyst of the spleen (5 patients) and splenic abscess (2 patients) with no evidence of portal hypertension or liver cirrhosis.

Liver biopsy was within normal in twelve patients (60%)of the control group while fatty liver was evident in the remaining eight patients (40%) .

Table (1): ***Routine laboratory investigations***

	Portal hypertension group (Mean ± SD)	*Control group (Mean ± SD)*
Hemoglobin Gm/dl	10.3 ± 1.9	12.51 ± 0.87
RBCs Million/ml	3.46 ± 0.67	4.88 ± 0.49
WBCs 1000/ml	2.80 ± 1.46	7.55 ± 1.99
Platelets 1000/ml	92.4 ± 14.66	225 ± 56.48
Prothrombin conc.%	68.25 ± 12.31	89.9 ± 9.04
S.bilirubin Mg/dl	1.25 ± 0.42	0.63 ± 0.16
S.G.P.T. i.u./dl	21.2 ± 8.21	12.4 ± 9.04
S.G.O.T. i.u./dl	25.53 ± 11.83	12.93 ± 8.89
S.Albumin Gm/dl	3.11 ± 0.72	3.82 ± 0.64
S.Glucose Mg/dl	85.33 ± 8.61	94.6 ± 20.78
Bl. Urea Mg/dl	30.27 ± 9.38	22 ± 4.92
S.creatinine Mg/dl	0.91 ± 0.33	0.61 ± 0.27

Table (2) : ***Plasma fibronectin (mg/l) and serum procollagen III (ug/l) pre and post-operatively***

	Portal hypertension group (Mean ± SD)	*Control group (Mean ± SD)*
Fibronection mg/dl pre-op	141.3 ± 8.1***	285.4 ± 5.3
Two weeks post	147.0 ± 6.7***	291.3 ± 5.4
Three months post	170.0 ± 4.6**	300.6 ± 3.9
Six months post	182.0 ± 7.1**	298.7 ± 3.2
Procollagen ug/l pre-op	7.30 ± 1.4***	2.51 ± 0.6
Two weeks post	8.06 ± 0.9***	2.91 ± 0.5
Three months post	7.80 ± 1.1**	3.21 ± 0.7
Six months post	6.11 ± 0.8**	2.7 ± 0.6

NS: Not significant
* : Significant P<0.05
** : Significant P<0.01
*** : Significant P<0.001

Table(3) : ***Hepatic total collagen content (mg/gm)and liver tissue fibronectin***

	Portal hypertension group (Mean ± SD)		*Control group (Mean± SD)*
	Pre-operative	*Post-operative*	
Total collagen mg/gm	30.7 ± 2.2***	28.6 ± 1.4	4.3 ± 0.81
T Fn parenchymal	+ NS	0/+	0/+
T Fn vascular	+++***	++	0/+
T Fn portal tract	+++***	+++	0/+

0/+ : Minimal
\+ : Mild
++ : Moderate
+++ : Marked
NS : Not significant
* : Significant P<0.05
** : Significant P<0.01
***: Significant: P<0.001

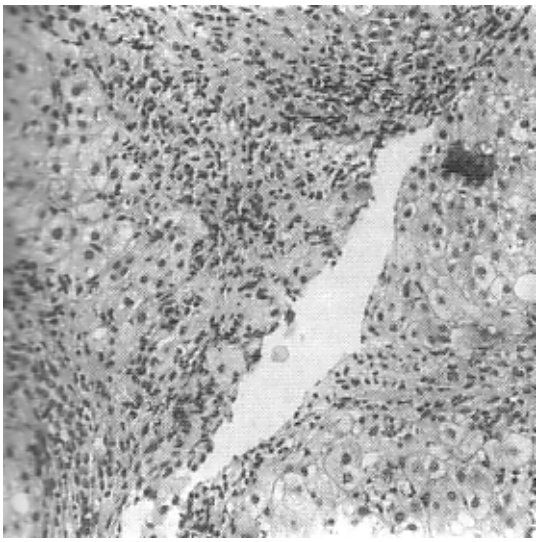

Fig. (1): Mixed infection with hepatitis B & C viruses, showing regenerating nodules (cirrhosis) and piece-meal necrosis (Hx. & E. x 200)

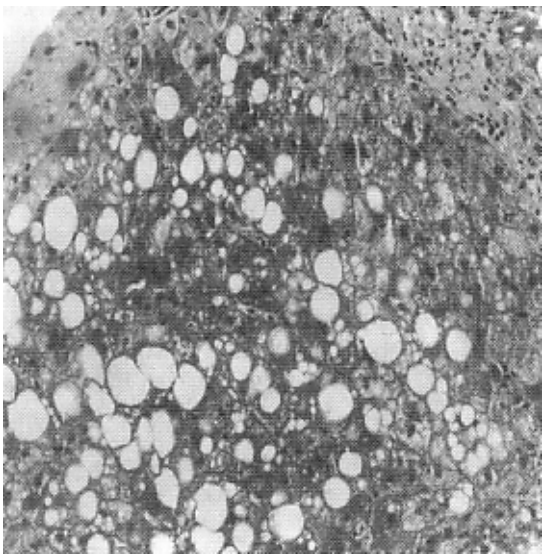

Fig. (2): Hepatitis C viruse Infection showing disturbed architecture, steatotic changesof hepatocytes and thick inflamed portal tracts (Masson trichrome x 200)

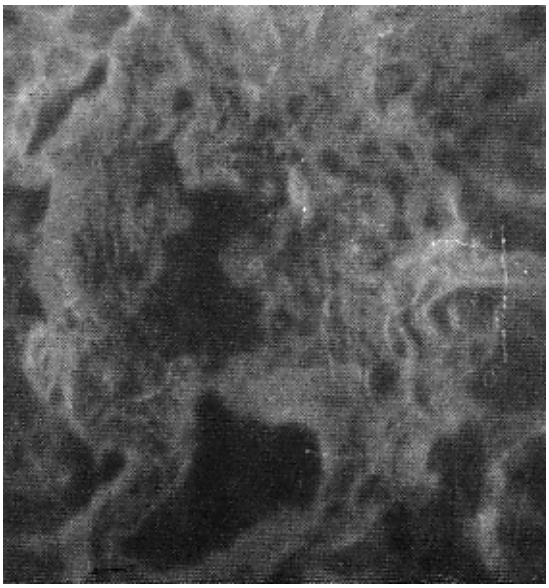

Fig. (3): Indirect immunofluorescet technique in stage IV hepatic fibrosis showing markedly thickened portal tracts with marked positive staining x 250.

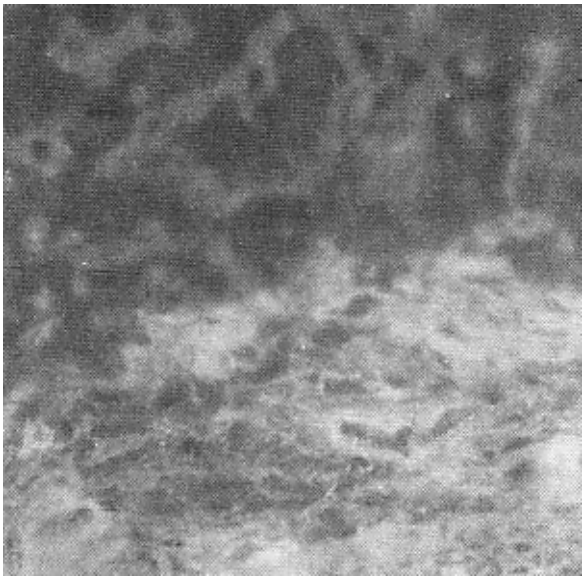

Fig. (4): Indirect immunofluorescet technique in stage III hepatic fibrosis showing moderate positive staining in thickened portal tract and in the wall of blood vessels also sinusoids showed increased positivity x 250.

DISCUSSION

Most liver diseases lead to a pathobiochemical reaction termed liver fibrosis. This is a dynamic process implying different rates of progression or regression. Thus, histological examination of a liver biopsy is essential for a diagnosis but biochemical tests are necessary for assessing the activity of the process and monitoring its evolution. [10]

Hepatic schiostosomiasis is a chronic endemic disease in Egypt. It represents the most prevalent health problem. Nowadays, pure bilharzial hepatitis became extremely rare and most of the hepatic fibrosis was either due to post viral hepatitis C and/or B, or mixed bilharzial and viral hepatitic pathology. Accumulation of collagen is a major pathological feature in a variety of fibrotic processes affecting liver cirrhosis.[11, 12, 13]

Recent advances in the understanding of the normal biochemistry of collagen have allowed to define specific levels of collagen biosynthesis and degradation at which a pharmacologic intervention could lead to reduced collagen deposition in tissues. [14]

In this study, total hepatic collagen in the control group was 4.3 $\pm$ 0.81mg/gm. It did not show any difference between normal and fatty livers.[15] The difference was highly significant in portal hypertension group (30.7 $\pm$ 2.2 mg/gm) (about 7 folds more than that of control group) with $p<0.001$. Other series observed 3 to 6 fold elevations of collagen content in cirrhotic liver.[16] However; no difference was elicited between different etiologies of hepatic fibrosis.[17, 18]

In accordance with our results, a positive correlation was observed between total collagen content of the liver and the amount of collagen fiber deposition.[19]

It was suggested that serum procollagen type III (PIIIP) could be a valuable, non-invasive monitor of hepatic fibrogenesis.[20]A close correlation has been observed between the serum PIIIP concentration and the degree of fibrosis in portal tracts.[21]

In this work, serum procollagen type III (PIIIP) showed no significant difference between the normal and fatty liver or between males and females. A temporary rise of its level was observed three months post-operatively in the control group because of healing process.

In the portal hypertension group, the preoperative level of serum PIIIP (7.30 $\pm$ 1.4 ug/l) was significantly higher than that of the control group (2.51 $\pm$ 0.6 ug/l). This marked increase in serum PIIIP could be attributed to increased synthesis by activated fibroblasts or impaired catabolism by the fibrotic liver.[10, 22, 23]

Serum level of PIIIP was elevated in fibrosing chronic liver diseases such as alcoholic cirrhosis, untreated chronic active hepatitis and primary biliary cirrhosis.[24] In contrast, Patients with chronic persistent hepatitis and fatty liver without fibrosis had normal or slightly elevated values. This

indicate that serum PIIIP may reflect the fibrogenesis or the disease activity of the liver. [25]

The liver was found to be the main source of plasma fibronectin and so its level might reflect the degree and severity of liver fibrosis or cirrhosis.[26] On contrary, other authors reported that determination of plasma FN concentration has no importance in evaluation of the degree of liver fibrosis.[27] This study showed a significant decrease of plasma fibronectin in portal hypertension group (141.3 $\pm$ 8.1mg/l) as compared with the control group (285.4 $\pm$ 5.3mg/l) $P<0.001$. This decrease was claimed to impaired hepatic synthesis, increased splenic consumption with high phagocytosis or increased catabolism mediated by accelerated fibrinolysis. [28, 29, 30]

There was significant increase of plasma fibronectin 3 months and 6 months post-operatively in portal hypertension group with $P<0.05$ and $P<0.01$ respectively. This may be explained either by removal of the enlarged spleen which was responsible for increased degradation of fibronectin and/or increased hepatic synthesis due to improvement of liver condition post-operatively.[31] This increase was statistically insignificant in the control group. This may be claimed to the role of fibronectin in wound healing through its incorporation to fibrin clot, incorporation to tissue wastes or binding to tissue debris.[27]

In conclusion, serum procollagen III peptide, serum fibronectin and tissue fibronectin are specific markers for fibrogenesis in chronic viral hepatitis and concomitant schistosomal hepatic fibrosis. Hepatic collagen deposition was insignificantly decreased following splenectomy when compared with its level pre-operatively. On contrary serum fibronectin showed a mild to moderate significant increase post-operatively. It is concluded that the role of the spleen in the development of hepatic fibrosis seems ancillary. Multifactorial influences, including etiology, timing of splenectomy, hepatic vascular adjustment, and factors associated with the biology of extracellular matrix of the liver, probably play a more significant role. Further studies were recommended to assess the role of splenectomy on liver fibrogensis at different stages of fibrosis.

REFERENCES

1. Benyon, R. C. and J. P. Iredale (2000). "Is liver fibrosis reversible?" Gut 46(4): 443-6.
2. Andrade, Z. A., L. M. Silva, et al. (1998). "Role of the spleen on the pathogenesis of schistosomal periportal (pipestem) fibrosis of the liver: an experimental approach." Am J Trop Med Hyg 59(4): 557-62.
3. Chen, D., W. Liu, et al. (1998). "Effect of splenectomy on CCl4-induced liver fibrosis in rats." Chin Med J (Engl) 111(9): 779-83.
4. Szantova, M. and V. Kupcova (1999). "[Biochemical markers of fibrogenesis in liver diseases]." Bratisl Lek Listy (English abstract) 100(1): 28-35.
5. Wilson, M., J. Fried, et al. (1977). "Evaluation of the indirect immunofluorescence and complement fixation tests for the serodiagnosis of schistosomiasis." Am J Trop Med Hyg 26(6 Pt 1): 1159-63.
6. Berg, R. A. (1982). "Determination of 3- and 4-hydroxyproline." Methods Enzymol 82 Pt A: 372-98.
7. Bradley, K., S. McConnell-Breul, et al. (1975). "Collagen in the human lung. Quantitation of rates of synthesis and partial characterization of composition." J Clin Invest 55(3): 543-50
8. Risteli, L. and J. Risteli (1987). "Analysis of extracellular matrix proteins in biological fluids." Methods Enzymol 145: 391-411.
9. Bowen, M. and T. Muller (1983). "Influence of sample preparation on estimates of blood fibronectin concentration." J Clin Pathol 36(2): 233-5.
10. Plebani, M. and A. Burlina (1991). "Biochemical markers of hepatic fibrosis." Clin Biochem 24(3): 219-39.
11. Abdel-Wahab, M. F., S. Zakaria, et al. (1994). "High seroprevalence of hepatitis C infection among risk groups in Egypt." Am J Trop Med Hyg 51(5): 563-7.
12. Arthur, R. R., N. F. Hassan, et al. (1997). "Hepatitis C antibody prevalence in blood donors in different governorates in Egypt." Trans R Soc Trop Med Hyg 91(3): 271-4.
13. El-Zayadi, A.,Dabbous, H., et al.(1998)."Current status of HCV infection in Egypt ." Cairo Liver Center, Cairo.
14. Peterson, T. C., P. Hodgson, et al. (2000). "Hepatic fibrosis and cytochrome P450: experimental models of fibrosis compared to AHR knockout mice." Hepatol Res 17(2): 112-125.
15. Pilette, C., M. C. Rousselet, et al. (1998). "Histopathological evaluation of liver fibrosis: quantitative image analysis vs semi-quantitative scores. Comparison with serum markers." J Hepatol 28(3): 439-46.
16. Takamatsu, S., H. Nakabayashi, et al. (1997). "Noninvasive determination of liver collagen content in chronic hepatitis. Multivariate regression modeling with blood chemical parameters as variables." J Gastroenterol 32(3): 355-60.
17. Poynard, T., V. Ratziu, et al. (2000). "Fibrosis in patients with chronic hepatitis C: detection and significance." Semin Liver Dis 20(1): 47-55.
18. Stone, P. J. (2000). "Potential use of collagen and elastin degradation markers for monitoring liver fibrosis in schistosomiasis." Acta Trop 77(1): 97-9.
19. Gu, S., H. Zhang, et al. (1999). "[Relationship between serum fibrosis markers and fibrosis quantitative analysis of liver

tissue]." Zhonghua Gan Zang Bing Za Zhi (English abstract) 7(4): 199-200

20. Imbert-Bismut, F., V. Ratziu, et al. (2001). "Biochemical markers of liver fibrosis in patients with hepatitis C virus infection: a prospective study." Lancet 357(9262): 1069-75.

21. Hayasaka, A. and H. Saisho (1998). "Serum markers as tools to monitor liver fibrosis." Digestion 59(4): 381-4.

22. Ding, H., Y. Chen, et al. (2001). "[Correlation between liver fibrosis stage and serum liver fibrosis markers in patients with chronic hepatitis B]." Zhonghua Gan Zang Bing Za Zhi (English abstract) 9(2): 78-80.

23. El-Mohandes, M., H. Hassanein, et al. (1987). "Serum concentration of N-terminal procollagen peptide of collagen type III in schistosomal liver fibrosis." Exp Mol Pathol 46(3): 383-90.

24. Luo, R., S. Yang, et al. (2001). "[Diagnostic value of five serum markers for liver fibrosis]." Zhonghua Gan Zang Bing Za Zhi (English abstract) 9(3): 148-50.

25. Schuppan, D., C. Jax, et al. (1999). "[Serum markers of liver fibrosis]." Dtsch Med Wochenschr (English abstract) 124(41): 1213-8.

26. Golubovic, M., N. Majkic-Singh, et al. (1999). "[Diagnostic importance of fibronectin in chronic liver diseases]." Med Pregl (English abstract) 52(1-2): 35-8.

27. Simon, K., M. Zalewska, et al. (1995). "[Plasma fibronectin in chronic liver disease--marker of fibrosis?]." Przegl Lek (English abstract) 52(4): 129-32.

28. Soresi, M., D. Di Martino, et al. (1993). "[Plasma fibronectin in chronic liver diseases]." Recenti Prog Med (English abstract) 84(9): 602-7.

29. Abdel-Rahman, HM., Kamel, LN., et al. (1988)."Fibronectin and orosomucoid in schistosomal hepatic fibrosis with and without haematemsis" Egypt.J.Bilh. 10 (2): 187-198.

30. Sidlo, J., J. Jakubovsky, et al. (1996). "[Fibronectin and the human spleen]." Cesk Patol (English abstract) 32(1): 14-8.

31. De Angelis, V., M. Zambon, et al. (1988). "Fibronectin decrease in liver cirrhosis is related to spleen size." Klin Wochenschr (English abstract) 66(12): 524-6.