

## Original Article

# Pathological changes and iNOS expression in portal vein and hepatic artery of patients with liver cirrhosis and portal hypertension

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**Abstract:** *Objective:* To investigate pathological alterations in morphologies of the portal vein, hepatic artery, and hepatic vein in patients with liver cirrhosis and portal hypertension (PHT), to study inducible nitric oxide synthase (iNOS) expression in the hepatic artery wall, and to explore roles of iNOS in pathogenetic mechanisms of portal hypertensive vasculopathy. *Methods:* Sections of the portal vein, hepatic artery, and left liver vein were obtained from 11 noncirrhotic patients during hepatectomy and 24 patients with liver cirrhosis and PHT during liver transplantation. Specimens were observed by optical microscopy. Expression of iNOS in liver artery walls was analyzed by immunohistochemistry. *Results:* In portal veins of cirrhotic patients, we found proliferative intima, extensive thrombi adhering to the venous walls, and thickened muscle fibers of veins with increased extracellular matrix. Thrombi mimicked arteriosclerotic plaques and were accompanied by smooth muscle cell hypertrophy. Internal elastic membrane and medial elastic fibers of the hepatic artery wall were broken and degenerated. Few changes in the hepatic vein were observed. Positive staining for iNOS was seen in the smooth muscle cell cytoplasm. Furthermore, iNOS activity was elevated in cirrhotic compared to noncirrhotic patients ( $P < 0.01$ ). *Conclusion:* PHT may be complicated by hepatic arterial and portal vein vasculopathy. There may be an interactive relationship among PHT, hyperdynamic splanchnic circulation, and splanchnic vasculopathy in the pathogenesis of PHT.

**Keywords:** iNOS, liver cirrhosis and PHT, portal hypertensive vasculopathy

## Introduction

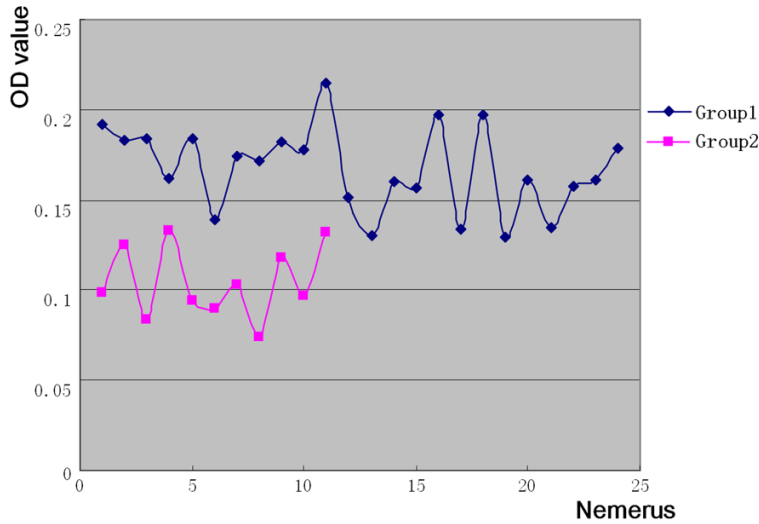
Liver cirrhosis and portal hypertension (PHT) are benign end-stage hepatic diseases that are commonly observed during hepatobiliary surgeries. Therapeutic measures for these conditions are limited. Research on pathological changes of visceral vessels in patients with PHT has mainly focused on splenic, gastric, and small intestine vessels [1-3]. Meanwhile, studies of the liver and portal vessels, as well as studies of inducible nitric oxide synthase (iNOS) expression in the liver artery wall, have been limited to animal experiments [4, 5]. Researchers [6] suggested that nitric oxide (NO) production and bioavailability may be diminished in cirrhotic livers, contributing to increased intrahepatic vascular resistance. Increased iNOS or reduced endothelial nitric oxide synthase (eNOS) levels induced development of

fibrosis in some animal experiments [7, 8]. To confirm the above findings in humans, we designed this study to observe the pathological intrahepatic vascular changes, including iNOS expression in the hepatic artery wall, in patients with liver cirrhosis and PHT.

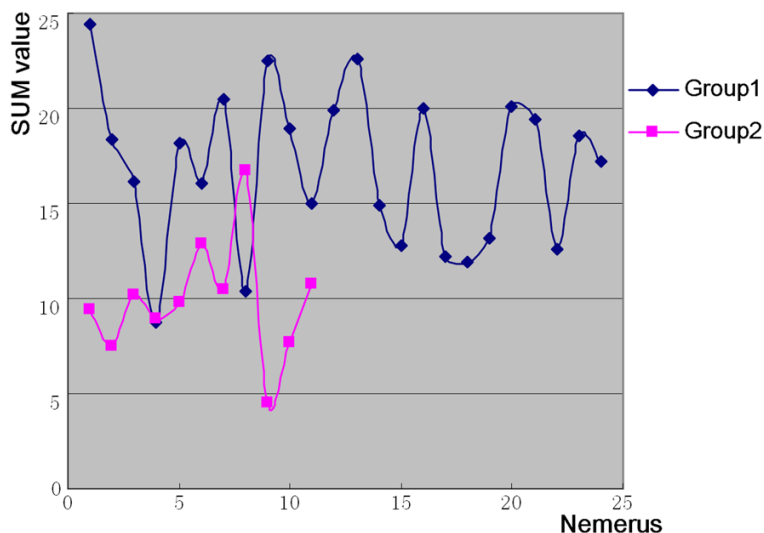
## Materials and methods

Clinical data of liver transplantation cases were collected. We obtained 1-cm sections of the left portal vein (from 1st hepatic hilus), left hepatic artery (from 1st hepatic hilus), and left hepatic vein (from 2nd hepatic hilus) from resected livers. Collected samples were free of oncothliposis or tumor embolus. Specimens in the control group were 1-cm sections of the portal vein, hepatic artery, and hepatic vein from similar positions as above, obtained from patients with traumatic hepatorrhesis who were undergoing hepatectomy.

## Pathological changes of portal vein and hepatic artery in cirrhosis



**Figure 1.** Curves of OD of the patient and control groups. Group 1: Patient Group; Group 2: Control Group.



**Figure 2.** Curves of the SUM of the patient and control groups. Group 1: Patient Group; Group 2: Control Group.

**Table 1.** Statistical results of average OD and total SUM

	Group Name	X±SD	F-analysis		T-test	
			F	Sig	t	Sig
OD	Patient group	0.167±0.023	0.428	7.743	7.743	0.000
	Control group	0.105±0.020				
SUM	Patient group	16.858±4.122	2.957	4.970	4.970	0.000
	Control group	9.894±3.131				

Specimens were serially sectioned and allocated to two groups, according to whether the specimen was obtained during liver transplan-

tation (patient group) or hepatectomy (control group). Specimens were submitted to formalin fixation and paraffin embedding. Hematoxylin and eosin (HE), Masson, and elastic fiber staining processes were performed. Analysis of iNOS was done through immunohistochemistry (IHC) staining of artery specimens. Staining was observed by optical microscopy. A pathological image analyzer was used to acquire and analyze images. Four visual fields from every slice were observed under a high-power lens (×400). Optical density (OD) and total staining-positive area (SUM) were obtained.

### Statistical analyses

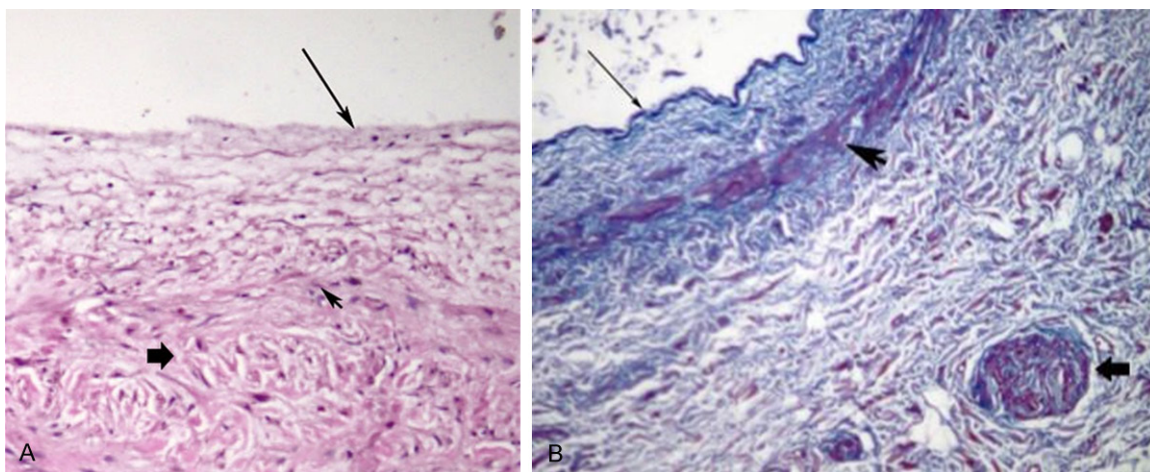
Statistical analyses were conducted with SPSS 19.0 software. Significance level was set at  $P < 0.05$ . Values of OD and SUM from IHC staining of hepatic artery sections of the two groups were recorded as mean ± standard deviation ( $X \pm SD$ ). Statistical comparisons were made by Levene's and Student's *t* tests.

### Results

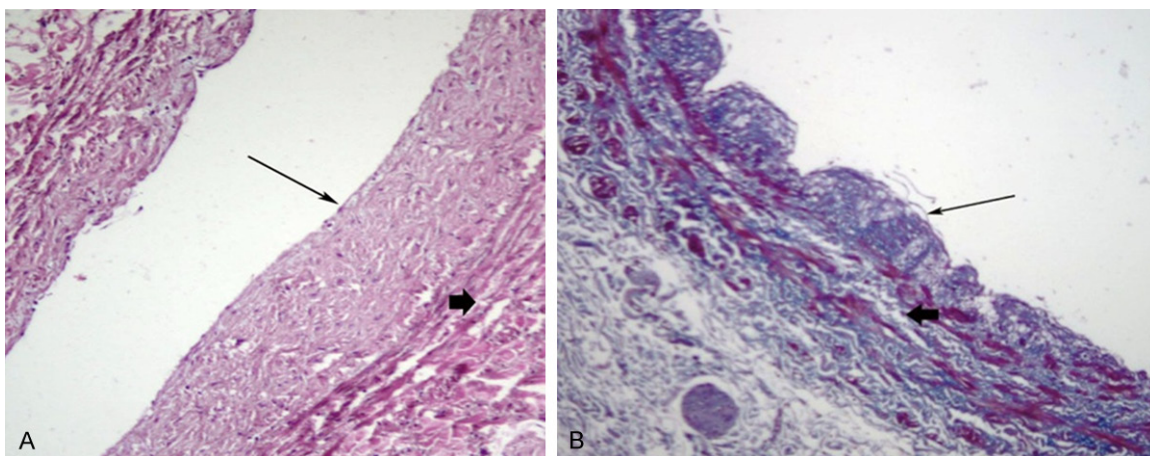
#### Basic information

The patient group comprised 23 men and 1 woman, with an average age of  $44.8 \pm 11.9$  years (range: 22-60 years). According to patients' preoperative Child-Pugh classification, there were 9 cases (37.5%) of grade A, 3 cases (12.5%) of grade B, and 12 cases (50.0%) of grade C liver function. All 24 cases were diagnosed with hepatocirrhosis, which was due to alcoholic cirrhosis (3 cases, 12.5%), hepatitis C (1 case, 4.2%), or hepatitis B virus (HBV) (20 cases, 83.3%). Ten cases

## Pathological changes of portal vein and hepatic artery in cirrhosis



**Figure 3.** HE and Masson staining of portal vein in control group ( $\times 200$ ). Intima appears thin (long arrow), with a thin layer of loosely arranged circular smooth muscle cells (short triangular arrow). Extima appears thick, with longitudinal smooth muscle cells (bold arrow).



**Figure 4.** HE and Masson staining of portal vein in patient group ( $\times 200$ ). Diffuse thickening and distinctive proliferation of the intima (long arrow) can be seen, with obvious proliferation of circular smooth muscle cells of the tunica media, thickened muscle cells, and increased number of layers (bold arrow).

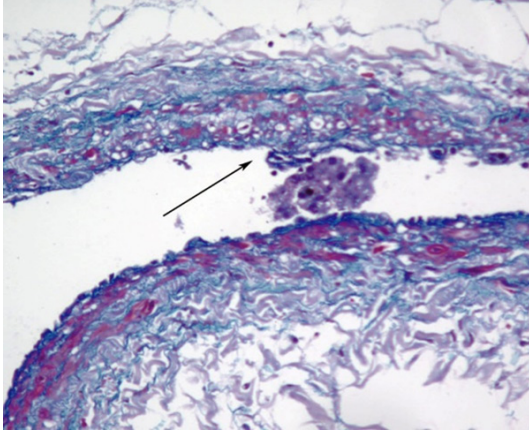
(41.7%), all carrying HBV, were accompanied by liver cancer. The control group comprised 9 men and 2 women, with an average age of  $31.5 \pm 8.4$  years (range: 23-47 years). Controls underwent hemihepatectomy due to liver trauma (8 cases) or giant hemangioma (3 cases). There were no instances of hepatitis B, hepatitis C, cirrhosis, or other liver diseases in the control group.

### Statistical information

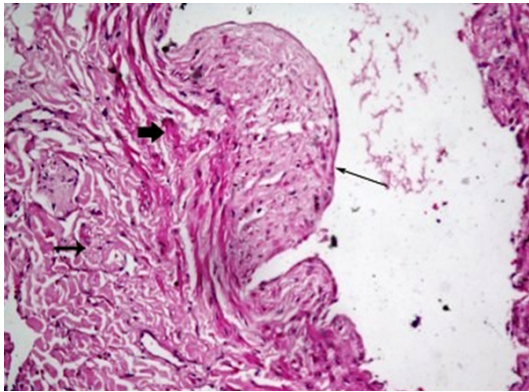
Average value of OD and total SUM acquired from the observation of slices in the patient and control groups are shown in **Figures 1** and

**2**, respectively. The OD and SUM data failed to show heterogeneity of variance (**Table 1**). Therefore, results manifesting homogeneity of variance were used (for OD:  $t = 7.743$ ,  $P < 0.05$ ; for SUM:  $t = 4.970$ ,  $P < 0.05$ ). The OD value was higher in the patient group ( $0.167 \pm 0.023$ ) compared to the control group ( $0.104 \pm 0.020$ ). Similarly, the SUM value was higher in the patient group ( $16.858 \pm 4.122$ ) compared to the control group ( $9.894 \pm 3.131$ ). We concluded that the average and total protein expression levels of iNOS in the artery wall were higher in the patient group than those in the control group.

## Pathological changes of portal vein and hepatic artery in cirrhosis



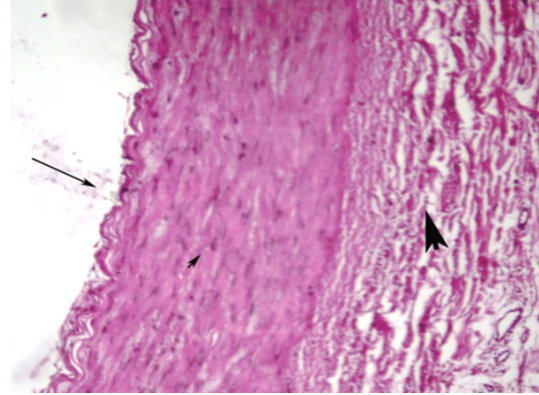
**Figure 5.** Masson staining of portal vein in patient group ( $\times 200$ ). Broken vascular endothelium (long arrow) can be observed, as well as proliferation of the intima (long arrow) and formation of minute thrombi.



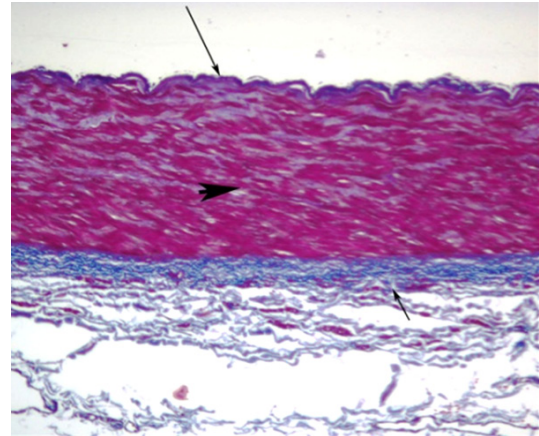
**Figure 6.** HE staining of portal vein in patient group ( $\times 400$ ). Irregularities mimicking atheromatous proliferative plaques can be observed (long arrow), together with twisted irregular thickening of the smooth muscle cells in the tunica media (bold arrow).

### *Clinical pathological changes*

**Arterialized remodeling of the portal vein:** Portal veins from the control group were observed under optical microscopy. Thin intima was observed, with the tunica media being formed by several layers of loosely arranged circular smooth muscle cells (SMCs). Thick extima was formed by connective tissues comprised of many longitudinal SMC bundles (**Figure 3A, 3B**). Features of portal veins from the patient group were as follows. Thickened intima resulted from diffuse and focal thickening. New intima was formed, which comprised proliferative collagen fibers and a large quantity of SMCs arranged in different directions. Buninoid proliferative



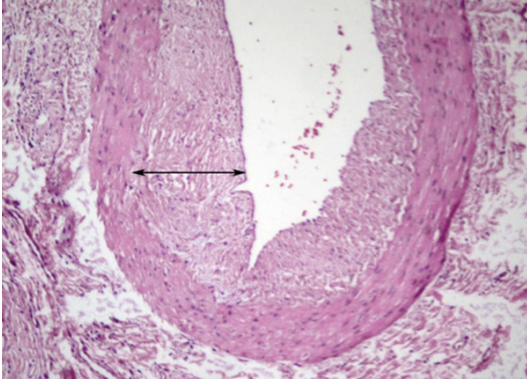
**Figure 7.** HE staining of hepatic artery in control group ( $\times 100$ ). Thin intima (long arrow) can be observed, with continuous internal elastic membrane in the form of ripples. Thick tunica media (small triangular arrow) can be seen. Extima (large triangular arrow) is a layer of thick loose connective tissue.



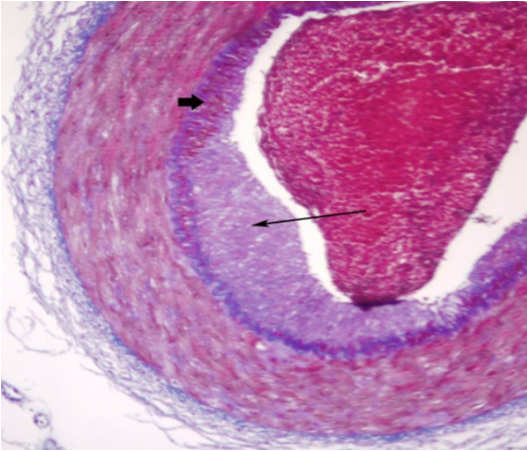
**Figure 8.** Masson staining of hepatic artery in control group ( $\times 100$ ). Long arrow, triangular arrow, and short arrow point to the intima, tunica media, and extima, respectively.

erative plaques protruded towards the inside of the vein. Plaques comprised a large quantity of collagen fibers and SMCs in a disordered arrangement. Platelets and blood cells adhered to the surface of the broken vascular endothelium. Their accumulation gradually led to the formation of minute thrombi, which resembled arteriosclerosis plaques. Thickened tunica media and internal muscle cells arranged in longitudinal, transverse, and oblique directions. The external longitudinal muscle was well-developed, with thickened and dense muscle fibers. Some SMCs were moving from the tunica media to the intima (**Figures 4A, 4B, 5, 6**).

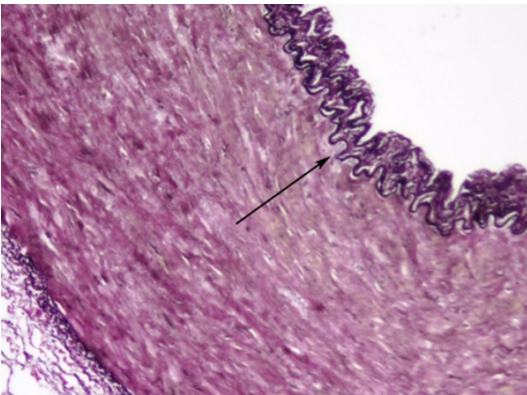
## Pathological changes of portal vein and hepatic artery in cirrhosis



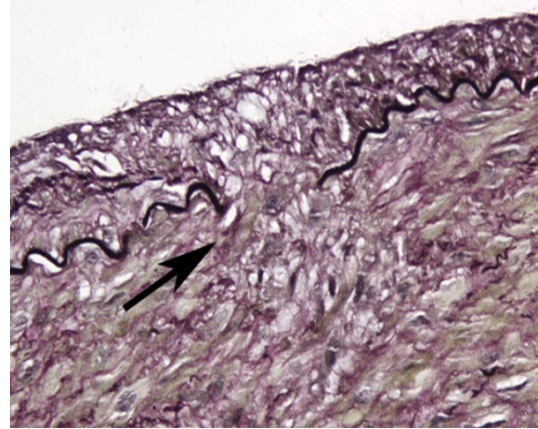
**Figure 9.** HE staining of hepatic artery in patient group ( $\times 100$ ). Obvious irregular thickening of the intima can be seen (arrow).



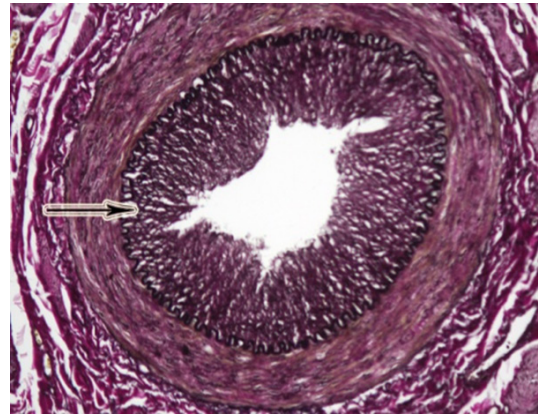
**Figure 10.** Masson staining of hepatic artery in patient group ( $\times 200$ ). Obvious proliferation of the intima (long arrow) can be seen. Some smooth muscle cells are growing towards the subintima and forming new intima (bold arrow).



**Figure 11.** Elastic staining of hepatic artery in control group ( $\times 200$ ). Thin intima appears, composed of a single layer of endothelial cells, with internal elastic membrane (arrow) immediately below, in the form of ripples, with good texture and elasticity.



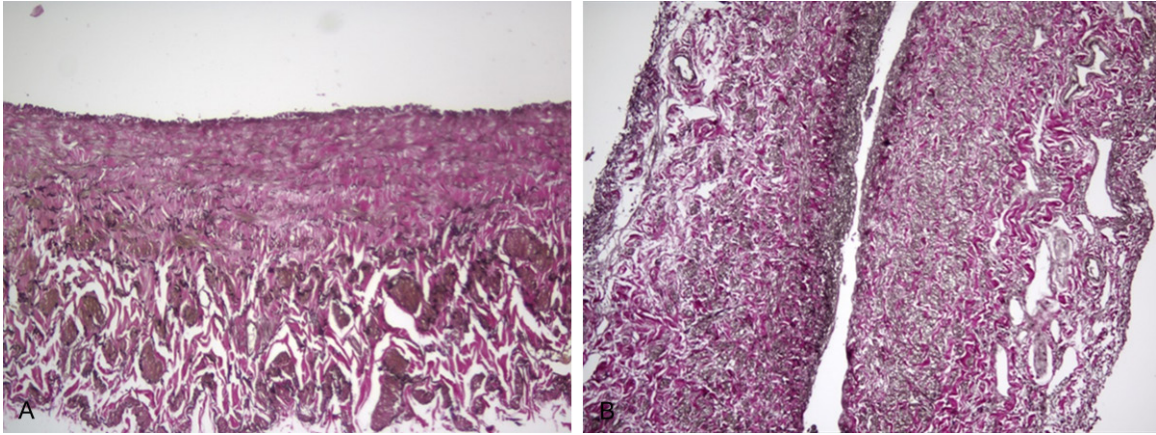
**Figure 12.** Elastic fiber staining of hepatic artery in patient group ( $\times 400$ ). Proliferation of intima and broken internal elastic membrane can be seen. Smooth muscle cells of the tunica media are moving toward the intima and form new intima (arrow).



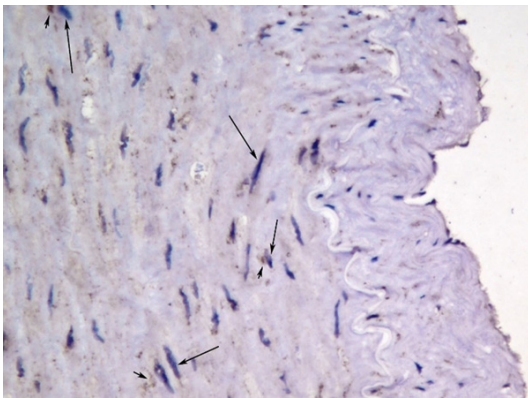
**Figure 13.** Elastic fiber staining of hepatic artery in patient group ( $\times 100$ ). Extensive proliferation of intima and a narrowed arterial lumen can be observed.

*Broken contractile structure of the hepatic artery wall:* Features of the hepatic artery wall of the control group were discerned under optical microscopy. We observed thin intima and wavy internal elastic membrane. Tunica media was relatively thick, formed by 10 to 40 layers of circularly arranged SMCs, with some elastic and collagen fibers among them. Extima was almost as thick as the tunica media and was formed by loose connective tissue (**Figures 7, 8**). Features of the hepatic artery wall from the patient group were as follows. Broken arterial endothelial cells (ECs) were observed, some of which were detached. Platelets adhered to the thickened intima. After elastic fiber staining, we observed flattened ripples, which were formed by folded internal elastic membrane. Some rip-

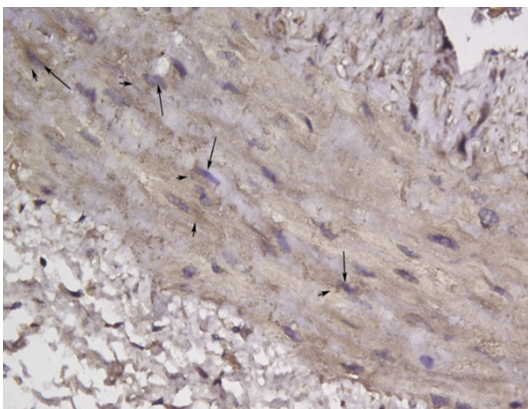
## Pathological changes of portal vein and hepatic artery in cirrhosis



**Figure 14.** HE staining of hepatic vein in control and patient groups ( $\times 200$ ). Few changes between the two groups can be observed.



**Figure 15.** iNOS staining of hepatic artery in control group ( $\times 400$ ). Cytoplasm of tunica media smooth muscle cells show pale staining that appears grainy (short arrow) and wrapped around the smooth muscle cell nuclei (long arrow).



**Figure 16.** iNOS staining of hepatic artery in patient group ( $\times 400$ ). Cytoplasm of tunica media smooth muscle cells show dark staining (short arrow), which appears brownish, platy, and embraces the smooth muscle cell nuclei (long arrow).

ples disappeared and broke into several layers or even broke down. Most of the medial elastic fibers were broken and degenerated. Some SMCs of the tunica media were moving to the intima. Some were atrophied and deformed, whereas others showed hypertrophy and proliferation. SMCs were separated by collagen fibers and arranged in a disordered fashion (**Figures 9-13**).

Few changes in the hepatic vein were observed between the groups (**Figure 14A, 14B**).

### *Expression of iNOS in hepatic artery*

Expression of iNOS in the control group was weakly positive, with yellow granular positive staining surrounding the nucleus. In hepatic arteries from the patient group, expression of IHC-positive substance was observed in the cytoplasm of SMCs from the tunica media, with color ranging from yellow to brown. The substance formed a plate-like structure and surrounded the blue-stained center nucleus (**Figures 15, 16**).

### **Discussion**

Under the influence of damage factors, such as shear stress [4], endotoxins, and inflammatory mediators, some vascular ECs were broken and detached, which led to exposure of collagen fibers, platelet aggregation, and thrombosis. ECs expressed adhesion molecules and released chemotactic factors to stimulate monocytes to bind ECs and penetrate the lower intima. These factors stimulated vascular SMCs to move toward the lower intima and to yield an

## Pathological changes of portal vein and hepatic artery in cirrhosis

extensive extracellular matrix by proliferation and synthesis. We observed a broken intima of the vascular wall in the portal vein and hepatic artery, as well as platelet adhesion, thrombosis, and proliferative intima.

In the portal vein, thickened intima was observed, which comprised proliferative collagen fibers and extensive SMCs in different orientations. Thickened tunica media was observed, with dense and thickened muscle fibers. Extima was infiltrated by inflammatory cells. Proliferation of SMCs heightens vascular contractility, doubling the effective contractility of the portal vein [9] and increasing the hepatic blood supply. This condition can be described as arterialized remodeling of the portal vein. In the hepatic artery, we observed a thickened intima and a flattened or broken internal elastic membrane. Atrophied and deformed SMCs in a disordered arrangement were observed in the tunica media. Transformation of SMCs from contractile to synthetic cells [10, 11] could lead to the observed broken contractile structure and weakened contractility of the hepatic artery wall.

We believe that the arterialized remodeling of the portal vein, weakened contractility of the hepatic artery, and portal hypertensive vasculopathy (PHV) should be attributed to a new concept of cirrhosis-related vasculopathy (CRV). This new concept includes all of the pathological changes of the intra- and extra-hepatic blood vessels. A possible mechanism for this concept is as follows.

PHT is known to originate from the increase of intrahepatic vascular resistance due to various causes. Within a certain range of pressure, vascular tension (T), transmural pressure (P), and geometric parameters of the blood vessels (vascular radius  $r$  and wall thickness  $W$ ) conform to La Place's Law [12],  $T = P (r/W)$ . When vascular tension is within the elastic range, an increase of transmural pressure can be compensated by an increased wall thickness and decreased vascular radius. In cases of cirrhosis and PHT, the vascular wall of the hepatic inflow vessel thickens, and the vascular lumen narrows, buffering the impact of transmural pressure on vascular tension. This process, to a certain degree, functions as compensatory regulation. When the resistance of hepatic blood

inflow increases, the body will compensate for the pressure increase by increasing the vascular wall thickness and decreasing the vascular radius.

When hepatic pathological changes develop to the extent that the transmural pressure exceeds the ability of the blood vessel to compensate, the pressure cannot be buffered by vascular autoregulation. Elastic membrane within the artery will fold, flatten, and even break into parts. Collateral circulation will form in the portal vein system, followed by spontaneous portal systemic shunting and reverse blood flow in the portal vein [4, 13]. As a result, the vascular wall will show an uneven thickness, hard texture, poor elasticity, and fibrosis. Decreased vascular adaptability and reactivity [14, 15] will also be seen. The vessels fail to react to neural or humoral regulation, and the vascular resistance will increase. Therefore, structural changes in the portal vein and hepatic artery are not only the outcomes of autoregulation in the body, but also factors that accelerate formation and development of PHV.

Patients with liver cirrhosis and PHT show increased blood levels of NO, dilated visceral arteries, and increased portal vein blood flow. Tung-Ming Leung et al. [16] showed that the adverse effects of NO in chronic liver injury might be due to increased iNOS levels. Zipprich et al. [17] found that NO was involved in arterial remodeling. In the absence of iNOS, mice exhibited decreased necrosis, increased apoptosis, and reduced liver fibrosis [7]. Hepatitis B or C infection induces iNOS expression in hepatocytes, suggesting that NO overproduction might have an important role in the progression of chronic viral hepatitis to cirrhosis [18].

We believe that iNOS expression is increased in chronic liver disease. Angeli et al. [19] showed that iNOS was upregulated in mesenteric arteries in decompensated cirrhosis. However, the situation in the hepatic artery was not assessed. In our research, we found increased iNOS expression in the hepatic artery wall in the patient group.

We hypothesize that at the early stage of cirrhosis, SMCs of the hepatic artery wall transform from contractile to synthetic cells. Increased iNOS expression leads to NO release

into the blood, stimulating vascular dilation. Subsequent arterial remodeling and increased blood flow increase the hepatic inflow, forming a hyperdynamic splanchnic circulation (HSC) [20]. Increased iNOS expression plays roles in both PHV and HSC. In conclusion, both NO and iNOS are involved in the formation and development of PHV and HSC, and both molecules aggravate PHT. On the other hand, PHV itself could promote PHT and HSC, which, in turn, could aggravate PHV. As a result, a viscous cycle forms among PHT, PHV, and HSC.

### Disclosure of conflict of interest

None.

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## Pathological changes of portal vein and hepatic artery in cirrhosis

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