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The process of liver fibrosis in chronic hepatitis C – histological and immunohistochemical study

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Abstract

Liver fibrosis is one of the most serious histopathological (HP) lesions that, together with the inflammatory process and the hepatocyte lesions, determine the change of the liver architecture, having as a clinical result the onset of liver failure phenomena. Hepatitis C virus represents one of the most frequent conditions leading to the onset of liver fibrosis and favors the progression of the disease towards hepatocellular carcinoma. We evaluated the HP and immunohistochemical (IHC) aspects on fragments of liver biopsies taken from 104 patients diagnosed with chronic hepatitis C and altered capacity of work. In our study, we observed a growth of the portal (Kiernan) spaces by the presence of a chronic inflammatory infiltrate, the presence of collagen fibers and conjunctive matrix. The density and dimensions of collagen fibers were correlated with the severity of the liver disease, in the severe forms being highlighted porto-portal and porto-central fibrous bridges. The IHC examinations highlighted the change of the phenotype of perisinusoidal dendritic cells, the growth of the myofibroblast cells in the portal spaces, the growth of the macrophage number in the inflammatory infiltrate and of the Kupffer cells in the liver parenchyma.

Keywords: chronic hepatitis C, liver fibrosis, inflammatory infiltrate, extracellular matrix, hepatic stellate cells.

Introduction

Liver fibrosis is one of the most severe histopathological (HP) lesions that, together with the inflammatory process and the hepatocyte lesions, determine the change of the liver architecture, having as a clinical result the onset of liver failure phenomena; untreated appropriately, liver fibrosis favors the disease progression to cirrhosis and hepatocellular carcinoma [1]. A large spectrum of chronic liver diseases (viral hepatitis, cholestatic liver diseases, alcohol abuse, non-alcoholic steatohepatitis, etc.) may cause chronic liver inflammation, hepatocyte lesions and liver fibrosis in various stages [2–5]. Chronic hepatitis C virus (HCV) represents one of the most frequent conditions leading to the onset of liver fibrosis. At present, the infection with HCV presents a high international interest, due to its substantial effect on morbidity and mortality [6–9]. Moreover, the disease represents a huge economical burden on national health systems, due to the infection hepatic and extrahepatic effects [10–12]. Epidemiological data showed that chronic hepatitis C affects about 71 million people around the world [13, 14].

The physiopathological mechanisms of liver fibrillogenesis are extremely complex and quite studied over the last years. At present, it is accepted that the onset of liver fibrosis is due to the increase of extracellular matrix (ECM) production by myofibroblast activation and proliferation [1, 15].

In the present study, we analyzed some microscopic aspects of the liver fibrosis process in the diagnosed patients with chronic hepatitis C.

Patients, Materials and Methods

Our study included 104 patients from Olt County, Romania, who presented between January 2008 and December 2012 in the Medical Work Expertise Office, Slatina, within the Olt Pension House. The patients were diagnosed with chronic post viral hepatitis C in the Clinic of Gastroenterology and Internal Medicine of the Emergency County Hospital of Craiova, in the Clinic of Infectious Diseases of "Victor Babeş" Hospital of Infectious Diseases and Pneumophthisiology of Craiova and in the Medical Clinic of "Filantropia" City Hospital of Craiova, Romania.

Based on the clinical and laboratory data (viremia, transaminases, protein blood count, complete blood count), there was decided the introduction of the specific antiviral treatment in this group of patients. In accordance with the criteria established by the Expert Board of the National Health House, the inclusion of the patients in the antiviral treatment was based on clinical and HP criteria (liver biopsy), absolutely necessary for establishing the severity and extension of liver lesions, quantified by various scores [Histology Activity Index (HAI) score, necrosis score, portal inflammation score, METAVIR].

That is why, in all the patients of our group there were performed percutaneous liver biopsies, under local anesthesia. The fragments of liver biopsy, immediately after harvesting, were fixed in 10% neutral buffered formalin solution for 24 hours and sent to the Laboratory of Pathology for paraffin embedding, staining and interpretation.

The histopathology and immunohistochemical (IHC) study was continued in the Research Center for Microscopic Morphology and Immunology within the University of Medicine and Pharmacy of Craiova, where there were performed the classical Hematoxylin–Eosin (HE) and Goldner–Szekely (GS) trichrome stainings for highlighting collagen fibers. Also, there were performed two IHC stainings, with anti-CD68 antibody (clone KP1, Dako, mouse anti-human, 1:100 dilution) for highlighting the macrophages and Kupffer cells; and with anti-alpha-smooth muscle actin (α-SMA) antibody (clone 1A4, Dako, mouse anti-human, 1:250 dilution) for highlighting myofibroblasts and liver dendritic cells.

Results

In our study, in patients with chronic hepatitis C, liver fibrosis was observed quite often. Thus, of the 104 patients with chronic hepatitis C, 97.33% presented various stages of fibrosis. Most patients (64.66%) presented microscopic aspects of moderate and severe liver fibrosis (stages 3–5).

The process of liver fibrosis was highlighted in most cases in the portal spaces, being always associated with the presence of a more or less intense inflammatory process. Still, there was not observed the presence of a direct relation between the intensity of the inflammatory process and the intensity of collagen fibrosis (Figures 1 and 2). Still, we observed the fact that collagen fibrosis in the portal spaces is secondary to the inflammatory infiltrate, as we also identified portal spaces with inflammatory infiltrates without fibrosis. Steatosis was also a common finding in these patients, with both micro- and macro-vesicular types, and with no predominant affinity for a certain region of the liver lobular structure.

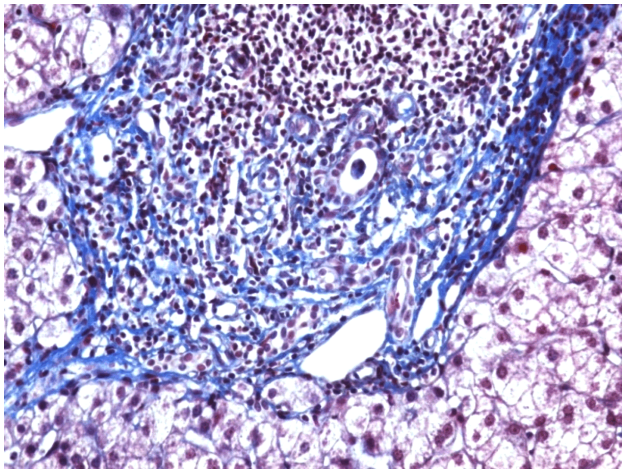

Figure 1 – ***Image of chronic hepatitis with an abundant inflammatory infiltrate in the Kiernan space, moderate fibrosis, moderate hepatocyte necrosis and dilated bile canaliculi with cholestasis (GS trichrome staining, ×200).***

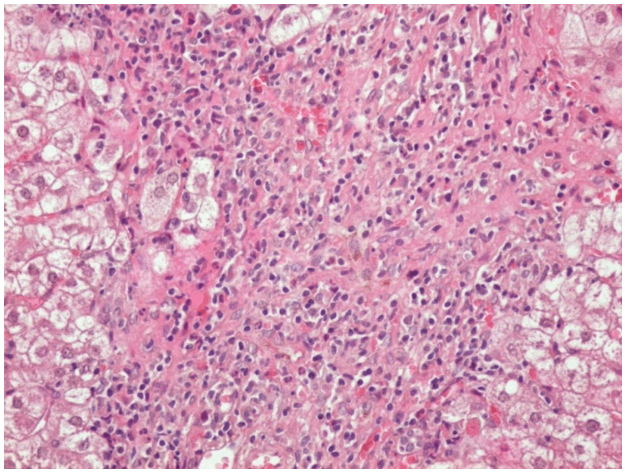

Figure 2 – ***Microscopic image of severe chronic hepatitis, with intense fibrosis in the portal space, intense hepatocyte necrosis and abundant inflammatory infiltrate (HE staining, ×200).***

In severe forms of chronic hepatitis, where there were identified large quantities of collagen fibers in the Kiernan spaces, there was identified the tendency of collagen fibers to progressively expand to other portal spaces, thus forming porto-portal fibrous bridges, or to the center of the liver lobe, forming porto-central bridges (Figure 3).

In our study, we observed that the collagen fibers from the porto-billiary space had quite variable dimensions and orientations. Some fibers replaced small parenchymal areas, with plexiform arrangement, while in other cases we identified collagen fibers organized in rough fascicles with a relatively homogeneous arrangement, forming porto-portal or porto-central fibrous bridges (Figure 4).

All the Kiernan spaces appeared of large dimensions due to the presence of the inflammatory infiltrate, to collagen fibers and also to other molecules of the ECM, as together with the collagen molecules, there also secrete high quantities of other proteins (laminins, fibronectins, elastins), glycosaminogycans or proteoglycans. All these constitute the pathological ECM, where the cells of the immune system are integrated and synthesize numerous cytokines and lymphokines, which, on one side, contribute to the healing process or, on the contrary, they may aggravate the hepatocellular lesions.

In our study, in the patients with massive hepatocyte necrosis, we also observed the presence of a more intense fibrosis process, which makes us believe that fibrillogenesis represents a "reparatory" process, still non-functional, of the liver parenchyma, a scar that tends to heal by forming a conjunctive tissue in the damaged liver area.

The IHC study evaluated the reaction of the liver dendritic and of myofibroblasts, by using the anti-α-SMA antibody. There was observed an intense staining of the liver perisinusoidal dendritic cells to anti-α-SMA antibody (Figure 5), and also an intense staining of the conjunctive cells from the porto-biliary spaces (Figure 6), most probably represented by the myofibroblasts. The analysis of microscopy specimens did not reveal a significant enlargement of Disse spaces. Also, the presence of collagen fibers has not been noted here.

The use of anti-CD68 antibody allowed us to observe the existence of a high number of macrophages in the inflammatory infiltrate of the portal spaces and a high number of Kupffer cells in the liver parenchyma (Figures 7 and 8). Both macrophages and Kupffer cells were of increased size and showed an intense IHC reaction, proving they had intense activity.

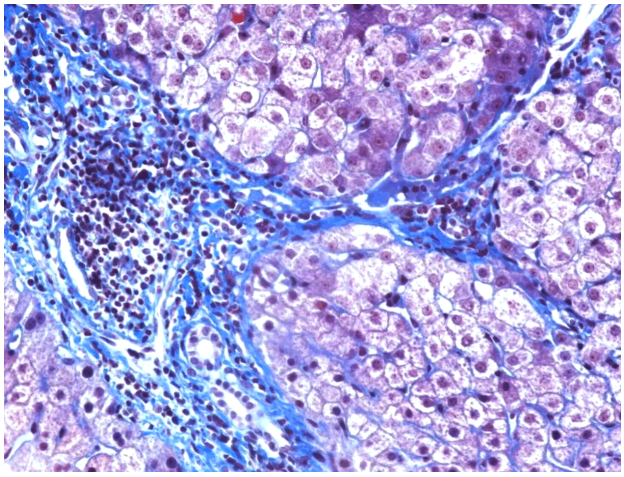

Figure 3 – ***Image of intense portal fibrosis, with formation of porto-portal and porto-central bridges in a case of severe chronic hepatitis (GS trichrome staining, ×200).***

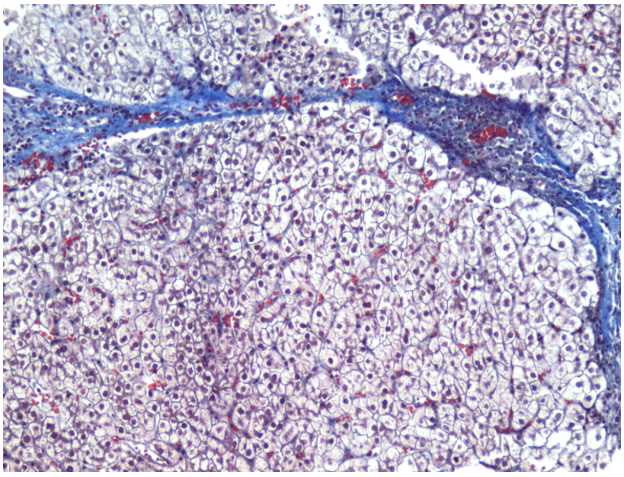

Figure 4 – ***Fibrous porto-portal bridges (GS trichrome staining, ×100).***

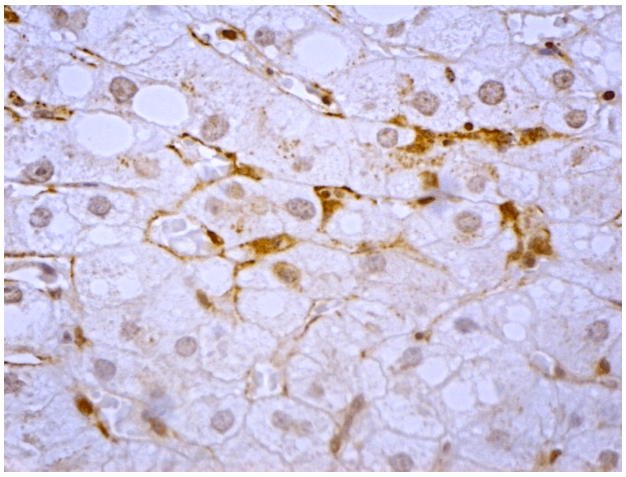

Figure 5 – ***Intralobular numerous large dendritic cells, intensely positive to α-SMA, which show an activated phenotype (Immunomarking with anti-α-SMA antibody, ×400).***

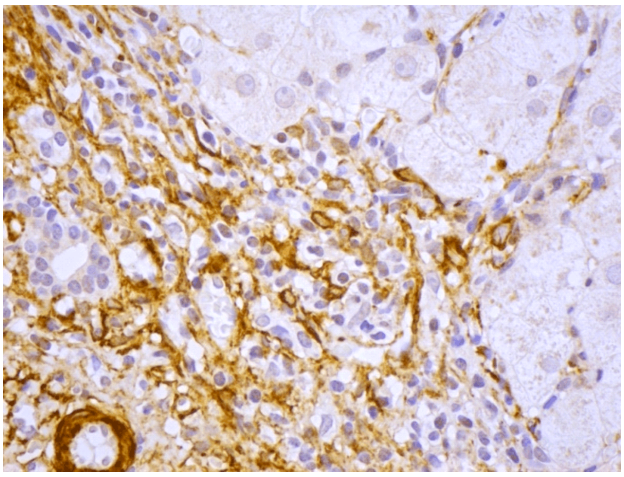

Figure 6 – ***Kiernan space, with multiple myofibroblast cells that give an intense reaction to anti-α-SMA antibody (Immunomarking with anti-α-SMA antibody, ×200).***

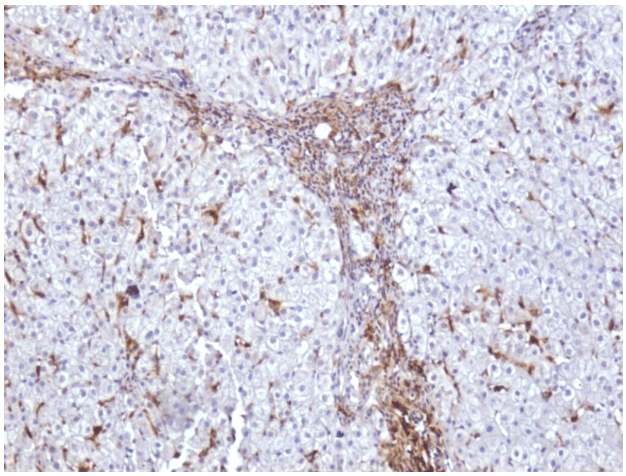

Figure 7 – ***Microscopic image of liver parenchyma with numerous macrophages present in the inflammatory infiltrate from the portal space and numerous Kupffer cells in the liver lobule (Immunomarking with anti-CD68 antibody, ×100).***

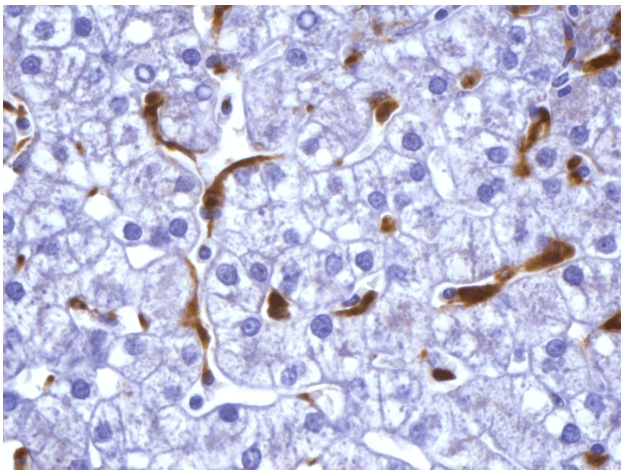

Figure 8 – ***Liver lobule with hyperplastic Kupffer cells, enlarged, arranged in the liver sinusoids, with an intense reaction to the anti-CD68 antibody (Immunomarking with anti-CD68 antibody, ×200).***

Discussions

Liver fibrosis is a complex fibrogenic and inflammatory process, a common pathological outcome of the aggression of various etiopathogenic factors (viral diseases, autoimmune diseases, drug, toxic or metabolic aggressions) that cause chronic, intense lesions on hepatocytes [16, 17]. Liver fibrosis is the result of a progressive synthesis and accumulation of extracellular conjunctive matrix that changes the normal architecture of the liver [18, 19], thus leading eventually to liver failure and even death.

Numerous studies state that liver fibrosis is a definite characteristic of chronic hepatitis. In our study, most patients with chronic hepatitis C (over 97%) presented liver fibrosis, in various stages.

Clarifying the mechanisms that are at the basis of liver fibrogenesis is of utmost importance for handling and preventing liver diseases in a terminal stage, namely of cirrhosis and hepatocellular carcinoma [20, 21].

We consider that the onset of liver fibrosis is correlated to the processes of hepatocellular necrosis and to the intensity of inflammatory processes. In our study, we observed that liver fibrosis was more intense in the individuals with a more severe liver necrosis. Based on these observations, we also consider, like other authors, that liver fibrosis is a regenerative process, a response of the body to the effort of healing the lesions caused by HCV or other pathogenic agents [2, 22]. Although it is a physiological process, by its intensity, it becomes an aggravating factor of the liver function and, if left untreated, it will lead to severe liver failure, as the accumulation of ECM distorts the liver architecture by forming some fibrous scars that eventually lead to the development of regenerative liver nodules, a HP aspect that defines cirrhosis [23, 24].

In our study, we observed that the intensity of liver fibrosis did not always correlate with the intensity of the inflammatory infiltrate in the portal spaces. We believe that liver fibrosis is preceded by the onset of the portal inflammatory infiltrate, its development being conditioned by a multitude of cytokines produced by the inflammatory cells.

It is well known the fact that, after an acute liver lesion (viral hepatitis), some hepatocytes die by necrosis or apoptosis, but they also regenerate, a process by which the damaged hepatocytes are replaced. This process is associated with an inflammatory response and a limited deposit of ECM. If liver lesions become chronic, hepatocyte regeneration is low or fails, the hepatocytes being replaced by abundant ECM [24, 25].

The enlargement of portal spaces observed by us is a proof of the synthesis of a high quantity of ECM in the liver. According to some studies, in advanced stages, the liver contains approximately six times more ECM than the normal situation, especially collagen (I, III and IV), fibronectin, elastin, laminin, hyaluronate and proteoglycans. Accumulation of ECM results both in the increase of the synthesis and in the decrease of its degradation [26, 27].

For a long time, liver fibrosis was considered a passive and irreversible process due to the collapse of the liver parenchyma and its replacement by a rich collagen tissue [28, 29]. In the last years, more clinical and experimental studies showed that the decrease of liver fibrosis score after an appropriate treatment in a variety of liver conditions, such as steatohepatitis, hemochromatosis, Wilson's disease, child cirrhosis, bile obstruction, autoimmune hepatitis, chronic viral hepatitis [30–38]. These studies gave hope to the patients with chronic liver conditions and opened up the way for studies on drugs with antifibrotic properties.

The studies of liver fibrosis were more numerous after 1980, when there were identified hepatic stellate cells (HSCs) (previously known as Ito cells, lymphocytes or perisinusoidal dendritic cells) as the main cells that produce collagen in the liver [39]. Localized in the perisinusoidal space, between the hepatocytes and the endothelial sinusoidal cells, in chronic liver conditions, HSCs undergo a major phenotype change, thus acquiring fibrinogenic properties [39]. In the normal liver tissue, HSCs have the main function of stocking retinoids and lipids, but also to synthesize some particular proteins (glial fibrillary acidic protein) [40–43]. In chronic liver lesions, under the influence of some autocrine and paracrine factors received from the Kupffer cells, hepatocytes and endothelial cells of sinusoidal capillaries, there is produced the transdifferentiation of HSCs in a myofibroblast phenotype, HSCs synthesizing high quantities of ECM [16, 44, 45].

In our study, by using the anti-α-SMA antibody, we showed that, in the patients with chronic hepatitis C, HSCs became positive to this antibody, similarly to the myofibroblasts, thus changing their phenotype profile. Still, the strongest reaction to anti-α-SMA antibody was observed in the portal spaces, where there was identified a very large number of myofibroblasts. Also, we showed that portal spaces were quite enlarged, the proof of the presence of a high quantity of ECM. Contrary to our expectations, the perisinusoidal (Disse) spaces did not have too large sizes, which show that, at his level, there is a lower quantity of ECM. Although most studies state that HSCs are the key cells of liver fibrosis [46–51], we consider that the main cells that are involved in this process are the myofibroblasts in the portal spaces, and less the HSCs. In this matter, we remind the fact that all the researchers showed that liver fibrosis develops in the portal areas, and as the disease aggravates, there appear the porto-portal bridges or the central bridges. Therefore, liver fibrosis starts in the portal spaces (and not in the Disse spaces, as supported by some studies [51]), from where it extends progressively. It is true that HSCs acquire a myofibroblast phenotype and synthesize ECM, but portal myofibroblasts also come from other sources: local fibroblasts or mesenchymal cells present in the liver, mesenchymal stem cells from the bone marrow, cholangiocytes or even hepatocytes [52–57].

In our study, by using anti-CD68 antibody, we also evaluated the reaction of macrophage cells in chronic hepatitis and we observed both an increase of the macrophage number and activity in the portal spaces, but also of the Kupffer cells from the liver parenchyma. This microscopic aspect suggests an intense phagocyte activity, and also some biochemical compounds of ECM. At present, it is considered that macrophages and Kupffer cells play essential roles in the process of liver fibrosis, having the capacity of influencing the progression and regression of fibrosis, by the paracrine regulation of HSCs activation, and also by a direct phagocyte activity [58–61]. The activation of macrophage cells is correlated and strongly influenced

with the cells of the immune system, mainly of the cells from the inflammatory infiltrate from the portal spaces.

We consider, like other authors, that liver fibrosis is a complex reparatory process, similar to other chronic fibro-proliferative processes [51, 62] that occur in some kidney diseases [63, 64], lung diseases [65, 66], heart diseases [67, 68], or skin diseases [69].

Conclusions

In chronic hepatitis with C virus, liver fibrosis was highlighted in over 97% of the cases. It represents a HP lesion favoring the progression of the disease to cirrhosis. Fibrosis was present in the portal spaces as collagen fibers with a heterogeneous, plexiform arrangement and only in the severe forms as thick fibrous strings. The density and size of the collagen fibers were correlated to the severity of the liver disease, in the severe forms being highlighted porto-portal and porto-central fibrous bridges. The portal spaces (Kiernan) had large sizes by the presence of a chronic inflammatory infiltrate, the presence of collagen fibers and conjunctive matrix. The IHC examinations highlighted the change of the phenotype of perisinusoidal dendritic cells, the increase of the myofibroblast number in the portal spaces, the increase of the macrophage number in the portal inflammatory infiltrate and of the Kupffer cells in the liver parenchyma.

Conflict of interests

The authors declare that they have no conflict of interests.

Author contribution

Octavian Ion Predescu has equal contribution and thus shares first authorship.

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