Histochemical and pathological Assessment of Iron and Polysaccharides in patients with Chronic Hepatitis C Infection with Varying Degree of Liver Damage.

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Abstract: The liver is the major iron storage organ in the body, and therefore, iron metabolic disorder is sometimes involved in chronic liver diseases. Chronic hepatitis C is one of the liver diseases that show hepatic iron accumulation, even though its level should be recognized to be basically mild to moderate and sometimes within the normal range. The mechanisms underlying hepatic iron accumulation in chronic hepatitis C have not been fully elucidated. Reduction of the hepcidin transcription activity by hepatitis C virus (HCV)-induced reactive oxygen species may in part account for it, but the regulation of hepcidin is very complex and may depend on many variables, including the particular stage of the systemic and/or hepatic inflammatory conditions and the circulating transferrin-bound iron and intracellular iron stores. This might explain the variations in hepatic iron concentrations reported among patients with HCV-related chronic liver disease. However, even mild-to-moderate iron overload in the liver contributes to disease progression and hepatocarcinogenesis in chronic hepatitis C probably by reinforcing the HCV-induced oxidative stress through Fenton reaction. The present review highlights the current concept of hepatic iron overload status in chronic hepatitis C and discusses how iron metabolic disorder develops in this disease and the impact of hepatic iron overload on disease progression and its relevance to hepatocarcinogenesis.

Introduction

Chronic infection with the hepatitis C virus (HCV) is highly prevalent. More than 170 million persons are infected worldwide "Global surveillance and control of hepatitis C, 1999". Mortality associated with chronic HCV infection is mainly attributable to progression of hepatic fibrosis and subsequent occurrence of cirrhosis with its complications of hepatocellular carcinoma and portal hypertension (Tong et al., 1995). Progression of HCV- associated hepatic fibrosis and occurrence of cirrhosis with its complications may be affected by several factors, including alcohol intake, age and time of infection, and sex Feeff., (1997). Liver iron accumulation in patients with chronic hepatitis C virus (CHC)

has received increasing in recent years (Fujita et al., 2007). Iron overload in HCV-infected patients has been proposed as a factor that may promote progression of liver disease, but its exact role remains unclear. Serum iron values (serum iron, serum ferritin, and transferring saturation) are commonly elevated in patients with chronic hepatitis C virus infections Riggio et al., 1997). Histological examination has revealed that chronic inflammation seems to play an important role in the pathogenesis of chronic hepatitis C, and excess iron, also is associated with increased morbidity and mortality (Keisuke et al., 2013).

Material and Methods

The current study involved fifty patients with chronic hepatitis C (35 males and 15 females; with a median age 45 (23 – 65) years) admitted to Mansoura Gastroenterology Center from January, 2009 through December, 2010.

Patients were excluded if the size of liver biopsy did not allow for accurate histological assessment of fibrosis, or liver iron, or in case of positive HbsAg or HIV infection. Further criteria for exclusion were previous treatment with interferon and/or ribavirin, known C282Y homozygosity, previous iron depletion therapy, or coexisting affection which could influence interpretation of iron measurements or liver fibrosis. A liver biopsy was obtained from all patients were cut into two parts. A portion of the tissue fragment was immediately frozen at-20°C for subsequent determination of iron concentration, and the other was used for histopathological examination. The latter

specimens were formalin-fixed and paraffin-embedded. Slides were stained with hematoxylin-eosin. Masson´s trichrome and Perl´s Prussian blue to assess liver iron content.

These patients were divided into three groups on the basis of degree of fibrosis (stage) which recorded according to (Metavir, 1994) scoring system for the assessement of fibrosis:
◆ Group I: 20 patients with Fibrosis (F≤3) with average (36.5 ± 10.1) years.
◆ Group II: 15 patients with Cirrhosis (F=4) with average age (44.5 ± 7.5) years.
◆ Group III: 15 patients with hepatocellular carcinoma with average age (49.3 ± 5.2) years.
◆ A group of 10 individuals (adult males and females) free from a disease taken as controls with average age (38.3±8.3) years.

Methods

Histochemical stains :(1) Total carbohydrates: Periodic Acid Schiff's Reaction (PAS).
(2) Acid mucopolysaccharides: Alcian blue.
(3) Prussian Blue Staining Protocol for Iron.
(4) Masson's Trichrome Staining Protocol for Collagen Fibers.
(5) Feulgen Stain for DNA.

Results

A photographic paraffin sections in the liver biopsies of control stage (F1), Fibrosis stage (F2), cirrhosis stage (F4) and hepatocellular carcinoma groups.
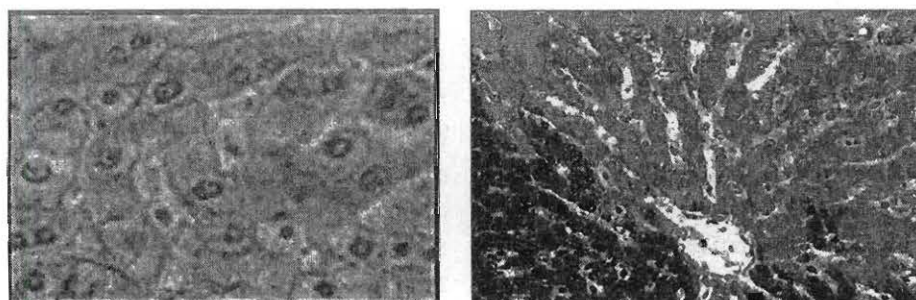

Fig. (1-2) : Cross sections in liver biopsy of control stage (F1) Showing Kupffer cells with Regular single nuclei and central vein. (X 200, X 100 respectively)
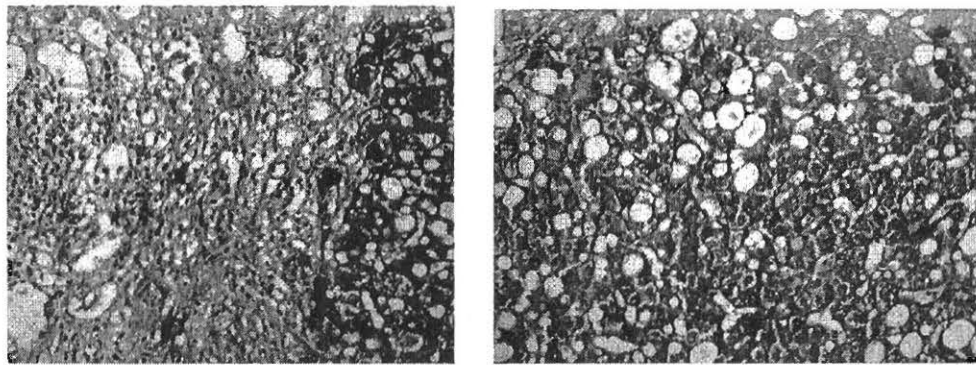
Fig. (3-4): Liver histopathological examination for groups of chronic hepatitis C patients with marked PAS +ve cytoplasmic material and early Capillarization of the sinusoid. (X 100)
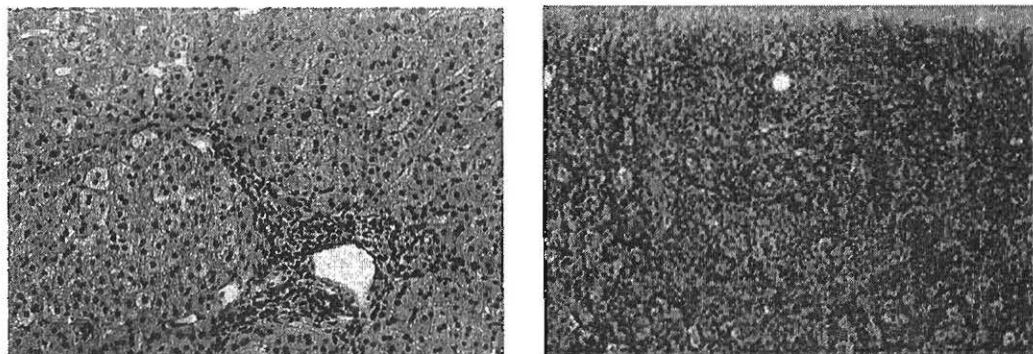

Fig. (5-6): The fragment of parenchyma with anamorphous hepatocytes and narrow sinusoids in liver of a patient with chronic hepatitis C*Acid mucopolysaccharides appear blue/Neutral mucopolysaccharides appear red (Alcian blue stain X100)
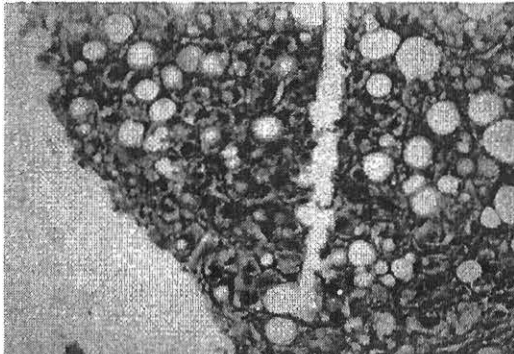

Fig. 7 *Perl's Prussian blue staining for ferric iron in liver biopsy. chronic hepatitis C infection with moderate Fe deposition. (X 200)
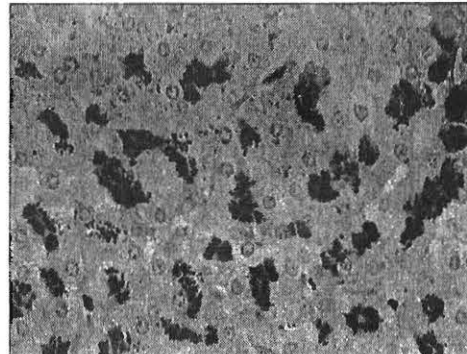
Fig. 8 Hemochromatosis of liver showing +ve iron staining in area of hepatocellular carcinoma with malignant hepatocyte. (PB X 100)
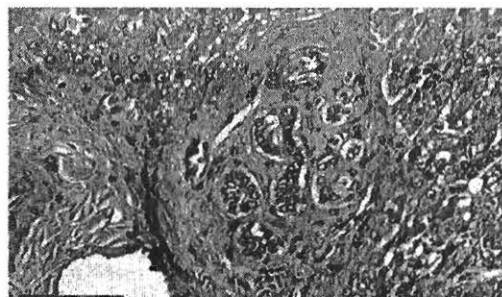

Fig. 9 ******+ve iron staining in Kupffer Cells of cirrhotic nodules. (PB X 100)
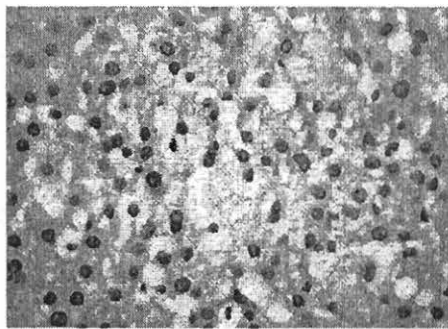
Fig. 10: Feulgen stain of many magenta nuclei and green cytoplasm are showed clearly in case of hepatitis C infection. (X 100)
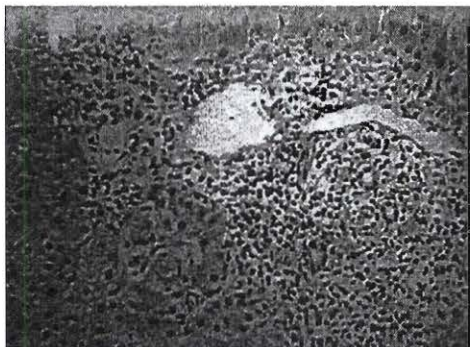

Fig. 11: cross section in portal areas heavy infiltrated by mononuclear cells with necrosis.(Masson's Trichrome stain X100)
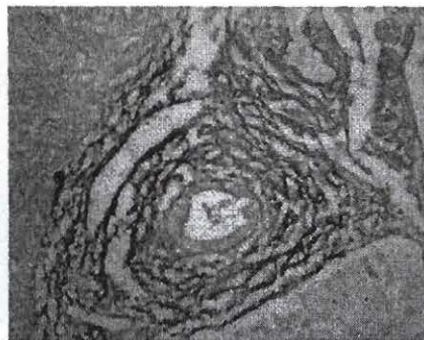

Fig. 12: Chronic hepatitis C with marked fibrosis in portal area with bile duct proliferation (Masson's Trichrome stain X200)

Discussion

There was significant relationship between histological grading of iron storage, mucopolysaccharides as well as hepatic iron concentration and other traditional biochemical tests for iron overload in patients with chronic hepatitis C.

In this study we demonstrated significant relationship between Metavir fibrosis score and histological grading of iron storage in patients with liver cirrhosis (G2), and with hepatocellular carcinoma (G2) in which the cirrhotic area beside malignant hepatocytes (G3) is present, this is agreed with (Guyader *et al.*, 2007), who reported that hepatic iron score correlated with fibrosis stage (P <0.001), in univariate analysis while in multivariate logistic regression model hepatic iron score was independently associated with age, male sex and excessive intake of alcohol and not correlated with Metavir fibrosis score. So, histological evaluation of iron staining provides complementary inflammation to traditional biochemical markers for iron overload.

Currently, it is difficult to determine whether the increased HIC seen in individuals with cirrhotic HCV may facilitate disease progression toward end-stage liver disease or simply result from increased iron deposition in the cirrhotic liver compared with the pre-cirrhotic liver. In order to address this question, we have recently performed a pilot study to determine if histological changes in serial liver biopsies would correlate with an increase in HIC. The results of this study showed that histological progression was not correlated with increased HIC at early stage of fibrosis, but iron accumulation appeared to occur after the development of cirrhosis. There is relatively good agreement that iron deposition in HCV-infected livers is found not only in hepatocytes, but also in the portal tracts and sinusoidal mesenchymal cells (Pirisi *et al.*, 2000). Increased histologic grade is associated with total iron deposition regardless of location, possibly due to portal inflammation and interface hepatitis (Giannini *et al.*, 2001). It is possible that excess iron within endothelial cells may represent

phagocytosed hepatocytes and may inhibit their normal role in cellular immunity, thus leading to increased severity of HCV (Kaji *et al.*, 1995).

The mechanism of liver damage by HCV alone is similar in some aspects to pathogenic iron overload. Both agents potentiate free radical formation, induce cytokine response through activation of NF-kβ, and ultimately result in fibrogenic and inflammatory conditions. However, HCV core protein has been shown to directly trigger apoptosis by upregulation of the tumor suppressor p53 and downregulation of the cell cycle regulators p21 and p38 (Schuppan, 2003). HCV core protein also directly alters lipid metabolism by 1) upregulation hepatic lipogenesis via activation of peroxisome proliferator-activated receptor- a (Tsutsumi *et al.*, 2002); 2) increasing lipoprotein flux by enhancing β oxidation of fatty acids (Schuppan, 2003); and 3) interacting with apolipoprotein A1 to downregulate microsomal lipid transfer protein (Perlemuter, 2002). The resulting steatosis may lead to ROS formation and lipid peroxidation.

The acute phase of viral hepatitis closely resembles that of toxic hepatitis clinically, biochemically, and histologically (Mitchell *et al.*, 1976); Wheeler *et al.*, 2001), and no early histological feature is specifically diagnostic of viral hepatitis (Koff, 1993). Coinfection of HIV with HCV accentuates liver damage and cirrhosis is 15 times more frequentin HCV patients abusing alcohol (Larson and Carithers, 2001).

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الملخص العربى

تعتبر الاصابه بالالتهاب الكبدى الفيروسى (سى) من اكثر الامراض انتشار فى العالم كذلك فان نسبه الاصابه بهذا المـرض فى مصر تصل الى اكثر من ٢٠ % من السكان وهذه النسبه تشكل خطرا على الصحه العامة . ايضا زيادة معـدل الوفيـات نتيجة مضاعفات هذا المرض من حدوث تليف ثم تشمع للكبد والذى ينتهى بدوره بحدوث ورم بالكبد .

وقد تم تقسيم المرضى بهذه الدراسة الى ثلاث مجموعات على حسب درجة التليـف الكبـدى تبعـا لتقـسيم ميتـافير (Metavir) الى مرضى بتليف كبدى من الدرجة اقل من او يساوى ٣ (٢٠) مريض ومرضى بتليف كبدى من الدرجة الرابعة (١٥) مريض وكذلك مرضى مصابين بورم بالكبد (١٥) مريض بالاضافة الى مجموعة مكونه من ٢٠ فرض من الاصـحاء كمجموعة ضابطة .

وقد تم الحصول على النتائج التالية والتى يمكن تلخيصها على الوجه التالى :

■ امكن ملاحظة نسبة وتركيز الحديد بالكبد فى مرضى التليف الكبدى (تليف اقل من او يساوي ٣) وتـشمع كبـدى (تليف يساوي ٤) وذلك بمقارنته بمجموعة الاشخاص الاصحاء وهذا يدل على ان الحديد يعتبر من اهم السموم الكبديـة فـى حالات الالتهاب الكبدى وايضا فان تراكم الحديد فى الخلايا الكبدية السرطانية كان اكثر وضوحا فى الخلايا الكبديـة الـسليمه وذلك يوضح ان زيادة الحديد يمكن ان يكون عامل مساعد فى تحول الخلايا الطبيعية الى خلايا سرطانية وبذلك يمكن اعتبـار نسبة الحديد فى الكبد دليل على تليف الكبد الحاد فى حالات الالتهاب الكبدى المزمن (سى) .