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Role Of Platelets-Derived Growth Factor Receptor ß and Tumor Necrosis Factor α In The Pathology Of Chronic Hepatitis C

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ABSTRACT:

Background: Chronic liver diseases are characterized by two fundamental aspects, persistent injury and fibrosis, which are cardinal features for the continuous progression towards cirrhosis and end stage liver failure**.**

Objective: To study the expression of platelets derived growth factor receptor ß (PDGFR- β) and tumor necrosis factor (TNF α) in the liver and their relation to the stage and grade of chronic HCV.

Patients and Methods: Analytical cross-sectional study was conducted on fifty patients with proven chronic hepatitis C. All patients were referred to Tropical Medicine and Gastroenterology Department, Sohag University Hospital. Patients were categorized into two groups according to the histological stages of chronic hepatitis C as follows, group 1 includes 40 patients in stages 0,1 and 2; whereas group 2 includes 10 patients in stage 3. All patients were then recategorized according to the grades of inflammatory activity into patients with minimal and mild inflammation (G1+G2=32 patients) and those with moderate and severe inflammation (G3 +G4=18 patients). All patients were subjected to full history taking, complete clinical examination, complete blood count, liver function tests. Liver biopsy was evaluated for immunohistochemical expression of PDGFR- β and TNFα.

Results: The included patients were 80 % males and 20 % females. Their mean age was 44.42±11.17 years. The mean average weighted scores (AWS) of Imunohisto-chemical expression of PDGFR- β, and TNFα were significantly higher in moderate and severe inflammation versus minimal and mild inflammation (P=0.05; 0.0001respectively). Similarly, the mean AWS of PDGFR- β, and TNFα were significantly higher in patients with stage 3 versus stages 0,1 and 2 (P=0.04; 0.0001 respectively).
Conclusion: Expression of PDGFR- β, and TNFα markers were associated with progression of disease activity and fibrosis.

Keywords: Chronic hepatitis C, staging , grading , PDGFR-β , TNF α .

Introduction:

Viral hepatitis is a global health problem affecting hundred millions of people worldwide and considered the main cause of liver cirrhosis, hepatocellular carcinoma and liver transplantation in developing countries[(1)].

HCV continues to represent the main causative agent of the hepatitis which, independently of patient's age, leads to chronic transformation of the process in over 80% of patients[(2)].

Hepatic fibrosis was historically thought to be a passive and irreversible process due to the collapse of the hepatic parenchyma and its substitution with a collagen-rich tissue [(3)]. Currently, it is considered a model of the wound-healing response to chronic liver injury [(4)].

Blood PLTs, besides hemostatic properties, have the features of inflammatory cells [(5)]. Blood PLTs, while activated in inflammatory processes, release active compounds: platelet-derived growth factors (PDGF), vascular endothelial growth factor (VEGF), transforming growth factor

(TGF)-b, and so forth. PLTs transport these active compounds to the target cells [6].

There are many reports presenting multipotential properties of blood PLTs, such as angiogenesis [7], wound healing [8], liver regeneration [9], and metastasis in cancer [10]. After virus infection, PLTs are recruited to the liver, delaying virus elimination and increasing immunopathological liver cell damage [11].

PDGF signal transduction pathways play a prominent role in fibrosis [12]. PDGFRs are thought to play a central role in activating HSCs and promoting liver fibrosis and cirrhosis, Whether PDGFR α and PDGFR-ß play independent roles in fibrogenesis is not known [13]. It has been suggested that PDGFRα signaling is more likely to induce fibrosis than PDGFR-ß. However this notion has not been conclusively demonstrated in the liver [14].

Tumor necrosis factor-α (TNFα) is a pleiotropic cytokine produced by a variety of immune cells including macrophages/monocytes [15]. TNFα is required for normal proliferation of hepatocytes in liver regeneration and it exhibits anti-apoptotic activity [16], but, on to the grade and stage of the disease.

Subjects and methods:

Before starting, the present study protocol was approved by the Ethics Review Committee of Sohag Faculty of Medicine and a written informed consent was obtained from all patients before their inclusion.

An analytical cross-sectional study was conducted on fifty patients with proven chronic HCV infection. All patients were referred to Tropical Medicine and Gastro-enetrology Department, Sohag University Hospital.

The inclusion criteria were chronic hepatitis C proven by positive HCV Ab and HCV RNA ≥ 6 months, and compensated liver disease. Exclusion criteria were

: patients who received or are receiving any anti-HCV treatment, patients with liver cirrhosis or coagulopathy, patients whose PLT count less than 100,000 /µl, patients whose prothrombin time was more than three seconds over the control, co-infection with hepatitis B virus, patients with autoimmune liver disease, alcoholic liver disease, drug induced liver disease, Wilson's disease, haemochromatosis, and hepatocellular carcinoma, and patients with chronic medical

the other hand, it represents a well recognized mediator of hepatocyte death **(17)**.

Previous studies examined the intracellular expression of cytokines in liver tissues of chronic hepatitis C patients. They reported that liver sinusoidal cells and cells of inflammatory infiltrates as the most common sources of the cytokines [18, 19, 20]. Under certain conditions, other cells, such as endothelial cells, HSCs, bile duct epithelial cells, and hepatocytes are able to synthesize TNF- and IL-1 [18, 21, 22]. Reports on the amount of cytokines detected in livers with chronic hepatitis C or on correlation of cytokine expression on one hand and grading and/or staging and/or intrahepatic viral load on the other are divergent [23].

The inflammatory phase is perpetuated by TNFα production, which results in the activation of resident HSCs into fibrogenic myofibroblasts [24].

The aim of the present work is to study expression of PDGFR- β and TNF α in patients with chronic hepatitis C and their relation

problems as cardiac and chronic renal failure.

All patients were subjected to, full history taking, full clinical examination, blood sample were obtained for complete blood count, and liver function tests (Alanine transaminase, Aspartate transaminase, Serum Albumin and Bilirubin). Abdominal ultrasound examination was done for evaluation of liver (size, surface, echogenecity, focal lesions and portal vein diameter), splenic size and the presence of any collaterals.

Liver biopsy was obtained from all patients for histopathological and immunohistochemical analysis. All specimens were stained with hematoxylin and eosin and examined under a light microscope for staging and grading of chronic hepatitis C patients. The severity of fibrosis was classified according to **Scheuer et al.,** [(25)] into:-Stage 0: No fibrosis, Stage 1: mild (portal fibrosis), Stage 2: moderate (periportal fibrosis), Stage 3: severe (bridging fibrosis with lobular distortion), Stage

4: cirrhosis. The inflammatory activity (grade of disease activity) was classified into:- Grade 0: No inflammation, Grade 1: Minimal inflammation, Grade 2: Mild inflammation, Grade 3: Moderate inflammation and Grade 4: Severe inflammation.

Monoclonal antibodies were used against PDGFR-β by perisinusoidal mesen-chymal cells, and TNFα expression by kupffer cells using staining kit (Catalogue # AEX080-IFU, ScyTek Laboratories).

Patients were then categorized into two groups according to the histological stages of chronic hepatitis C as follows, group 1 includes 40 patients in stages 0,1, and 2; whereas group 2 includes 10 patients in stage 3. All patients were then recategorized according to the grades of inflammatory activity into patients with minimal and mild inflammation (G1 & G2=32 patients) and those with moderate and severe inflammation (G3 & G4=18 patients).

Statistical analysis: data were analyzed using Statistical Package for Social Sciences (IBM SPSS) software package version 20. Data were expressed as mean ± standard deviation (SD) for continuous variables and comparison of mean values of 2 groups was done using *student's t-test*. Categorical variables were expressed as frequency and percentage and the comparison between these variables was carried out using *Chi-square* (x^2) test. The level $P<0.05$ was considered the cut-off value for significance. Presentation of the collected data in tables and figures was carried out.

Results

Table (1) shows the results of histopathological examination of 50 liver biopsies according to **Scheuer et al. [25].** Most patients (60%) were mild grade of inflammation. Also the same table reveals that less than half (48%) of patients were moderate (periportal fibrosis; stage 2)

Table (2) compares stages of fibrosis (0, 1 and 2) versus stage (3) regarding PLTs count, PDGFR- β and TNFα. Mean PDGFR-β score in perisinusoidal mesenchymal cells was significantly higher in stage (3) compared to earlier stages (9.2±2.7 *vs* 6.7±3.6; P=0.04). Similarly, mean TNFα score in Kupffer cells was significantly higher in stage 3 compared to earlier stages (7.6±3.53 *vs* 2.25±2.31; P<0.0001).

Table (3) compares grades of inflammatory activity (1 & 2) versus grades (3 &4) regarding TNFα in infiltrating mononuclear cells in portal area. Mean TNFα score was higher in grades (3 & 4) compared to lower grades (6.28±3.64 *vs* 1.66±1.58; P<0.0001).

Table (1): Grading and staging of HCV patients (Total no=50)

Grades of inflammatory activity according to	
Scheuer et al. [25]:	
Minimal Grade (1)	**2(4%)**
Mild Grade (2)	**30(60%)**
Moderate Grade (3)	**8(16%)**
Severe Grade (4)	**10(20%)**
Stages of liver fibrosis according to Scheuer et al. [25]:	
None (stage 0)	**2(4%)**
Mild (portal fibrosis; stage 1)	**14(28%)**
Moderate (periportal fibrosis; stage 2)	**24(48%)**
Severe (bridging fibrosis with lobular distortion; stage 3)	**10(20%)**

Table (2): Comparison between stages of fibrosis (0-1-2) versus **stages (3) regarding PLTs count, PDGFR- β and TNFα expression.**

	Stages (0-1-2)	**Stage (3)**	**P-value**
	N=40	**N=10**	
PLTs	**280±100.88**	**237.2±133.83**	**0.23**
PDGFR- β	**6.7±13.6**	**9.2±2.7**	**0.04**
TNFα	**1.35±0.53**	**2.2±0.63**	**<0.0001**

Significant P values are in Bold.

Table (3): Comparison between grades of necroinflammation regarding mean PLTs count, PDGFR- β and TNFα expression**.**

	Grades (1,2)	**Grades (3,4)**	**P-value**
	N=32	**N=18**	
PLTs	**290.44±91.57**	**243.83±124.74**	**0.13**
PDGFR- β	**6.9±3.6**	**5.27±3.92**	**0.05**
TNFα	**1.19±0.39**	**2.12±0.6**	**<0.0001**

Significant P values are in Bold.

Discussion:

Liver fibrosis is one of the leading causes of morbidity and mortality worldwide, but very limited therapeutic options are currently available for this condition. Indisputable evidence now exists that HSCs play a central role in hepatic fibrogenesis secondary to virtually all types of liver injury [(26)].

Our finding of a significantly higher immunohistochemical expression of both TNFα $_2$ and PDGFR- β in grades 3& 4 compared to lower grades are consistent with **Furie** [(27)] who highlighted on the role of TNFα and PDGFR- β in hepatic inflammatory activity and fibrosis as the same mechanism occurring in blood vessels. The vessel wall, with its inner lining of endothelium, is crucial to the maintenance of a patent vasculature. The endothelium contains thrombo regulators nitric oxide, and prostacyclin, which together provide a defense against PLT thrombus formation. When the endothelium is disrupted, collagen and tissue factor become exposed to the flowing blood, thereby initiating the formation of thrombus. Endothelium is also an important target for tumor necrosis factor (TNF) and interleukin-1 (IL-1). The endothelium synthesizes and releases platelet activating factor (PAF) in response to TNF. This activity of TNF overlaps that of IL-1, which also induces PAF production in endothelium. These vessel wall alterations result in a change in endothelium from antithrombotic to thrombotic. The disrupted endothelium is the first reaction in PLT adhesion to the vessel sub endothelium under physiologic blood flow. In the presence of TNF α-induced sinusoidal alteration, PLTs adhere to sinusoidal endothelial cells in the same way as to vessel walls. Characteristic pathological features of chronic HCV infection are chronic inflammation and apoptosis of infected and bystander hepatocytes.

Our results of a significantly increased mean average weighted score (AWS) of Imunohistochemical expression of TNFα in infiltrating mononuclear cells in portal area in advanced stage of fibrosis and higher grades of inflammatory activity is in agreement of previous reports. Fong et al. (1996) studied the immunohistochemical expression of TNF-α and its receptors (TNFR-A and TNFR-B) in the liver of chronic hepatitis B patients. They reported that TNF-α was detected exclusively in infiltrating mononuclear cells (MNC) and its expression in these cells was correlated with liver histology. TNFRs were detected in hepatocytes, infiltrating sinusoidal cells and infiltrating MNC. The expression of the receptors was also correlated with liver histology. Also**, Kasprzak et al.** [(22)] found a positive correlation between expression of TNF-α (localized mainly in liver sinusoidal cells) and staging in adults with chronic hepatitis C.

Orfila et al. [(28)] studied the immunohistochemical expression of TNF-α in carbon tetrachloride (CCl4)-induced chronic liver injury in rats. They found that after 3-9 weeks ingestion of CCl4 and accompanying the increased necrosis, an increased cellular expression of TNF-α was observed. This was associated with the development of fibrosis and may contribute to disease severity.

In our study, the mean average weighted score (AWS) of PDGFR- β as a marker of liver fibrosis was significantly higher in stage 3 compared to earlier stages. Similarly, **Kondo et al.** [(29)] identified a dense population of cells expressing PDGFR-β in the periportal areas of cirrhotic liver, whereas only a few mesenchymal cells stained for this peptide were seen in patients at the lower stage of chronic hepatitis and in normal control liver. In addition, most of the PDGF receptor- β expressing cells were also stained for smooth muscle actin. These cells, which play a central role in liver fibrosis, are believed to be transformed from HSCs, and their proliferation is stimulated

by PDGFR- β. HSCs are increasingly being seen as key mediators in the progression of liver fibrosis [30].

Borkham-Kamphorst et al. [31] studied Liver fibrosis induced by hepatic over expression of PDGF-B in transgenic mice. They reported that PDGFR- β has a stimulating influence on the fibrogenesis and mitogenesis of HSCs in the liver. Moreover, **Kocabayoglu et al.** [32] stated that depletion of β-PDGFR in hepatic stellate cells decreased injury and fibrosis *in vivo*, while its auto-activation accelerated fibrosis.

Ikura et al. [33] studied the role of PDGFR-ß and its receptor in livers of patients with chronic liver disease and reported PDGFR-ß in the periportal area as myofibroblast-like cells. These cells, which play a central role in liver fibrosis, are believed to be transformed from Ito cells [34] and their proliferation is stimulated by PDGF-ß [35]. In addition, the number of the cells with PDGF-ß was correlated with both the inflammatory activity and the degree of fibrosis. These findings suggest that PDGF-ß is released by macrophages in inflamed areas, and may act on myofibroblast-like cells to induce the fibrogenesis related to inflammatory liver injury.

When HSCs expressing PDGF receptor- β are present in the liver, the liver may be susceptible to PDGF contained in PLTs. The accumulation of PLTs in the liver with chronic hepatitis may be involved in liver fibrosis through the activated HSCs [29].

Conclusion

The results obtained in the present study indicate that expression of PDGFR- β, and TNFα markers play central roles in pathogenesis CHC. Expression of these 2 markers is markedly elevated in association with progression of disease activity and fibrosis. Further studies of the biological characteristics and function of these cytokines in the liver may help in identifying new treatment strategies for hepatic fibrosis in chronic hepatitis C patients.

Conflict of interest: The authors declare that they have no conflict of interest.

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