Platelets Accumulation In The Liver : A novel Mechanism Of Thrombocytopenia In Chronic Hepatic C Patients

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ABSTRACT

Background and aim: Thrombocytopenia is a common complication of chronic liver diseases and is due to various causes.The effect of thrombocytopenia on liver damage and the exact mechanisms that lead to thrombocytopenia in chronic liver disease and cirrhosis are still unclear. As Platelets Derived Growth Factor ß (PDGF-ß) is released from platelets (PLTs) upon activation. We tried to identify PLTs and PDGFR- ß in the liver to shed some light on the pathophysiology of thrombocytopenia and liver fibrosis in chronic hepatic C patients.

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. Group 1 includes patients with PLT count less than 150000/µL (thrombocytopenia). Group 2 includes patients with normal PLT count (150000-450000/µL). All patients were subjected to full history taking, complete clinical examination, complete blood count, liver function tests. Liver biopsy was obtained for histological staging and grading. Immunohistochemical study of PLTs and PDGFR- ß were done using monoclonal antibodies against PLT's surface marker CD41 and PDGFR- ß.

Results: The included patients were 80 % males and 20 % females. Their mean age was 44.42± 11.17 years. The mean average weighted score (AWS) of Imunohisto-chemical expression of CD41was significantly higher in thrombocytopenic group compared to normal PLTs group (5.8±2.86 *vs* 3.43±3.03; P<0.001). There was a significant negative correlation between CD41 expression and peripheral PLT count (r=-0.4; P=0.007). PDGFR- ß expression was significantly stronger in thrombocytopenic patients than patients with normal PLT count (6.9±3.6 vs 5.27±3.92; P=0.001). It showed also a significant negative correlation with peripheral PLT count (r=-0.34, P=0.045). Both CD41 and PDGFR- ß expression were significantly elevated in patients with advanced stage of fibrosis than in those
with earlier stages (7.26±5.23 *vs* 5.88±3.24; P=0.04 &9.2±2.7 *vs* 6.7±3.6; P<0.01 respectively).

Conclusion: The accumulation of PLTs in the liver in patients with chronic hepatitis C may be involved in thrombocytopenia and liver fibrosis.

Keywords: chronic hepatitis C, thrombocytopenia, CD41, PDGFR- ß.

Introduction

Egypt has the highest Hepatitis C virus prevalence in the world [(1)]. Among the hematological derangement in chronic hepatitis C the decrease of PLTs number seems to be the most common [(2)]. Thrombocytopenia has negative impact on the evolution ofthe disease, mainly in the advanced stages when the PLTs number falls below 50000/µl [(3)].Thrombocytopenia is associated with a poorer prognosis, and it frequently prevents patients from receiving crucial interventions such as medications, as well as invasive diagnostic or therapeutic

procedures [4]. Blood PLTs, besides haemostatic properties, have the features of inflammatory cells [5]. There are many reports presenting multipotential properties of blood PLTs, such as angiogenesis [6], wound healing [7], liver regeneration [8], and metastasis in cancer [9].

In acute viral hepatitis, PLTs mediate cytotoxic T lymphocyte-induced liver damage [10]. After virus infection, PLTs are recruited to the liver, delaying virus elimination and increasing immunopathological liver cell damage [11].

Thrombocytopenia is a marked feature of chronic liver disease and cirrhosis, it occurs in 64-76% of patients with cirrhosis and/or fibrosis, compared with 6% of non-cirrhotic patients with chronic liver disease [12].

The clinical relevance of thrombocytopenia is determined partly by the low PLT count and partly by abnormal haemostasis or PLT dysfunction, all of which impact patient management and clinical outcome. For example, severe thrombocytopenia is associated with life-threatening complications that occur in end-stage liver disease [13].

The pathophysiology of thrombocytopenia in patients with HCV-related chronic liver disease is not completely understood. However, it is universally agreed that multiple pathogenetic mechanisms that arecomplementary and often act in concert are involved [14].

According to previous reports assessing the feasibility of PLTscintigraphy, an accumulation of PLTs in the liver was observed in patients presenting with thrombocytopenia [15,16].

Based on these findings, thrombocytopenia with chronic hepatitis and cirrhosis may be caused by hypersplenism, as well as by the capture of PLTs by the liver. However, the PLT kinetics of patients with chronic liver disease is not well characterized. PDGF ß is a potent mitogen that stimulates proliferation and migration of mesenchymal cells [17].The overexpression of PDGF and PDGFR in livers of an experimental injury model was previously reported [18].

Therefore, the aim of the present study is to: 1-Investigate the relationship between intrahepatic PLTs and thrombocytopenia in chronic hepatitis C patients.

2- Study the relationship between intrahepatic expressions of CD41, PDGFR ß and liver fibrosis in these patients.

Patients and Methods:

efore starting, the 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 inclusion in the study. The aim of the research and procedures that were used were explained briefly to the patients to get his/her confidence and cooperation. Patients were informed that analysis and interpretation of data were restricted to scientific purpose only.

Analytical cross-sectional study was conducted fifty patients with proven chronic HCV infection referred to Tropical Medicine and Gastroenterology Department, Sohag University Hospital. Patients were categorized into two groups.

Group 1(10 patients) with PLT count less than $150x10^3/\mu L$ (thrombocytopenia).

Group 2(40 patients) with normal PLT count ($150x10^3$-$450x10^3/\mu L$).

The inclusion criteria were proven chronic hepatitis C patients by positive HCV Ab and HCV RNA ≥ 6 months, and compensated liver disease. Whereas the exclusion criteria were, patients who received or are receiving any anti-HCV treatment, patient with liver cirrhosis or coagulopathy, (patients whose PLT count less than $100x10^3$ /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 problems as cardiac and chronic renal failure.

All patients were subjected to: full history taking, full clinical examination, blood samples were obtained for complete blood count, and liver function tests (Alanine transaminase, Aspartate transaminase, Serum albumin, Bilirubin, Prothrombin time and concentration).

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 microscopy.

The histological liver damage of these specimens was evaluated for fibrosis and inflammation, regarding the severity of fibrosis was classified according to **Scheuer et al., [19]** 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.

Patients were then recategorized 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.

Immunohistochemical study of Monoclonal antibodies were used against CD41 in hepatocyte area as a marker of PLTs and PDGFR- β by perisinusoidal mesenchymal cells as a marker of fibrosis using staining kit (Catalogue # AEX080-IFU, ScyTek Laboratories). The intensity of expression of each marker was presented by calculation of the average weighted score (AWS) on a (0-12 scale).

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. Comparison of mean in 2 groups was carried by *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*. *Pearson's correlation* was used to study the correlation. 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 a comparison between the HCV patients groups, GI (thrombocytopenic group) and G II (Normal PLTs group) regarding some demographic parameters. No statistically significant difference regarding age, gender, and residence (**P>0.05)** was observed.

Table (2) illustrates comparison between the HCV patients GI (thrombocytopenia group) and G II (Normal PLTs group) regarding some liver functions tests. Mean ALT, AST and Total Bilirubin were significantly higher in group I compared to group II (**P<0.05).** On the other hand, mean serum albumin was significantly lower in group I compared to group II (**P<0.05).** Table (2) and figure (1) show that the mean AWS of CD41 and PDGFR- ß were significantly higher in group I compared to group II **(P<0.001 for each).**

Table (3) clarifies that the mean peripheral PLTs number was non significantly lower in advanced stage (3) of fibrosis than earlier stages (0-1-2) **(280±100.88 *vs* 237.2±133.83; P=0.23).** The same table clarifies that the mean AWS of immunohistochemical expression of CD41 was significantly elevated in patients with stage (3) fibrosis than patients with lower stages **(7.26±3.53 vs 5.88±3.24; P<0.01)**. Similarly, we found that the mean AWS of immunohistochemical expression of PDGFR- ß was significantly higher in stage (3) fibrosis than lower stages **(9.2±2.7 *vs***
6.7±3.6; P=0.04)

Figure (2) reveals a significant negative correlation between AWS CD 41 in hepatocyte area and PLTs number among HCV patients **(r= - 0.41, P=0.007).**

Figure (3) reveals a significant negative correlation between AWS PDGFR- β in fibrous septa extending from portal area and PLTs number among HCV patients (**r= -0.34, P=0.045).**

Table (1): Comparison between the 2 studied groups regarding some parameters

Parameters:	Group I N=10	Group II N=40	P-value
Age (years)	48.1±5.8	34.5±12.02	0.248
Gender (M/F)	7/3	33/7	0.314
Residence (Rural/Urban)	7/3	30/10	0.515

Table (2): Comparison of liver functions tests between the 2 studied groups of patients.

Parameters:	Group I N=10	Group II N=40	P-value
ALT (IU/L)	95.1±55.76	48.48±29.86	<0.001
AST (IU/L)	91.93±47.9	56.7±34.2	0.01
PT(Sec)	13.2±1.06	12.57±0.94	0.072
TBL(mg/dL)	1.25±0.37	1.06±0.34	0.045
Albumin(g/dl)	3.6±0.71	4.49±0.7	<0.001
CD41	5.8±2.86	3.43±3.03	<0.001
PDGFR- β	6.9±3.6	5.27±3.92	<0.001

Significant P values are in Bold.

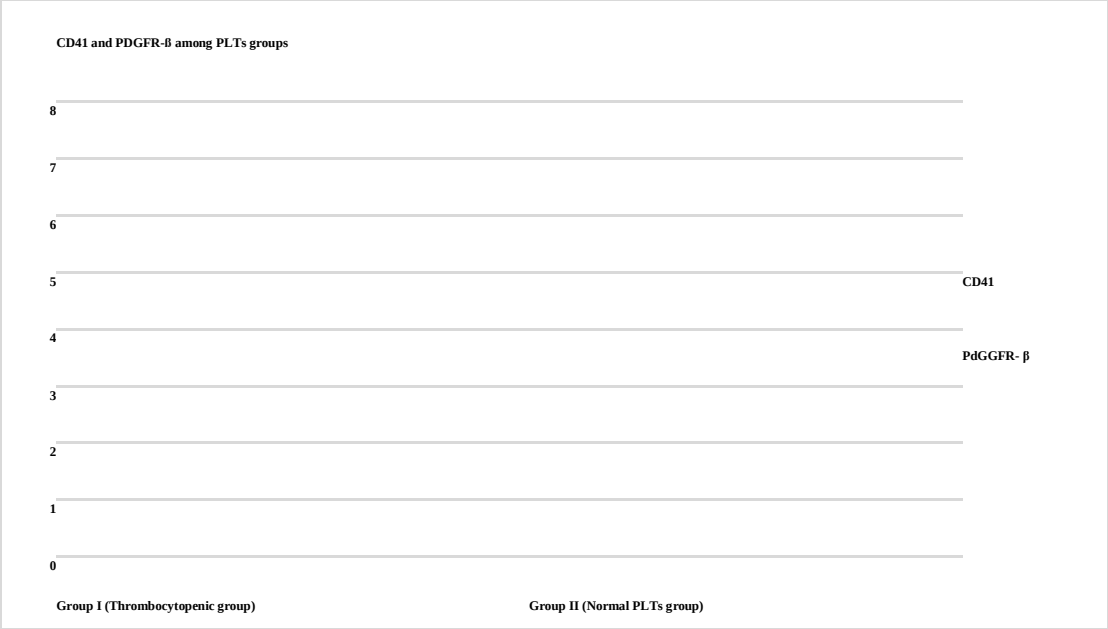

Fig (1): Comparison of Immunohistochemical expression of CD41 and PDGFR ß among PLTs groups**.**

Table (3): PLTs count, AWs of immunohistochemical expression of CD41, and PDGFR ß in different stages of chronic hepatitis**.**

	Stages (0-1-2) N=40	**Stage (3) N=10**	**P-value**
PLT count	280±100.88	237.2±133.83	0.023
CD41	5.88±3.24	7.26±3.53	**<0.01**
PDGFR- β	6.7±3.6	9.2±2.7	**0.04**

Significant P values are in Bold.

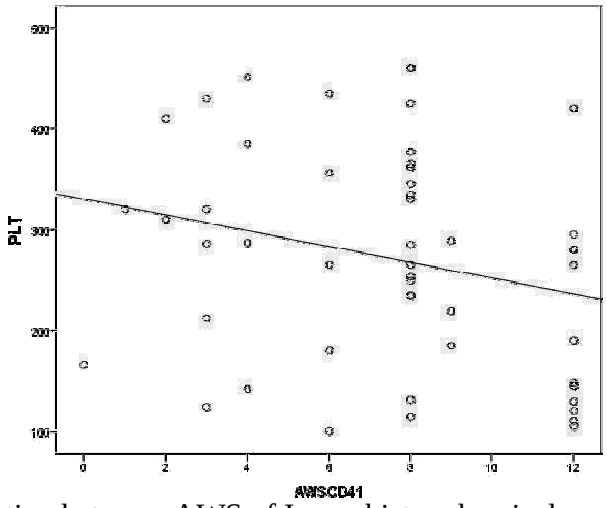

Fig (2): Correlation between AWS of Imunohisto- chemical expression of CD41 in Hepatocytes and PLTs count**.**

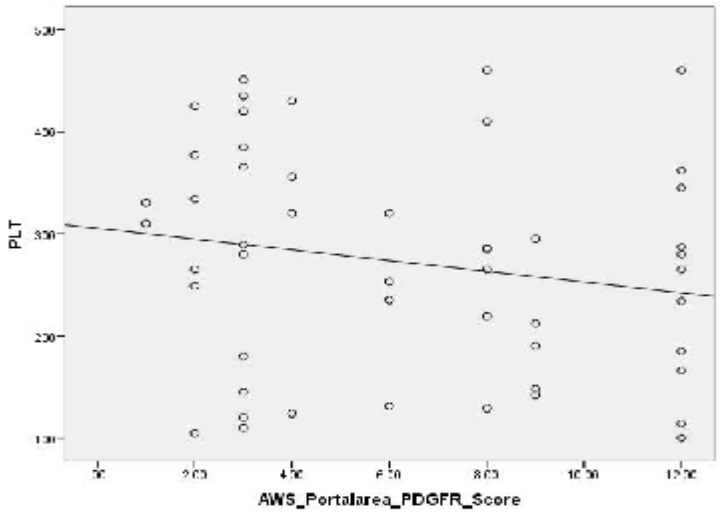

Figure (3): Correlation between AWS of Imunohistochemical expression of PDGFR- β in fibroblasts in fibrous septa extending from portal area and PLT count.

Discussion:

Thrombocytopenia is especially common in patients infected with HCV through a variety of mechanisms, one of which is direct bone marrow suppression. Patients with HCV without splenomegaly show depressed platelet production, and production increases after successful treatment of the infection [(20)]**.** Furthermore, Chronic HCV infection is associated with a plethora of autoimmune disorders. Approximately 38% of patients with HCV infection exhibit at least one immune-mediated, extra hepatic manifestation during the course of their disease. Patients with chronic liver disease due to HCV develop a thrombocytopenia that parallels the severity of their disease and is mirrored by increasing titers of Platelet-Associated Immunoglobulin G (PAIgG) [(21, 22)]**.**

Here, we studied whether the intrahepatic PLTs accumulation could contribute to

thrombocytopenia in chronic HCV patients. We examined the immunohistochemical expression of PLTs surface markers CD41 in the livers of these patients.

Our findings of a significantly increased immunohistochemical expression of CD41 in liver tissue of HCV patients with thrombocytopenia than those with normal PLTs count is in agreement with **Kondo et al.** [(23)] who reported an accumulation of PLTs in the liver tissue of patients who underwent hepatectomy for hepatocellular carcinoma (HCC) secondary to chronic HCV. They measured the immunohistochemical expression of CD41 in the non-cancerous areas in patients with chronic hepatitis or cirrhosis where it increased along with an increase in histological liver damage, although the blood PLTs count significantly decreased. They reported that PLTs were predominantly present in the sinusoidal spaces of periportal area with inflammation. Similarly, **Yan et al.** [(24)] reported intra- hepatic PLTs accumulation in patients with chronic hepatitis B using monoclonal Ab against CD61 PLTs surface antigen.

Sata et al. [(25)] used 111Indium-Labelled PLT to clarify the mechanism of thrombocytopenia observed in patients with chronic hepatitis B treated with interferon. They reported intrahepatic PLTs accumulation in 50% of the studied patients.

Starlinger et al. [(26)] performed an immunohistochemical study for detection of platelets markers (CD61) in the liver; they described PLTs accumulation with in the liver during the initial periods of liver resection (During hepatectomy). They also reported a decrease of PLT's count in the with earlier stages of fibrosis. **Ikeda et al.** [(34)] reported significantly lower PLT count in patients with advanced fibrosis and showed that human PLTs contribute to suppression of both HSC activation

liver veins when compared to the portal vein, indicating that PLTs adhere in the hepatic vasculature and remain in the liver. Viral infections have been associated with Immuno-Thrombocytopenic Purpura (ITP), especially HCV. Up to 30% of patients with ITP without evidence of advanced liver disease are seropositive for HCV [(27)]**.** The rate of ITP among patients infected with HCV is 30.2 per 100,000 person/year compared to 18.5 per 100,000 person/year for non-HCV-infected individuals [(28)]**.**

Liang et al. [(29)] performed an immunohistochemical study on spleens of patients with ITP and they demonstrated the presence of PLT antigen CD41 in the germinal centers of foamy macrophages, which may reflect the sites of immune reaction and PLT destruction in immune TP.

Recent studies highlighted that PLT Desialylation (cleavage of terminal sialic acids from the glycoconjugates on the PLT's surface) leads to PLT clearance via hepatocyte asialoglycoprotein receptors (ASGPs). Over the past decades, PLT desialylation has been shown to be responsible for PLT clearance in many contexts, such as destruction of chilled PLTs [(30)], free radicals and infections related thrombocytopenia [(31, 32)] and the clearance of senescent PLTs [(33)]**.**

Based on these findings, it would be reasonable to consider intra hepatic PLTs accumulation as one of the possible mechanism of thrombocytopenia in chronic HCV patients.

There is complex interaction between PLTs and liver fibrosis, we found that patients in stage (3) of fibrosis had non significantly lower mean PLT count than those8

and type I collagen production via cyclic AMP signaling pathway *in vitro*. In addition, platelet derived hepatocyte growth factor (HGF) plays a critical role

in suppression of type I collagen gene expression in cultured HSCs [35].

Our findings of a significantly increased expression of PDGFR -ß in liver tissues of patients with advanced stage of fibrosis than those with earlier stages is in agreement with previous reports [23, 36]. They identified a dense of population of cells expressing PDFR -ß in the periportal area as myofibroblast like cells. These cells which play a central role in liver fibrosis and are believed to be transformed from Ito cells [37] and their proliferation is stimulated by PDGF [17]. When HSCs expressing PDGFR- ß are present in the liver, the liver may be susceptible to PDGFR contained in PLTs [23].

Conclusion:

In conclusion, we have demonstrated that the accumulation of PLTs in the liver in patients with chronic hepatitis C could be an important factor involved in pathogenesis of thrombocytopenia in these patients. Intra-hepatic PLTs may also contribute to liver fibrosis through the action of PDGF ß on hepatic stellate cells. Further studies of the biological characteristics and function of these cells will contribute to improving the treatment of thrombocytopenia and liver fibrosis.

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

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