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Antiplatelet Antibodies in Hepatitis C Virus Associated Thrombocytopenia

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ABSTRACT

Background: Thrombocytopenia is, likely, the most common extra hepatic manifestation that can affect patients of chronic hepatitis C virus (HCV) infection. More than one third of HCV patients develop apparent thrombocytopenia.

Aim of the Study: To investigate the role of antiplatelet antibodies in HCV-associated thrombocytopenia and correlate that with the development of thrombocytopenia, the level of viremia and ALT level.

Patients and Methods: The study was applied on ninety subjects. Seventy of them were chronic HCV infection patients divided into group (A) composed of 50 patients with thrombocytopenia and group (B) of 20 patients with normal platelet count. Also, there was a control group composed of 20 apparently normal healthy individuals. Quantitative indirect anti-platelet antibodies were detected by ELISA for all groups. Also, full blood count, liver function tests and level of viremia by real time quantitative PCR were done for all patients.

Results: There were negative significant correlations between platelet count and both of antiplatelet antibodies and level of viremia (r=–0.32, p=0.007 and r=–0.57, p=0.001 respectively). Also, positive significant correlation between antiplatelet antibodies and viral load was found (r=0.27, p=0.022).

Antiplatelet antibodies were significantly higher in HCV thrombocytopenic patients compared to those with normal platelet count and normal control (p=0.002 and p=0.001 respectively).

Conclusion: Antiplatelet antibodies were detected in thrombocytopenic HCV patients suggesting an immunological role for HCV infection in the development of thrombocytopenia. The level of viremia may be a predictive indicator for development of antiplatelet antibodies.

Key Words: *HCV – Thrombocytopenia – Antiplatelet antibodies.*

INTRODUCTION

The percentage of hepatitis C virus (HCV) infection among Egyptians is 14.7%. This is ten times greater than any other country in the world **[1]**.

Thrombocytopenia is one of the most common extrahepatic complications in patients with chronic HCV infection. Severe thrombocytopenia is associated with life-threatening bleeding risk **[2]**.

The pathogenesis of thrombocytopenia in patients with chronic HCV is complex and multifactorial **[3]**. Pathological mechanisms include immune dysfunction, bone marrow suppression, decreased thrombopoietin levels/ activity, hypersplenism and antiviral treatment related side effects **[4]**.

In HCV associated thrombocytopenia, autoantibodies against platelet surface antigens can enhance platelet sequestration and destruction by the reticuloendothelial system but this occurs in association with one or more of the other mechanismsof thrombocytopenia **[4]**.

The aim of this study was to investigate the presence of antiplatelet antibodies in patients with HCV associated thrombocytopenia and to correlate this with degree of thrombocytopenia, the level of viremia and ALT level.

PATIENTS AND METHODS

Patients:

Our study was applied on seventy patients with chronic HCV infection and twenty apparently healthy controls who presented to the

Hepatology and Hematology Clinics at Assiut University Hospitals and National Committee for Control of Viral Hepatitis at Health Directorate of Assiut Governorate and signed written informed consent was obtained from all patients. Patients were divided into: Group (A) composed of 50 HCV patients with thrombocytopenia (platelet count <150X10^9/L **[5]** and group (B) of 20 patients with normal platelet count (platelet count ≥150X10^9/L).

Methods:

All patients were subjected to history taking and clinical examination. Laboratory investigations included complete blood count (CBC), liver function tests: Alanine transferase (ALT), Aspartate transferase (AST), total bilirubin (T. Bil), prothrombin time (PT), prothrombin concentration (PC), serum albumin and quantitative HCV-RNA by real-time PCR. Quantitative determination of anti-platelet antibodies in serum was done by human platelet antibodies ELISA kit (Cat No# 30256, Glory science Co., USA).

Statistical analysis:

Statistical analyses were performed with the IBM SPSS 22.0 software. Quantitative data were described using mean and standard deviation. The distributions of quantitative variables were tested for normality using Kolmogorov-Smirnov test, Shapiro-Wilk test.

For normally distributed data, comparison between two independent populations was done using independent *t*-test. Correlations between two quantitative variables were assessed using Pearson coefficient.

RESULTS

Statistically significant lower values of hemoglobin, Total leucocytic count (TLC), serum albumin and prothrombin concentration with statistically significant higher values of viral load, prothrombin time, total Bilirubin, ALT/PLT ratio and AST/PLT ratio were detected in thrombocytopenic patients (Group A) compared to non-thrombocytopenic patients (Group B) (Table 1).

HCV patients with thrombocytopenia showed platelet antibody level of 255.2± 85.5ng/ml. This was significantly higher than the corresponding figure for HCV patients with normal platelets (196.3±56ng/ml, p=0.002) and for normal control (162.7±33.4ng/ml, p= <0.001). The difference between HCV patients with normal platelets and normal control was insignificant (p=0.138).

Antiplatelet antibodies had statistically significant weak positive correlation with platelet count, prothrombin time, total bilirubin and viral load (r=0.32, 0.24, 0.28 and 0.27 respectively; Table 2).

Platelet count showed significant negative correlation with viral load (r=–0.57) prothrombin time (r=–0.283) and total bilirubin level (r=–0.325) and significant positive correlation with hemoglobin level (r=0.468) and serum albumin level (r=0.368); correlation with TLC was border line with r<0.25 and p-value of 0.05 (Table 3).

ALT showed strong positive correlation with AST (r=0.72, p=0.005) and no correlation to other parameters.

Table (1): Comparison of laboratory parameters between thrombocytopenic (Group A) and non–thrombocytopenic (Group B) HCV patients.

Parameter	Group A: No 50	Group B: No 20	
TLC X 10^9/L	5.1±1.8	5.9±0.9	0.018*
Hb: g/dl	11.9±2.2	13.6±1.3	<0.001**
PT: seconds	13.5±2.1	12.4±0.7	0.002**
PC: %	78.4±16.3	91.2±9.5	<0.001**
Albumin: g /L	35.1±7.5	40.8±4.6	<0.001**
ALT:U/L	54.9±16.2	49.5±14	0.194
AST: U/L	53.6±12.5	52.1±9.6	0.629
Bilirubin: mg/dl	1.3±0.8	0.8±0.3	0.04*
AST/ALT	1.15± 0.4	0.9±0.37	0.09
ALT/PLT	0.6±0.5	0.2±0.3	0.000**
AST/PLT	0.6±0.7	0.16±0.26	0.000**
Viral load: IU/L	1697900± 714143.8	338436.5± 100407.3	<0.001**

TLC : Total Leucocytic Count.
Hb : Hemoglobin.
PT : Prothrombin Time.
PC : Prothrombin Concentration.
ALT : Alanine transaminase.
AST : Aspartate transaminase

Table (2): Correlation between Antiplatelet antibodies and other parameters in 70 HCV patients.

Parameter	*r*	*p*. value	Parameter	*r*	*p*. value
Platelet count	–0.32	0.007*	T.Bil	0.28	0.019*
TLC	–0.13	0.122	ALT	–0.03	0.795
Hb	–0.19	0.112	AST	–0.05	0.669
PT	0.24	0.046*	Albumin	–0.16	0.175
Viral load	0.27	0.022*			

TLC: Total Leucocytic Count. ALT: Alanine transaminase.
Hb : Hemoglobin. AST: Aspartate transaminase.
PT : Prothrombin Time.

Table (3): Correlation between Platelet count and different parameters in 70 HCV patients.

Parameter	*r*	*p*	Parameter	*r*	*p*
TLC	0.231	0.05*	Albumin	0.368	0.002**
Hb	0.468	0.000**	Total Bilirubin	–0.325	0.006**
PT	–0.283	0.017*	ALT	–0.041	0.260
Viral load	–0.57	0.001**	AST	–0.18	0.223

TLC: Total Leucocytic Count. ALT: Alanine transaminase.
Hb : Hemoglobin. AST: Aspartate transaminase.
PT : Prothrombin Time.

DISCUSSION

The results of the current study revealed significantly higher level of antiplatelet antibodies in thrombocytopenic HCV patients compared to HCV patients with normal platelet count and normal control group. This may indicate that immune-mediated processes are one of the mechanisms involved in the pathogenesis of thrombocytopenia in HCV patients. In HCV patients, autoantibodies directed against platelet surface antigens can promote platelet sequestration and destruction by cells of the reticuloendothelial system **[6]**.

However, the presence of antiplatelet antibodies in HCV patients is not always associated with thrombocytopenia and even rather high titers can be found in patients with normal platelet counts **[7]**. Though there was significant association between antiplatelet antibodies and thrombocytopenia in patients with HCV patients, it could not be demonstrated that these antibodies were the real cause of thrombocytopenia denoting the complex and multifactorial mechanisms of thrombocytopenia in chronic HCV patients **[8]**.

In the current study, the level of antiplatelet antibodies showed statistically significant negative correlation with platelet count. This result concluded that antiplatelet antibodies contribute to thrombocytopenia associated with chronic hepatitis C virus infection **[6,9]**. Also, some studies concluded that HCV infection plays an important role in development of antiplatelet antibodies leading to immunological destruction of the platelets **[10]**. However, other studies failed to show any correlation between antiplatelet antibodies and platelet count in subgroups of HCV patients **[8]**.

In the current study, antiplatelet antibodies showed significant positive correlation with viral load. Direct cytopathic involvement of HCV can be hypothesized. HCV has been reported to bind to platelet membranes by multiple cell surface receptors. In patients with high HCV viral loads, platelet membranes may be heavily coated with HCV. Anti-HCV antibodies may bind to the platelet surface-associated HCV, leading to phagocytosis of the antibody-coated platelets. A relationship between the HCV plasma viral load and thrombocytopenia is supported by the observation that reduction of HCV viral load with INF therapy can result in increased platelet counts **[6]**.

In the current study, the viral load in the serum by PCR was significantly higher in the thrombocytopenic group compared to the group with normal platelet count. This may indicate that HCV infection plays an important immunological role in the development of thrombocytopenia through development of anti-PLT antibodies leading to immunological destruction of the platelets **[10]**. However, another study reported insignificant difference so they suggest that HCV can be detected in platelets of chronically HCV-infected patients independent of circulating HCV load. Studies on HCV dynamics are needed to provide new insights into HCV binding to platelets **[12]**.

In the current study, there was significant negative correlation between viral load and platelet count. Our findings suggest an immune-based mechanism of thrombocytopenia in chronic HCV as aberrations of the immune system result in formation of anti-PLT antibodies and/or immune complexes that bind to PLTs and facilitate their premature clearance **[10,14]**.

The current data showed that both ALT and AST had no significant difference between

thrombocytopenic and non thrombocytopenic HCV patients. While, Another study stated that Serum ALT level is a well-known indicator of hepatocellular damage **[6]**.

The results of the current study revealed that ALT had significant positive correlation with AST but no significant correlation with platelet count or viral load **[10]**.

A limitation of this study is the small size of the individual subgroups, which limits interpretation of results. Further study of larger groups of patients would be required to confirm our findings.

Conclusion:

- The anti-PLT antibodies were significantly correlated to PLT counts, viral load but not ALT.
- HCV infection plays an important immunological role in the development of thrombocytopenia through development of anti-PLT antibodies leading to immunological destruction of the PLTs in the reticuloendothelial system.
- Antiplatelet antibodies were detected in the studied thrombocytopenic patients; exposure to HCV may be a causative factor for the production of platelet associated immunoglobulins.

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