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Evaluation of Hematological and Biochemical Changes observed in HCV Patients with Different Level of Viremia

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HCV viremic status is associated with many hematological and biochemical, immunological abnormalities in patients with chronic hepatitis C. Aim of the study: to describe different patterns of changes accompanying viremic status in HCV chronic liver diseases. 50 patients with chronic hepatitis C were enrolled in this study. Qualitative and quantitative PCR by real time PCR was done to determine the level of viremia before induction of therapy. In addition, complete blood count, prothrombin time and liver function tests were performed. The results showed that leucopenia and thrombocytopenia are more frequent in HCV patients with moderate degree of viremia than patients with mild viremia than those with no viremia ($p<0.05$). Moderate neutropenia (< 1000 /ul) and moderate lymphopenia (< 1000/ul) are observed in all patients with different levels of viremia in comparison with those patients with no viremia. Only 10 percent of patients with moderate viremia had mild anemia (Hb < 10 g/dl). Red cell indices are normal in different degree of viremia and in those with no viremic status. Platelets indices shows increased platelet distribution width in those with moderate viremia than patients with mild viremia than those with no viremia ($p<0.05$). ALT and AST are increased 3 fold normal range in patients with mild degree of viremia and more elevated than in patients with moderate viremia.

Key words:

INTRODUCTION

Hepatitis C virus (HCV) as a common cause of chronic liver disease is associated with major health consequences in almost one fifth of infected patients. HCV can lead to liver cirrhosis with consequent development of hepatocellular carcinoma **(Deutsch and Dourakis, 2004).**

HCV is a single stranded RNA virus **(Choo *et al.*, 1989),** belonging to the family of flaviviridae **(Lauer and Walker, 2001).** It is estimated that the prevalence of HCV infection exceeds 100 million people worldwide **(Lavanchy, 1999).**

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HCV infection is not restricted to the liver but it can involve also extrahepatic sites as the lymphoid cells leading to extrahepatic dissemination and acting as a reservoir for continuous hepatocytes infection (**Zignego *et al.*, 1992**).

Numerous studies reported the association between HCV infection and the autoimmune disorders (**Cacoub *et al.*, 2000**) with more emphasis on the hematology-hepatology linkage. Evidence of HCV replication has been reported in peripheral blood cells (**Lerat. *et al.*, 1996**) and abnormal blood counts have been noted in patients with HCV infection. There are several reports of neurtopenia, thrombocytopenia or pancytopenia associated with HCV infection (**Streiff *et al.*, 2002**). However, the hematological and biochemical changes in hepatitis C patients and its correlation with the level of HCV viremia were not fully examined.

This study was conducted to describe the different patterns of hematological and biochemical changes observed in HCV patients in correlation with different levels of viremia.

MATERIALS AND METHODS

Fifty patients with chronic hepatitis C were enrolled in this study. All patients were subjected to the following investigations; complete blood count, prothrombin time and liver function tests. Qualitative and quantitative PCR by real time PCR was done to determine the level of viremia before induction of therapy.

RNA extraction was performed using MagNA pure LC total nucleic acid isolation kit and amplification was done using a Roboscreen TaqMan reagent mix for HCV by using LightCycler real time PCR.

Data analysis was performed via SPSS, version 10. The degree of HCV viremia was subdivided into the following categories; mild = up to 0.25×10^6 IU/ml, moderate = 0.25×10^6 IU/ml- 2.5×10^6 IU/ml, high = 2.5×10^6 IU/ml- 10×10^6 IU/ml and very high = $> 10 \times 10^6$ IU/ml. Cases revealing less than 600 IU/ml were considered negative.

Experimental Protocol:

Reaction conditions:

Description		Volume (20 UL)
4 X RT-PCR Buffer		5.00
4 X HCV reagent	Ready to use	5.00
Enzyme 5U/UL		0.30
PCR grade water	RNA/DNA-PCR	5.00
RNA		5.00

	RT	Hold	45 Cycles	
Temperature	60 C'	95 C'	95 C'	61 C'
Time	30 min	5 min	5 Sec	1:00min

Fluorescence settings:

LED Power	CALIB	Display Mode	3.5 Compatible
Color	N/A		
Compensation	N/A		
Car. Movement	Continous		

Quantification settings:

Channel Setting	F1
Program Name:	Amplification

RESULTS

This study included fifty patients. All patients were subjected to the following investigations; complete blood count, prothrombin time and liver function tests. Qualitative and quantitative PCR by real time PCR was done to determine the level of viremia before induction of therapy.

The hematological changes observed in HCV patients are shown in table (1). The mean hemoglobin level was 13.96 g/dl, the mean value of red and white cell count (WBC) was 4.88/ul and 5964/ul respectively. The mean platelet count was 198.14/ul Regarding the hepatic enzymes, ALT mean value was 66 and AST mean value was 75. The degree of HCV viremia as stimated by quantitative PCR ranged from 0-11 $X10^6$ IU/ml (mean 2.1 $X10^6$IU/ml). These data are shown in table (3).

Table 1. Hematological changes in HCV patients

	Min	Maximam	Mean	SD
Hb (g/dl)	8.5	16.7	13.960	1.728
Red cell count (X^6/ul)	3.13	6.56	4.8846	.630
RDW (%)	11.10	20.80	14.4424	1.838
WBCs /ul	2400	13700	5964.00	2250.42
Platelets /ul	51	383	198.14	79.28

Table (2) presents the pattern of differential white blood cell counts. The mean values of the different cells were, neutrophils, 3590.4/ul; lymphocytes, 2017.52/ul; monocytes, 189.72/ul; eosinophils 90.26/ul and basophils, 6.62/ul.

Table 2. Differential of white blood cell counts in HCV patients

	Minimum	**maximum**	**Mean**	**SD**
Neutrophils/ul	912	8905	3590.40	1610.2
Lymphocytes/ul	1012	5280	2017.52	810.8
Monocytes/ul	48	411	189.72	85.0
Eosinophils/ul	0	276	90.26	65.5
Basophils/ul	0	83	6.62	17.7

Table 3. Hepatic enzymes estimates and PCR findings in HCV patients

	Min	**Maximam**	**Mean**	**SD**
ALT	45	145	66	13
AST	55	245	75	18
Quantitative PCR(IU/ml)	<100 (below detectable level)	$11X10^6$	$2.1\ X10^6$	$2.5\ X10^6$

The correlation between Hb level, white cell count, red cell count and platelets count with quantitative PCR revealed a significant correlation between Hb and PCR ($r = .361$, $p = .014$). Also, the correlation between the red cell count and PCR was significant ($p < .005$). However, the correlation between white cell count and PCR was not significant ($p = .58$) and

the correlation between platelets count and PCR was not significant (p = .32). These results are illustrated in figures (1 to 3). Figure 4 and 5 show the standard curve and patients' samples positive for HCV RNA with different levels by lightcycler PCR. The results showed that leucopenia and thrombocytopenia are more frequent in HCV patients with moderate degree of viremia than patients with mild viremia than those with no viremia ($p<0.05$). Moderate neutropenia (< 1000 /ul) and moderate lymphopenia (< 1000/ul) are observed in all patients with different levels of viremia in comparison with those patients with no viremia. Only 10 percent of patients with moderate viremia had mild anemia (Hb < 10 g/dl). Red cell indices are normal in different degree of viremia and in those with no viremic status. Platelets indices shows increased platelet distribution width in those with moderate viremia than patients with mild viremia than those with no viremia ($p<0.05$). ALT and AST are increased 3 fold normal range in patients with mild degree of viremia and more elevated than in patients with moderate viremia. These data are shown in table (4).

Table 4. Hematological & Biochemical parates among different category of viremia

Hematological Parameters	Mild viremia	Moderate viremia	Aviremic state	P value
leucopenia	30%	15%	0	<0.035
Thromcytopenia	25%	40%	0	< 0.045
Neutropenia	30%	20%	0	0.05
Anemia	0	10%	0	
Biochemical parameter				
ALT	46 ± 14	25 ± 10	60± 12	<0.04
AST	48 ± 9	36± 6	67 ± 13	<0.03

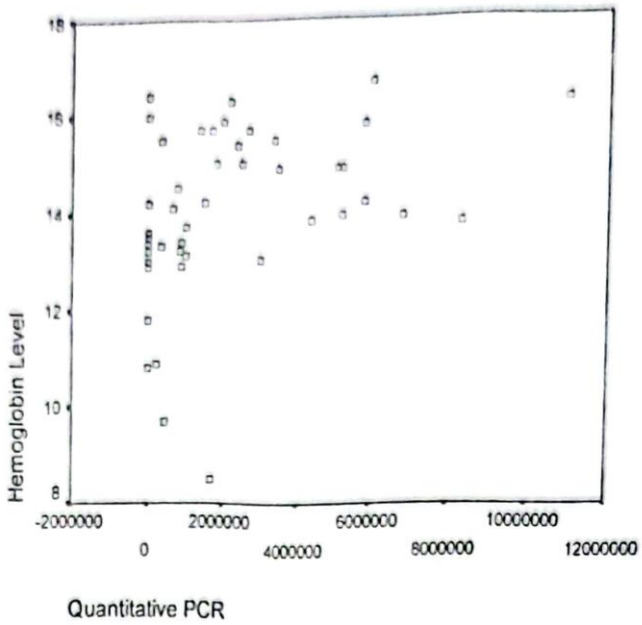

Fig. 1. Correlation between quantitative PCR and hemoglobin level (r = 0.361, p = 0 .014).

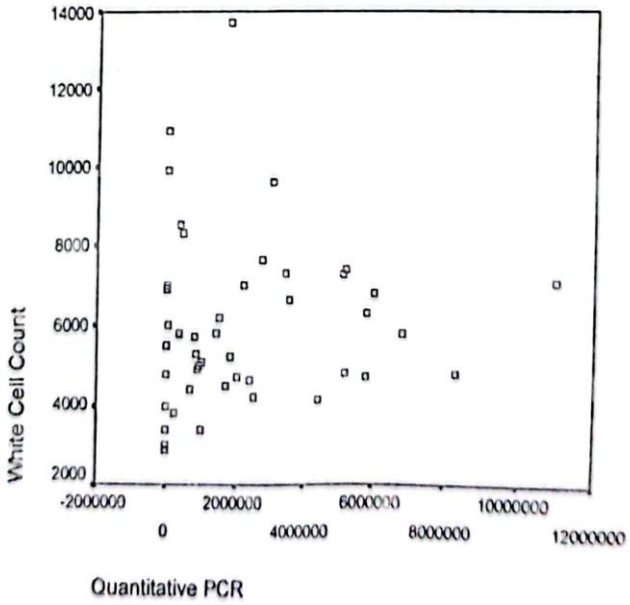

Fig. 2. Correlation between quantitative PCR and white cell count (p = 0 .58).

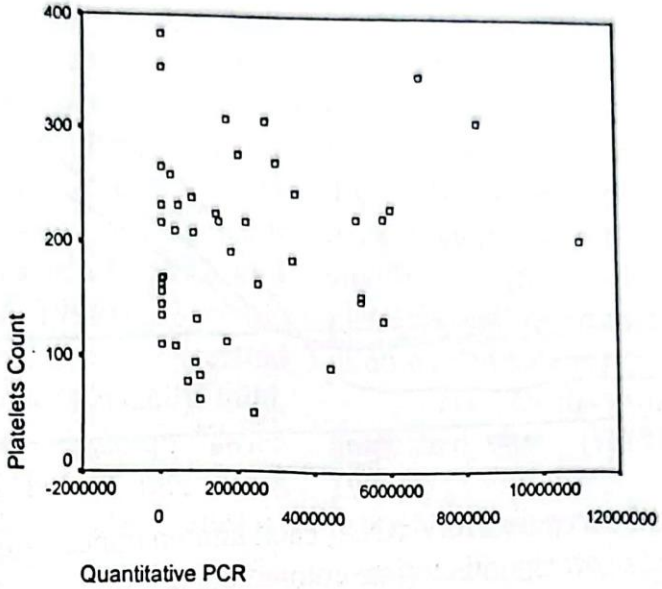

Fig. 3. Correlation between quantitative PCR and platelets count (p = 0 .32).

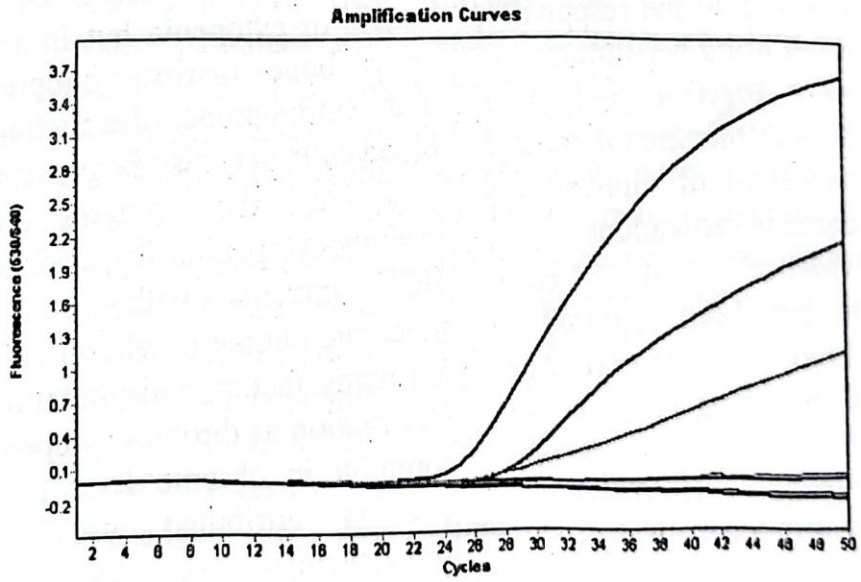

Fig. 4. Positive samples for HCV RNA, each curved line corresponds to number of cycle of amplification. Straight line indicates negative samples (eg green line).

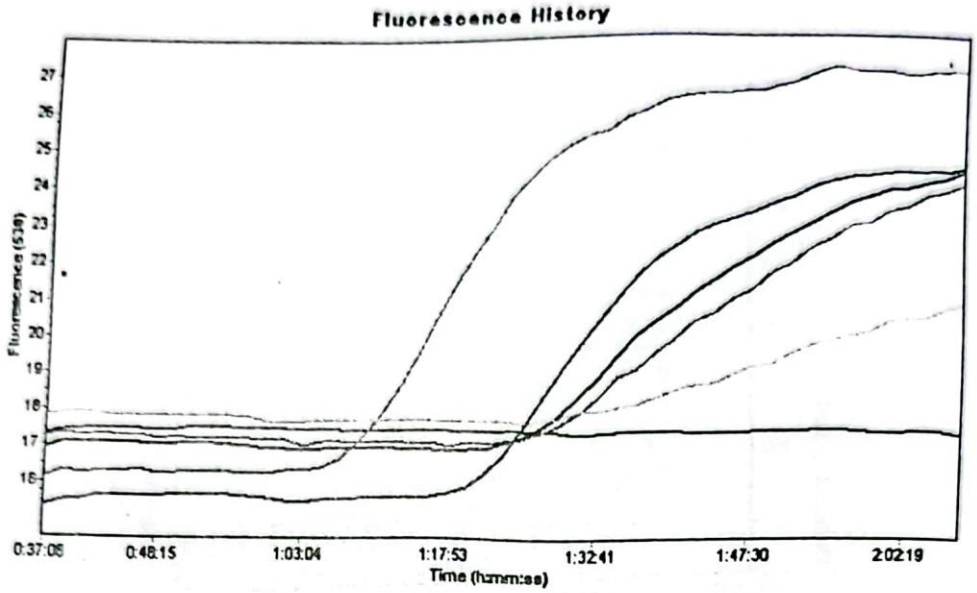

Fig. 5. Standard curve for HCV RNA, each known concentration of standards corresponds to one colored curved line.

DISCUSSION

The hepatitis C virus seems to be one of the responsible agents which bring hepatology and haematology together and it is obvious that in this area there is a great perspective of clinical and basic research investigations.

Evidence of HCV replication has been reported in peripheral blood cells (**Lerat *et al.*, 1996**) and abnormal blood counts have been noted in patients with HCV infection (**Streiff *et al.*, 2002**). There are several reports of neutropenia, thrombocytopenia or pancytopenia associated with HCV infection. Cirrhosis and hypersplenism **Bashour *et al.*, (2000)** are the most common causes of cytopenia but in several cases bone marrow suppression and autoimmune destruction of blood cells may also be present.

In the present study thrombocytopenia is evident in HCV patients with mild and moderate degree of viremia. There are many factors contribute to this observation as thrombocytopenia is common in chronic liver disease and is attributed usually to hypersplenism. Nevertheless rare cases of immune thrombocytopenia associated with positive anti-

platelet antibodies (PAIgG) have been described in association especially with chronic HCV infection. On the other hand, in ITP patients a higher frequency of HCV positivity has been documented than in general population **(Silva *et al.*, 1992 and Pawlotsky *et al.*, 1995).**

HCV related thrombocytopenia is usually mild, with platelet count >5 $x10^3$platelets/ul but in rare cases thrombocytopenia with clinical relevant bleeding tendencies has been reported. In a Japanese study thrombocytopenia (<$15x10^4$ platelets/ul) was found in 41% out of 368 patients with hepatitis C with a significant difference when compared with patients with chronic hepatitis B (18.9%). Moreover, elevated titres of platelet associated immunoglobulin G (PAIgG) were found in 88.1% of the patients with hepatitis C and HCV-RNA by RT-PCR was detected in the platelets from 11 out of 14 tested patients. A positive correlation between the PAIgG titre and the severity of architectural changes in liver histology could also be documented. In contrast with these findings another study could not find positive PAIgG in 11/13 HCV-positive thrombocytopenic (without hypersplenism) patients [14]. Nevertheless, in six out of these patients a trial of recombinant á2b-interferon at a dose 3 MU three times a week for 6-24 months was performed, and a significant and durable increase in platelet count could be documented in all treated patients.

The mechanism of HCV associated not **(Hadziyannis , 1996)** hypersplenic thrombocytopenia has not yet been clearly defined. The findings that HCV-RNA could be detected in platelets and in megacaryocytes lead to the suspicion of a mechanism in which HCV is directly involved in a possible underproduction of platelets. The other hypothesis is the possibility of a HCV induced disregulation of the host immune system (HCV binds to immunoglobulin which leads to the peripheral destruction of the platelets. On the other hand a progressive decrease of thrombopoietin production in patients with HCV infection in parallel with the decline in liver function could also been documented **(Hijikata *et al.*, 1993).** Decrease in thrombopoietin serum levels may partly be responsible for the

thrombocytopenia in chronic hepatitis C **(Hijikata *et al.*, 1993).**

In the present study correlation tests revealed negative significant correlation between HCV RNA levels and platelets counts and this result is approved by others who reported that after stratification by demographic data, hepatitis status, and health-related behavior in backward elimination from a multiple logistic regression analysis, anti-HCV positivity was revealed to be most strongly associated with thrombocytopenia (OR, 6.0; 95% CI, 3.211.2). ALT levels of >40 U/L (OR, 2.1; 95% CI, 1.13.9) and older age (>65 years; OR, 4.3; 95% CI, 2.09.5) were also strongly associated with thrombocytopenia **(Chong-Shan *et al.*, 2004).**

From the obtained data in this paper there is leucopenia mainly neutropenia, and had thrombocytopenia and these findings together point to acquired aplastic anemia. In accordance with these findings bone marrow depression associated with hepatitis has been first reported in 1955. Since then >200 cases of aplastic anaemia have been recognized. The overall incidence of this rare complication is estimated at 0.1-0.2% implicating hepatitis A, B and especially C. The described patients were all young (mean age 20) rather male than female and aplastic anaemia occurred especially in cases of acute hepatitis with a mean eight weeks after the onset of symptoms **(Giannini *et al.*, 2003).**

Finaly, in conclusion, hematological changes are more prominent in HCV patients in viremia status than those with no viremia. However, liver enzymes are increased in patients with low viremia in comparison with those with moderate viremia. This issue needs more clarification.

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