Correlation between CD4+, CD8+ T Cells Count and Liver Function Tests in Chronic Hepatitis C Infection.

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Abstract: T cells are believed to be involved in the pathogenesis of important liver diseases including both autoimmune liver diseases and viral hepatitis. **The aim** of this study was to find if there is a correlation between liver function tests and levels of CD4 and CD8 in patients with chronic hepatitis C virus (HCV) infection to determine the role of T cells in the pathogenesis of HCV. **Patients and methods:** This study was conducted on 60 patients with chronic HCV infection proved by PCR. The patients were divided into 3 groups; Group 1: included 20 patients with normal liver function tests and normal abdominal ultrasound. Group 2: included 20 patients with abnormal liver function tests and normal abdominal ultrasound. Group 3: included 20 patients with abnormal laboratory results and abnormal abdominal ultrasound. Immunophenotyping of peripheral blood lymphocytes by flow cytometry for CD4 & CD8 was done for all groups. **Results:** This study showed a highly significant positive correlation between CD4 T-cell counts and liver enzymes, also there was a highly significant positive correlation between CD8 T-cell count and bilirubin level and a negative correlation between CD4 T-cell count and CD8 T-cell count. **Conclusion:** CD4 and CD8 T-cell counts may represent a non invasive method to determine the immune response against chronic HCV infection.

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1. Introduction

T lymphocytes play a central role in inflammatory diseases of liver such as viral hepatitis, autoimmune hepatitis through their diffuse effector functions and their regulatory effect on other immune cells **(Law *et al.*, 2003).** Hepatitis C virus (HCV) is the most common cause of chronic liver disease worldwide as replication of the virus takes place primarily in the liver ***(Thimme et al., 2002).*** However, liver damage is not directly caused by the virus, rather is by the interplay between the virus and the immune system which results in the replacement of healthy liver tissue with fibrous scar tissue ***(Tedeschi, 2009)***.

Antibodies directed against several HCV proteins can be detected in chronic patients. A variety of autoimmune or immune complex-mediated diseases have also been associated with chronic HCV infection ***(Tedeschi, 2009).***

In chronic hepatitis C patients, HCV-specific CD4+ T cells were functionally impaired and their activity was not sustained, which was in clear contrast with resolved cases. Inoculation studies of infectious HCV to recovered chimpanzees demonstrated that CD4+ T cell help was indispensable for the development of effective CD8+ T cell response to protect from HCV persistence ***(Ulsenheimer et al., 2003).***

With regard to HCV-specific CD8+ T cells observed during the chronic stages of disease, conflicting results have been reported for their roles in HCV replication and liver inflammation. Several investigators have shown that the HCV specific cytotoxic T lymphocytes (CTL) response is inversely correlated with viral load, suggesting its inhibitory capacity on HCV replication. Also, HCV-specific CD8+ T cells in chronic hepatitis C patients were found to possess lesser capacity to proliferate and produce less IFN-γ in response to HCV antigens ***(Wedemeyer et al., 2002).***

Since CD8+ T cells are reported to be involved in HCV-induced liver inflammation, inefficient CD8+ T cells may evoke only milder hepatocyte injury, which level is not sufficient for HCV eradication ***(Wedemeyer et al., 2002).*** Several mechanisms have been proposed for T cell functional failure observed in chronic HCV infection:

1) HCV-escape mutation.
2) Primary T cell failure or T cell exhaustion.
3) Impaired antigen presentation.
4) Suppression by HCV proteins.
5) Impaired T cell maturation. ***(Ulsenheimer et al., 2005).***

Aim of the study

To find out a correlation between the laboratory results and level of CD4+ and CD8+ T cells to determine the role of T cells in patients having chronic HCV infection.

2.Patients and Methods

This study was conducted on 60 patients with chronic HCV infection proved by PCR at Ain Shams University Hospitals in the period from January 2012 to June 2012. The patients were divided into 3 groups as follows:

Group 1 included twenty patients with normal liver function tests and normal abdominal ultrasound.

Group 2 included twenty patients with abnormal liver function tests [including elevated liver enzymes, and/or increased bilirubin levels and/or prolonged prothrombin time (PT)] and normal abdominal ultrasound.

Group 3 included twenty patients with abnormal liver function tests [including elevated liver enzymes, and/or increased bilirubin levels and/or prolonged prothrombin time (PT)] and abnormal abdominal ultrasound in the form of hepatomegaly, hepatosplenomegaly.

All patients involved in the study were subjected to:

1) Full History taking and clinical examination.
2) Investigations:

- Liver function tests:
  - ALT (Alanine aminotransferase).
  - AST (Aspartate aminotransferase).
  - Serum Bilirubin (total and direct).
  - Serum albumin.
- Prothrombin time (PT).
- Abdominal Ultrasound.
- Immunophenotyping by flow cytometry for measurement of CD4+ & CD8+ T cell counts. For lymphocytes staining, 50 uL of whole blood (with leucocytic count adjusted to 10,000 cell/uL) was placed into polystyrene tubes and was simultaneously stained with 5 ul of FITC-labeled anti-human CD4 and PE-conjugated anti-human CD8 monoclonal antibodies (Immunotech, USA). After 15 min incubation in the dark, at room temperature, 1.0 ml of laboratory prepared ammonium chloride based lysing solution was added and RBC lysis was allowed for 10 min at room temperature. Samples were washed once and re-suspended in 0.5 mL of phosphate buffered saline (PBS).

Cells were analyzed using Coulter EPICS XL flow cytometer with System II software (Coulter, USA). Lymphocytes were electronically gated in a linear forward scatter / log side scatter histogram. The percentages of cells expressing CD4 and CD8 were estimated in a two-parameter histogram. Then the absolute count was calculated.

Statistical Analysis

Statistical package for Social Science (SPSS 15.0.1 for windows; SPSS Inc, Chicago, IL, 2001) program was used to analyze and calculate the statistics of our results.

3.Results

A total of 60 patients with HCV infection were included in this study. They were divided into three groups according to laboratory findings of liver function test. They were 15 males (75%) and 5 females (25%) in group I, 14 males (70%) and 6 females (30%) in group II and 15 males (75%) and 5 females (25%) in group III with no statistically significant difference between the three groups regarding gender (p >0.05).

The mean age of studied groups was 48.2±7.2 years in group I, 42.9±10.5 years in group II and 48.7±10.3 years in group III with no statistically significant difference between the three groups regarding age (p > 0.05).

There was no significant difference between the three studied groups regarding the level of viremia (Table 1).

Table (1): Comparison between the three studied groups as regard HCV- PCR.

	Group			*P**	Sig	Post Hoc test
	Normal lab and US (Gr1)	Abnormal lab and Normal US (Gr2)	Abnormal lab and US (Gr3)			
	Mean ± SD	Mean ± SD	Mean ± SD			
PCR (IU/L)	187900 ± 114420.23	187550 ± 157251.88	160675 ± 135216.33	.067	NS	Gr1 Vs Gr3 Gr2 Vs Gr3 Gr1 Vs Gr2

There was a highly significant difference between the three studied groups as regards CD4 count **(Table 2)**.

Table (2): Comparison between the three studied groups as regard CD4 count (% of total lymphocytic count).

	Group			*P**	Sig	Post Hoc test
	Group 1	Group 2	Group 3			
	Mean ± SD	Mean ± SD	Mean ± SD			
CD4	31.53 ± 8.66	41.66 ± 11.87	36.32 ± 8.83	.008	HS	Gr1 Vs Gr2

*ANOVA

There was a highly significant difference between the three studied groups as regards CD8 count **(Table 3)**.

Table (3): Comparison between the three studied groups as regard CD8 count (% of total lymphocytic count).

	Group			*P**	Sig	Post Hoc test
	Group 1	Group 2	Group 3			
	Mean ± SD	Mean ± SD	Mean ± SD			
CD8	23.05 ± 3.57	22.75 ± 10.64	34.78 ± 7.89	.001	HS	Gr1 Vs Gr3 Gr2 Vs Gr3

A highly significant positive correlation was found between CD4 counts and liver enzymes (AST and ALT), but no significant correlation could be detected between CD4 count and serum albumin, total Bilirubin, direct Bilirubin, Prothrombin time or PCR in the three groups (Tables 4,5,6).

Table (4): Correlation between CD4, liver function tests and PCR in Group 1

		S albumin	AST	ALT	Total Bil	Direct Bil	PT	PCR
CD4	r	0.04	.472	.415	.013	.006	0.33	.204
	P	0.76	.0001	.001	.919	.962	0.1	.117
	Sig	NS	HS	HS	NS	NS	NS	NS

Table (5): Correlation between CD4, liver function tests and PCR in Group 2

		S albumin	AST	ALT	Total Bil	Direct Bil	PT	PCR
CD4	r	0.05	.543	.422	.019	.008	0.30	.255
	P	0.7	.001	.001	.721	.852	0.12	.208
	Sig	NS	HS	HS	NS	NS	NS	NS

Table (6): Correlation between CD4, liver function tests and PCR in Group 3

		S albumin	AST	ALT	Total Bil	Direct Bil	PT	PCR
CD4	r	0.04	.401	.501	.029	.003	0.2	.298
	P	0.76	.004	.001	.601	.799	0.15	.718
	Sig		HS	HS	NS	NS		NS

There was a highly significant positive correlation between CD8 count and both Bilirubin (total and direct) and prothrombin time but there was no significant correlation between CD8 count and serum albumin, liver enzymes (AST and ALT) or PCR in the three groups (Tables 7, 8 and 9).

Table (7): Correlation between CD8, liver function tests and PCR in group 1

		S albumin	AST	ALT	Total Bil	Direct Bil	PT	PCR
CD8	r	0.03	.038	-.052	.424	.407	0.43	-.027
	P	0.75	.772	.695	.001	.001	0.001	.840
	Sig	NS	NS	NS	HS	HS	HS	NS

Table (8): Correlation between CD8, liver function tests and PCR in group 2

		S. albumin	AST	ALT	Total Bil	Direct Bil	PT	PCR
CD8	r	0.035	.046	-.043	.353	.510	0.5	-.031
	P	0.76	.892	.451	.001	.001	0.001	.779
	Sig	NS	NS	NS	HS	HS	HS	NS

Table (9): Correlation between CD8, liver function tests and PCR in group 3

		S. albumin	AST	ALT	Total Bil	Direct Bil	PT	PCR
CD8	r	0.052	.041	-.059	.632	.532	0.51	-.029
	P	0.695	.903	.567	.001	.001	0.001	.557
	Sig	NS	NS	NS	HS	HS	HS	NS

There was a significant negative correlation between CD4 and CD8 count in the three groups (Table 10 and Fig 1).

Table (10): Correlation between CD4 and CD8 in the three studied groups

		CD8 group 1	CD8 group 2	CD8 group 3
CD4	r	-.266	-.324	-.212
	P	.040	.032	.022
	Sig	S	S	S

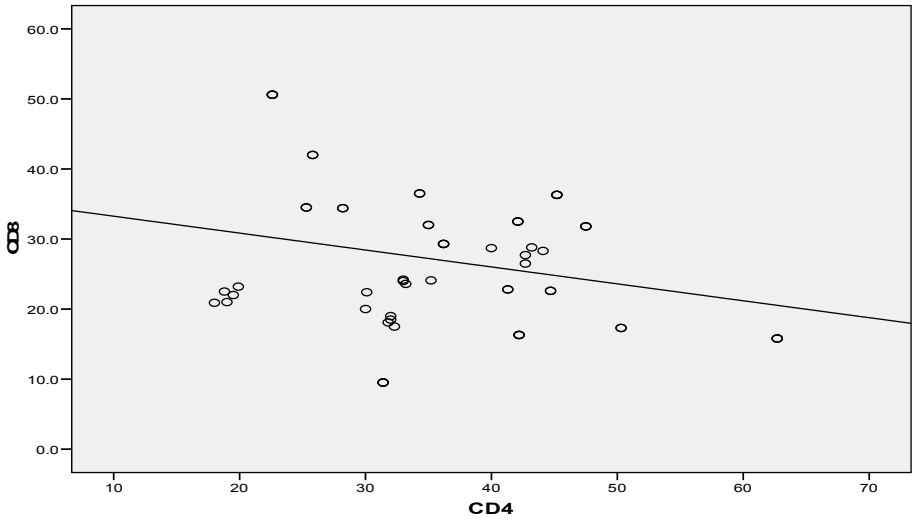

Figure (1) Correlation between CD4 and CD8 counts in the three groups

4. Discussion

Hepatitis C virus (HCV) is a major cause of liver disease globally **(Alter, 2007)**. The virus is able to evade host innate and adaptive immune responses in immunocompetent adults and to set up persistent infection in the majority of people. Those persistently infected are at risk of progressive liver fibrosis, cirrhosis and cancer **(Dazert *et al.*, 2009).**

The immunological response to HCV has an important bearing not only on the acute outcome (i.e. persistent infection versus spontaneous resolution), but also potentially on the long-term outcome in chronic carriers. Successful outcome is associated with the maintenance of broadly directed CD4+ and CD8+ T-cell responses, with maintained functionality **(Bowen and Walker, 2005)**.

In our study, there was a highly significant difference between the three studied groups as regard the CD4+ cell count. But no significant correlation could be detected between CD4 count and serum albumin, total and direct Bilirubin, and prothrombin time. However a highly significant positive correlation was found between CD4+ counts and ALT levels. This agreed with both **Rico *et al.*(2002)** who analyzed CD4+ T cell reactivity in liver and peripheral blood from HCV patients segregated by their ALT levels, and found that HCV-specific-induced T cell proliferation was less often in patients with normal ALT, compared to patients with elevated ALT levels and **Bolacchi *et al.*(2006)** who found that the proliferation of HCV specific CD4+ cells were significantly greater in patients with elevated ALT level than in patients with normal ALT. While, **Barbara *et al.*(2007)** highlighted unreported association between low absolute CD4+ T cell counts and cirrhosis in the absence of HIV infection and they believed that low CD4+ T cell counts are the result of global sequestration of blood cell lines related to portal

hypertension. This hypothesis was supported by the strong association between low CD4+ T cell counts and splenomegaly, leucopenia, and severe thrombocytopenia-conditions that are commonly seen in patients with cirrhosis, regardless of etiology.

Indeed, this study found that there was a highly significant positive correlation between CD4+ T cell count and AST level. This result could be explained by **Aslan *et al.* (2006)** who stated that the presence of CD4+ counts was associated with elevated AST levels based on the fact that not only CD8+ T cells but also CD4+ T cells may act as cytotoxic T cells.

The current study showed no significant correlation between CD4+ T cell counts and HCV viraemic load which came in agreement with **Shen *et al.* (2011)** lwho found that no correlation was found between HCV RNA level and the CD4+ cell count while HCV core antigen concentration was negatively correlated with the CD4+ cell count. On the other hand, **Beld *et al.* (1998)** and **Janice *et al.* (2001)** found an inverse correlation between CD4+ T cell count and HCV RNA plasma levels which were higher in HIV co-infected individuals than in HIV sero- negative patients with HCV.

As regards the CD8 counts, the current study found a highly significant difference between the three studied groups. Using the post hoc test, the significance was between group 1 & 3 also between group 2 & 3 being the highest in group 3 (with both abnormal liver functions & abnormal ultrasound) compared to the other 2 groups. This could be explained by **Leroy *et al.* (2003)** who have found a strong correlation between CD8 gene expression and both ALT serum level and histological activity index (HAI). Their immunohistochemical analysis clearly showed CD8 staining in lobular and piecemeal necrosis areas providing evidence that conventional CD8+ T lymphocytes are the main cell effectors implicated in the pathogenesis of HCV infection.

The current study found a highly significant positive correlation between CD8 T-cell count and both serum bilirubin level and prothrombin time (parameters that correlate with the severity of the disease) These findings agreed with **Rodriguez *et al.*(2007)** who studied the expression of CD8 T-lymphoctes by immunohistochemistry in portal and peri-portal areas of hepatic biopsies from patients with chronic HCV and found a positive correlation between CD8 T-cell densities and intensity of interface hepatitis. However, we didn't find any correlation between CD8+ T cell count and (AST) levels. This was in agreement with **Roe *et al.* (2009)** who studied the phenotypic characterization of lymphocytes in HCV infection and found no correlation between CD8 and AST. On the contrary, **Freeman *et al.* (2003)** and **Rodriguez *et al.* (2007)** studied the expression of CD4 and CD8 T-lymphocytes in peri-portal areas of hepatic biopsy from patients with HCV and found that AST was correlated significantly to CD8 and interface hepatitis.

Also, in our study, no correlation was found between CD8 T cell counts and HCV viraemia levels which came in agreement with **Roe *et al.* (2009) and Shen *et al.* (2010)**

In this study there was a negative correlation between CD4+ cells and CD8+ cells count which came in agreement with **Nascimbeni *et al.* (2011)** who performed a phenotypic analysis of CD4 & CD8 double positive T-cells in blood and liver from patients chronically infected by HCV or HBV and found that CD4 T-cells were high but CD8 T-cells were low in patients infected by HCV while in HBV infected patients, the CD4 T-cells were low and the CD8 T-cells were high. However, **Kim *et al.* (2005)** found that HCV specific CD8 T-cells responses decline with diminishing absolute CD4 T-cell count which provided a possible explanation for the more rapid HCV disease progression in the setting of the HIV co-infection.

Conclusion:

CD4 and CD8 T-cell counts might correlate significantly with liver function tests in chronic HCV infection. Also CD8 T-cell cytotoxicity was significantly correlated with the severity of liver disease but not with the level of HCV vireamia. So CD4 and CD8 T-cell counts might represent a non invasive method to determine the immune response against chronic HCV infection.

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