Effect of direct antiviral agents on neutrophils to lymphocytes ratio in patients with chronic hepatitis C virus

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Abstract— Neutrophils to lymphocytes ratio (NLR) is one of the most usefulness ratio to be systemic inflammatory markers to predict secondary occult hepatitis C infection (OCI) development after direct-acting antivirals (DAAs) therapy had been assessed in the blood of chronic hepatitis C patients (CHC) genotype 1. Herein, the usefulness of this ratio will be addressed in CHC genotype 4 Egyptian patients. Also, the correlation of this ratio with the parameters of liver functions will be studied. **Patients and Methods:** blood samples were withdrawn from non-treated CHC patients (n=31), treated patients with DAAs (n=40) as well as a group of healthy individuals (n=13). HCV-RNA, HCV-antibodies and the parameters of liver function tests together with platelets count and differential white blood cells assay were done for each individual. **Results:** The percentage of neutrophils in the blood of the treated group was highly significantly increased but the lymphocytic percentage was significantly decreased when compared with those of the non-treated patients ($P<0.0002$ and $P<0.034$, respectively).After DAAs treatment, neutrophils/lymphocytes ratio (NLR) was significantly increased when compared with that of the non-treated patients ($P<0.01$). In **conclusion**: as that of CHC patient's genotype 1, NLR can be used to monitor response to DAAs in CHC patients' genotype 4 as well in spite of absence of significant correlation with liver functions tests. The possible mechanism of ratio elevation involves viral eradication via DAAs.

Key words: lymphocytes, direct antiviral agents, HCV genotype 4, genotype 1, viral eradication.

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1 Introduction

Chronic liver damage and regeneration result in the formation of liver fibrosis[1].Residual HCV-RNA can persist in liver cells and also in peripheral blood mononuclear cells (PBMCs) even after antiviral therapy of chronic hepatitis C even in patients who reached sustained virological response (SVR) after direct-acting antivirals (DAAs). This is called secondary occult hepatitis C infection (OCI). This phenomenon could associates with impairment in immune response with subsequent progression in the severity of the liver disease [2].

HCV infection mediates production of reactive oxygen species (ROS) and oxidative stress and inflammation [3].Also, viral proteins can mediate the interaction between macrophages with hepatocytes and stellate cells [4-6].The latter cells type induce hepatic fibrosis, cirrhosis and; possibly, hepatocellular carcinoma (HCC) [7].

Neutrophil/to lymphocyte ratio (NLR) was studied in the blood of chronic hepatitis C genotype1 [2], Here in, the ratio will be evaluated in the blood of a group of chronic hepatitis C genotype 4 Egyptian patients who were treated with DAAs therapy compared with those in the blood of patients who were not treated. In each case the ratio will be compared with that of a group of healthy control. Its ability to predict response to DAAs treatment was tested.

2 Patients and Methods

2.1 Patients and Blood sampling

2.1.1 Patients:

The present study was conducted on 84 Participants. Of them, 31 CHC patients from the outpatient hepatology clinics, in Egyptian Liver Research Institute and Hospital (ELRIAH), Sherpin, Aldakahlia, Egypt, showed positive results for both HCV antibodies and for HCV RNA in their sera and negative results for HBV and any other liver diseases. On the other hand, 40 treated patients showed positive results for HCV antibodies but negative for HCV RNA in their sera. In addition, 13 individuals which showed negative results for HCV, HBV and any other liver-related diseases were used as a healthy control group.

2.1.2 Blood sampling:

2.1.2.1. Whole blood sample collection

Three ml venous blood sample were withdrawn from each individual then, they were poured onto EDTA for the hematological assays.

2.2 METHODS:

2.2.1 White blood cells (WBCs):

WBCs were done using D-cell 60 automated hematology analyzer (KT-6400, China).

2.2.2 NEUTROPHILS:

Neutrophils were done using D-cell 60 automated hematology analyzer (KT-6400, China).

2.2.3 LYMPHOCYTES:

Lymphocytes were done using D-cell 60 automated hematology analyzer (KT-6400, China).

2.2.4 NEUTROPHILS TO LYMPHOCYTES RATIO (NLR)

NLR calculated by dividing Neutrophils percentage on Lymphocytes percentage.

3 RESULTS:

3.1 WHITE BLOOD CELLS (WBCS) WITH THEIR DIFFERENTIAL COUNT IN THE BLOOD OF NON-TREATED HCV PATIENTS, TREATED PATIENTS AND HEALTHY CONTROL GROUPS:

3.1.1 WHITE BLOOD CELLS (WBCS):

The mean WBCs count of the healthy control group was 6.6± 1.4 $x10^3/\mu$Land it was 6.7± 2.4$x10^3/\mu$L in HCV non-treated patients. In the blood of the treated chronic hepatitis C patients, group, this count was 6.7 ± 1.9$x10^3/\mu$L. Statistically; the count of the non-treated HCV patients and treated patients groups was non-significantly differing than that of the healthy control group **(Table 1 and figures 1).**

3.1.1.1 NEUTROPHILS (%):

The mean percentage of neutrophils (%) of the blood of the healthy control group was 57.2± 8.5% and it was 53.4±10.7% in HCV-non-treated group. Also, it was 61.5±6.9% in the blood of the treated patients. The latter percentage of neutrophils in the blood of the treated group was highly significantly increased **(P<0.0002)** when compared with that the non-treated one **(Table 1 and figures 1)**.

3.1.1.2. LYMPHOCYTES (%):

The mean percentage of lymphocytes (%) of the blood of healthy control group was 37.0± 8.6% and it was 36.3± 8.8% in the blood of HCV-non-treated patients,. Conversely, it was only 32.2 ± 7.2% in the blood of the treated group. Thus, the lymphocytic percentage of the treated group was significantly decreased when compared with that of HCV non-treated patients and that of the healthy control **(P<0.034 and P<0.04, respectively, table 1 and figure 1).**

3.1.2 NEUTROPHILS TO LYMPHOCYTES RATIO:

The mean neutrophils to lymphocytes ratio in the blood of the healthy control group was 1.7 ± 0.57. This ratio was 1.6 ± 0.68 in the blood of the non-treated HCV patients group but it was 2.1 ± 0.9 in DAAs-treated group. The mean values of this ratio in treated patients group was significantly increased **(P<0.01)** when compared with that of non-treated HCV patients group **(Figure1and table1)**.

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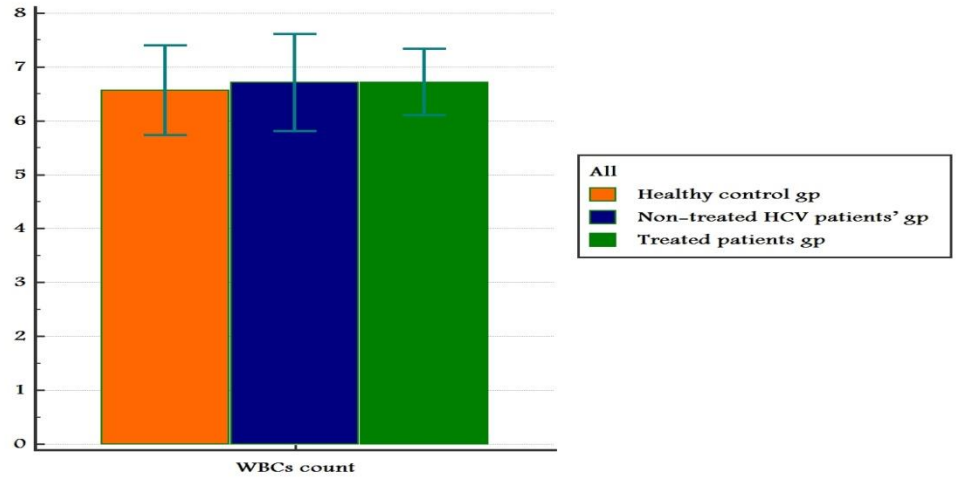
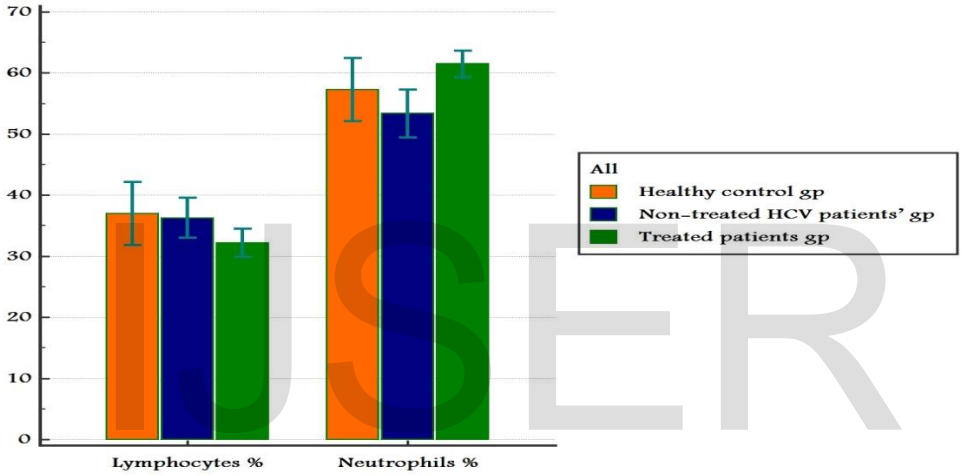
TABLE 1 WHITE BLOOD CELLS (WBCS) WITH THEIR DIFFERENTIAL COUNT IN THE BLOOD OF HCV PATIENTS BEFORE AND AFTER TREATMENT AS WELL AS IN THE BLOOD OF THE HEALTHY CONTROL.

PARAMETERS	HEALTHY CONTROL		NON-TREATED		TREATED PATIENTS	
	N	MEAN ± SD	N	MEAN ± SD	N	MEAN ± SD
WBCS COUNT (X103/µL)	13	6.6± 1.4	31	6.7± 2.4 P=NS	40	6.7 ± 1.9 P=NS *P=NS
NEUTROPHILS (%)	13	57.2± 8.5	31	53.4± 10.7 P=NS	40	61.5 ± 6.9 P=NS *P=0.0002
LYMPHOCYTES (%)	13	37.0± 8.6	31	36.3± 8.8 P=0.05	40	32.2 ± 7.2 P=0.04 *P=0.034
NLR	13	1.7 ± 0.57	31	1.6 ± 0.68 P=NS	40	2.1 ± 0.9 P=NS *P=0.01

N= number, P= probability, values were expressed as mean± standard deviation (mean ± SD), P= the value of significance when the mean values of the HCV patients group and treated group were compared with those of the healthy control group and *P= the value of significance when the mean values of the non-treated HCV patients group were compared with those of the treated group.



(a)



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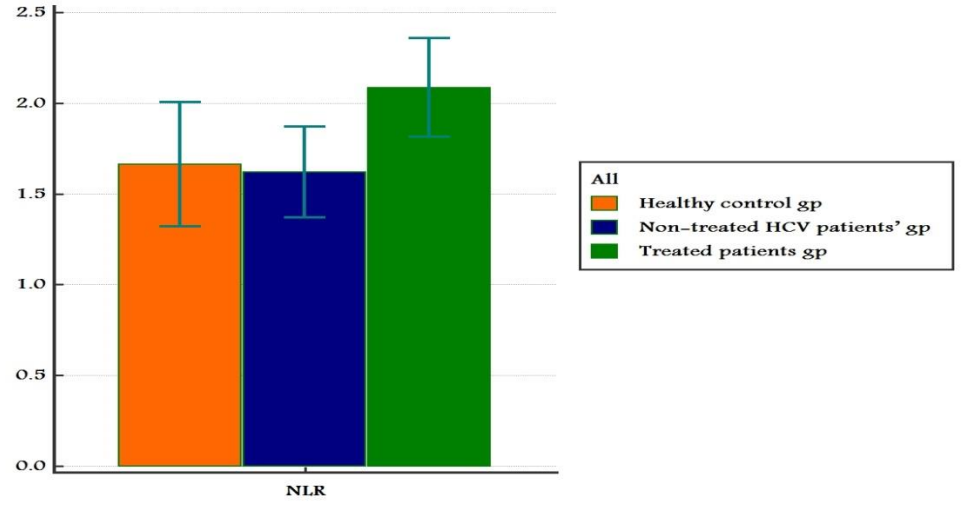

(c)

Figures 1: The mean total white blood cells (WBCs) count (a), percent of lymphocyte and neutrophils (b) and Neutrophils to Lymphocytes Ratio (NLR, c) in the blood of non-treated HCV patients, treated patients and healthy control groups.

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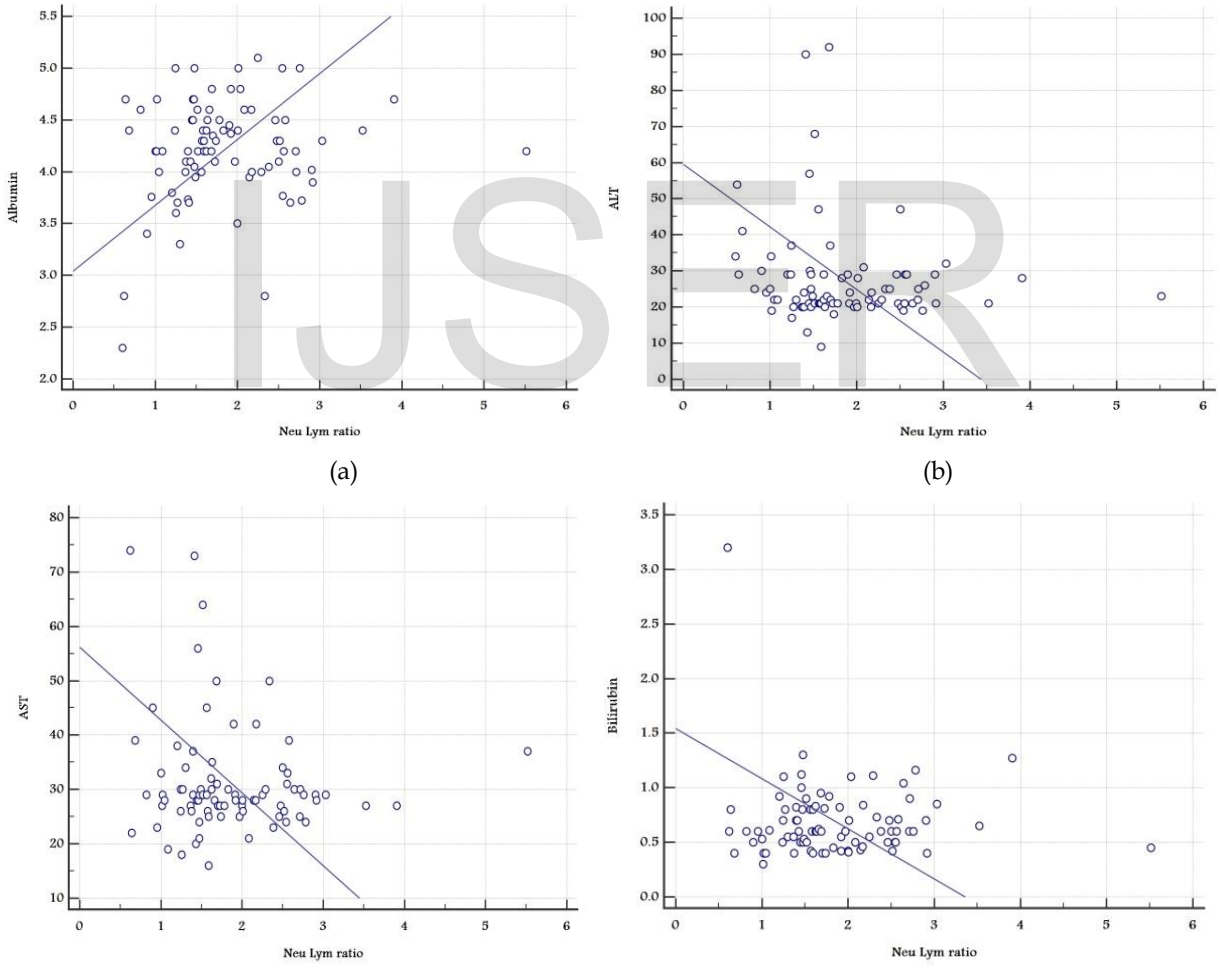
3.2 Correlations between Neutrophils to lymphocytes ratio (NLR) and Liver functions tests:

Table 2 showed the correlations of NLR with liver functions tests (Figures 2). NLR showed negative correlations with AST, ALT and total bilirubin. But, it was positively correlated with albumin.

Table 2: Correlations of Neutrophils to lymphocytes ratio (NLR) with Liver functions tests in all individuals.

Parameters	Albumin	ALT	AST	Total Bilirubin
NLR				
R	0.160	-0.150	-0.114	-0.071
P	0.15	0.18	0.3	0.52
N	84	83	82	84

N= Number of individuals, R= Pearson Correlation coefficient, P= Probability.



Figures 2: Correlations of NLR with albumin, ALT, AST and total bilirubin.

4 DISCUSSION

The findings of immune activation accompanying persistence of HCV RNA in PBMCs after SVR raises the possibility that some patients might be at increased risk of developing immune-mediated extrahepatic complications of HCV infection[8].The role of the immune system seems to play an essential role [9]. To confirm HCV invasion of PBMCs in CHC patients, HCV NS3 protein was detected by [10]. In the present study, the mean percentage of neutrophils in the blood of the treated CHC patients was highly significantly increased when compared with that of the non-treated one ($P<0.0002$). On the other hand, the lymphocytic percentage of the treated group was significantly decreased when compared with that of HCV non-treated patients or with that of the healthy control ($P<0.034$ and $P<0.04$, respectively).

Sarhan [11] revealed that, T cell susceptibility to HCV requires CD5 which increase cell susceptibility to HCV. The persistence of HCV-RNA in lymphocytes [2] and viral proteins in PMNCs, homogenate[10] on the level of positive staining of viral proteins in PMNCs, homogenate can explain why the percent count of lymphocytes in the peripheral blood of CHC patients was increased. This is because the infection may activate bone marrow to produce more lymphocytic. The significant reduction in the percent of lymphocytic count in the blood of the treated HCV patients than that of the non-treated individuals confirms such possibility. Also, our results confirm the previous results of Pham [12] who reported that, PBMC-derived gene transcription profiles in SVR patients were different from those in healthy individuals.

From the mean percent of neutrophils and lymphocytes in the individual groups, their ratio in the blood of the healthy control group was 1.7 ± 0.57. This ratio was 1.6 ± 0.68 in the blood of the non-treated HCV patients group but it was 2.1 ± 0.9 in DAAs-treated group. The mean value of the treated patients was significantly increased than that of non-treated patients, group ($P<0.01$).In spite of genetic variability of HCV genotype 1 than that of genotype 4 of the Egyptian patients, unexpectedly similar results after DAA treatment were obtained [13]. Unfortunately, there are no significant correlations between the ordinary functions tests with NLR. The causative factors may include the fact that the cell membranes of liver cells cannot leak enzymes or any cellular products unless significant hepatic damage occurs. If this case, one can expect that the synthetic function of the liver will not be impaired i.e., this mean that the hepatic functions are compensated . Further, one can expect severe liver damage involvement if HCV infection persists.

In conclusion, NLR can be used to monitor response to DAAs therapy in CHC patients' genotype 4 as well at the time in which liver functions tests remain normal.

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