Evaluation of CD64 Index in HBV and in Chronic HCV Infections

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Abstract

Background and aims: CD64 [Fc gamma receptor 1 (FcγRI)] is a promising biomarker used in predicting severe bacterial infection. The study was designed to assess their level in all stages of HBV infection and in chronic HCV infection before and after treatment with direct acting antiviral therapy as a possible biomarker of inflammation. Patients and methods: A case-control study was conducted, 50 patients with different disease stages of HBV infection (10 acute, 15 chronic hepatitis, 15 liver cirrhosis (LC) and 10 with hepatocellular carcinoma (HCC)), twenty patients with chronic HCV and 15 as a control group. Laboratory and imaging studies were evaluated. The levels of CD64 expressions in peripheral blood and CD64 Index were measured for all patients by flowcytometry using fluorescein isothiocyanate (FITC)-conjugated anti-CD64 monoclonal antibody. Results: The levels of CD64 expressions in peripheral blood and CD64 index were significantly higher in patients with HBV and HCV than in control group (P value = 0.01, 0.01 and 0.000, 0.000 respectively). They were increased significantly with disease progression in patients with HBV infection, acute hepatitis B infection showed the highest values. Their levels were significantly decreased in patients with HCV infection post treatment than before treatment. Conclusions: The levels of CD64 expressions in peripheral blood and CD64 index are considered good biomarkers of inflammation in viral hepatitis both B and C and could detect disease progression and also suppression of inflammation after antiviral therapy.

Keywords

CD64, HBV, HCV

1. Introduction

Hepatitis C virus (HCV) infection is one of the main causes of chronic liver disease worldwide; 170 million people are chronically infected with HCV [1], and its long term impact is highly variable, ranging from minimal histological changes up to cirrhosis with or without the development of HCC [2].

The main goal of treatment of HCV is to cure the infection by viral eradication. The sustained virological response (SVR) is associated with resolution of liver disease in patients without cirrhosis and reduces; but not eliminates, the risk of life-threatening complications such as hepatic failure and portal hypertension in patients with cirrhosis [3] [4].

Worldwide, about one third of population has serological evidence of past or recent infection with HBV. It is estimated that 240 million people are chronically infected with HBV [5] with a highest prevalence in Africa and Asia [6].

The natural history of chronic HBV infection can be divided into five phases depending on HBV replication, HBeAg, aminotransferase and the degree of hepatic necro-inflammation [7] [8].

Failure of detection and persistence of HBV or HCV infections increases the incidence of HCC; it constitutes the fifth most common cancer of all cancers [9].

The common markers used clinically to detect the degree of HBV inflammation are alanine aminotransferase (ALT) and HBV-DNA, but they cannot describe the actual state of the liver, some cases have HBV associated LC or hepatocellular carcinoma with normal levels of ALT and undetected HBV-DNA [10].

CD64 [Fc gamma receptor 1 (FcγRI)] is a promising biomarker used in predicting severe bacterial infection and could be used as a simple and also as a predictive test for diagnosing and monitoring bacterial infections in hospitalized patients [11]. Wang *et al.*, 2015 reported that, a neutrophil CD64 expression is a helpful marker in the early diagnosis of sepsis in critically ill patients [12].

Not only bacterial infection, but also, CD64 index could be used in diagnosis of viral infections, as Human Cytomegalovirus (CMV) [13], Epstein-Barr virus (EBV) [14] or other DNA virus [15].

A recent study focused on evaluating the relation between CD64 index in peripheral blood in different stages of chronic HBV and the degree of liver cell damage [10].

2. Aim of the Study

The current study aimed to assess CD64 expressions and CD64 index levels in all disease stages of HBV infection and in chronic HCV infection before and after treatment with direct acting antiviral therapy as a possible biomarker of inflammation.

3. Patients and Methods

3.1. Patients

This prospective (case-control) study was carried out on 70 patients; whom at-

tending outpatient clinic of Tropical Medicine & Gastroenterology department, Qena University Hospital; 50 with HBV, 20 with HCV infection and 15 subjects as a control group, from January 2016 to January 2017.

Patients with HBV infection were categorized according to their presentation into; 10 patients with acute hepatitis, 15 with chronic hepatitis, 15 with LC and 10 patients with HCC related HBV infection. Twenty patients with chronic HCV infection were included and were treated according to the Egyptian protocol of the national committee for control of viral hepatitis. Their diagnosis was based on combination of history, examination, laboratory and imaging investigations including fibroscan.

According to the Egyptian protocol for treatment of chronic HCV infection; which is based on the EASL guidelines for treatment of HCV [16]; patients were divided into two groups: patients easy to treat (naïve patients with serum bilirubin ≤ 1.2 mg/dl, serum albumin ≥ 3.5 gm/dl, INR ≤ 1.2 and platelet count ≥ $150 \times 10^3/\mu l$ and they received sofosbuvir 400 mg and daklatasvir 60 mg once daily for 3 months) and those difficult to treat (serum bilirubin ≥ 1.2 mg/dl, serum albumin ≤ 3.5 gm/dl, INR ≥ 1.2 and platelet count < $150 \times 10^3/\mu l$ and interferon experienced patients and they received sofosbuvir 400 mg and daklatasvir 60 mg and dose-weighted ribavirin (800 - 1200 mg) daily for 3 months. The duration of this regimen should be extended to 6 months if failed previous treatment with sofosbuvir and ribavirin).

The exclusion criteria for this protocol were: serum bilirubin > 3 mg/dl, serum albumin < 2.8 gm/dl, INR ≥ 1.7 and platelet count < $50 \times 10^3/\mu l$, pregnancy and presence of dynamic CT imaging of HCC or inadequately controlled diabetes mellitus; HbA1c > 9%. Also patients were excluded from the study if they experienced any type of infection or bleeding varices.

All included patients underwent: history taking, clinical examination and laboratory investigations including: complete blood count, complete liver function test, HBs Ag, HCV Ab, PCR HBV DNA, PCR HCV RNA, serum glucose and serum creatinine. Serum alpha fetoprotein protein was measured for suspected patients with HCC. Abdominal US examination was done for all patients.

3.2. Sampling

Eight ml of venous blood were collected from patients and control groups under aseptic precautions. Two samples were collected from patients with HCV infection; one at base line; before treatment and the other at 3 months after treatment (SVR, 3 months). Samples were divided as follow: 2 ml was placed in EDTA (Ethylene Diamine Tetra-acetic Acid) containing vacutainer tube to do complete blood count using automated hematology analyzer cell dyne-1800 (Abbott diagnostics, USA). 4 ml was taken in plain glass tube and after clotting, the tube was centrifuged at 2000 rpm for 3 min at the room temperature (37°C) and the serum was separated for estimation of liver function using Cobas c311 automated chemistry analyzer (Roche diagnostics, Germany). The rest of the sample was placed in EDTA containing vacutainer tube for flowcytometric determina-

tion of CD64 using FACS caliber flowcytometer (BD, Bectom Dickinson). All antibodies were obtained from Bectom Dickinson

CD64 expression and by flow cytometry: CD64 expression was measured using FITC CD64 monoclonal antibodies as follow: 100 μL from blood samples and 10 μl of FITC CD64 conjugated monoclonal antibody or matched isotype controls was added each in prepared tube then vortex and incubated in dark for 15 min at room temperature. Following incubation, red blood cells lysing solution 1 ml; 1× (BD Biosciences) was added and incubated for an additional 10 min at room temperature. Cells were centrifuged at 3500 rpm for 5 minute. The pellet was washed twice with phosphate buffered saline and then they were suspended in BD Biosciences fluid sheath for analysis on a FACS Calibur flow cytometer, BD Biosciences. Acquisition and analysis were performed using cell Quest software. The acquisition and analysis of immuno-marked cells was Standardized for 10,000 events per sample. Lymphocytes, monocytes and neutrophil were gated in the R1, R2 and R3 region according to the characteristic linear forward and side scatter features. CD64 expression was evaluated on a logarithmic scale for three regions (Figure 1).

Neutrophil CD64 index was calculated as follow: CD64 index = (neutrophil CD64 average fluorescence intensity/lymphocyte CD64 average fluorescence intensity)/(monocyte CD64 average fluorescence intensity/neutrophil CD64 average fluorescence intensity). We used CD64 expression on lymphocytes as internal negative control, while CD64 expression on monocytes was used as internal positive control.

3.3. Ethical Approval

The study protocol was approved by the ethical committee of our institution. All selected patients provided informed consent before enrollment in the study.

3.4. Statistical Analysis

The data first were coded and verified before its entry. Computer program Statistical Package for Social Sciences (SPSS) (ver.21) Chicago, USA was used for analyzing the collected data and for drawing figures. Data expressed as mean ± standard deviation and number, percentage. Student-*t*-test and ANOVA tests were used to determine the significant difference for numeric variable. Chi. Square was used to determine the significant values for categorical variable. Person's correlation was used for correlations between groups. P value is considered significant when P value < 0.05 and not significant when P value > 0.05.

4. Results

4.1. The Demographic Data of the Two Groups

Patients with HBV were 39 males (78%) and 11 females (22%) with their mean ages were 45.16 ± 15.38. Patients with HCV were 15 males (75%) and 5 females (25%) with their mean ages were 42.5 ± 12.84 and 15 subjects matched for age and gender as a control group; this is summarized in Table 1.
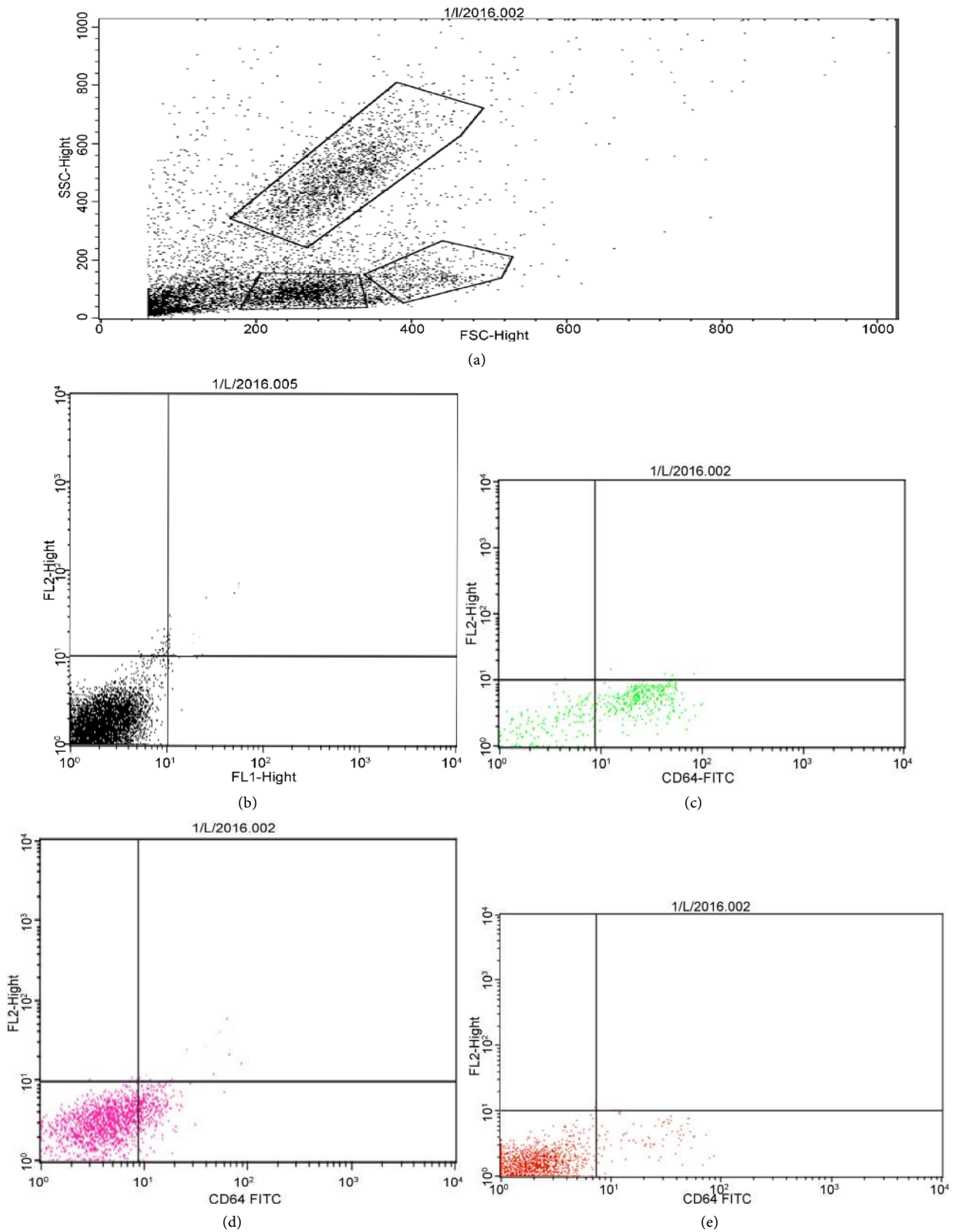
Figure 1. Peripheral blood expression of CD64: (a) Forward scatter, side scatter of peripheral blood; (b) Isotype control to exclude non-specific background staining; (c) Expression of CD64 onneutrophils; (d) Expression of CD64 onmonocytes; (e) Expression of CD64 onlymphocytes.

Patients with HCV infection, treated with direct acting antiviral therapy, were as follow; 15 (75%) patients were easy to treat and 5 (25%) were difficult to treat; only one of them was sofosbuvir and ribavirin experienced and no interferon experienced patients. All patients with HCV infection showed negative PCR HCV RNA at the end of treatment with end treatment response (ETR = 100%) and SVR; 3 months = 100%.

4.2. The Mean Values of Laboratory Data for Patients with HBV

Laboratory data for patients with acute, chronic hepatitis, LC-HBV and HCC-HBV are illustrated in Table 2.

4.3. The Mean Values of Laboratory Data for Patients with HCV

Laboratory data for patients with HCV before and after treatment (3 months) are illustrated in Table 3. Statistically significant reduction in bilirubin, ALT, AST, INR and increase in Hemoglobin level and Prothrombin concentration were detected after treatment with direct acting antiviral therapy for HCV infection.

Table 1. Characteristic demographic data for patients and control.

Parameter	Patients and control		
	control	HBV	HCV
Number	15	50	20
Age	49 ± 10.5	45.16 ± 15.38	42.5 ± 12.84
Gender			
Male	7 (70)	39 (78)	15 (75)
female	3 (30)	11 (22)	5 (25)

Data expressed as mean ± SD or number (%).

Table 2. Laboratory data for patients with HBV infection.

Parameter	HBV disease stage			
	acute	chronic	cirrhosis	HCC
Bilirubin (mg/dl)	4.90 ± 3.21	1.27 ± 0.23	1.76 ± 0.63	1.96 ± 0.60
Albumin (g/dl)	3.76 ± 0.08	4.0 ± 0.28	3.2 ± 0.71	3.12 ± 0.56
ALT (U/L)	607 ± 353	25.4 ± 15.5	66.9 ± 20.4	48 ± 15.6
AST (U/L)	675 ± 501	21.4 ± 12.9	53 ± 16.1	40 ± 18.1
PT (seconds)	14.4 ± 3.3	11.6 ± 0.5	18.9 ± 10.0	14.8 ± 2.9
PC (%)	72.1 ± 23	92.6 ± 4.7	64.34 ± 32.8	65.6 ± 18.9
INR	1.3 ± 0.27	1.12 ± 0.04	1.74 ± 0.89	1.39 ± 0.252
WBC (10^3/μl)	7.22 ± 4.9	5.0 ± 1.6	6.84 ± 3.19	6.76 ± 3.77
PLT (10^3/μl)	134.6 ± 41.6	241 ± 119	167 ± 52.6	84.4 ± 50.6
HGB (g/dl)	9.9 ± 3.9	12.7 ± 2.0	12.6 ± 1.66	11.7 ± 1.09
Creatinine (mg/dl)	0.78 ± 0.2	0.9 ± 0.11	0.94 ± 0.26	1.12 ± 0.34

Data expressed as mean ± SD.

4.4. Peripheral Blood CD64 Expressions and Neutrophil CD64 Index in Patients and Control

Both patients with HBV and HCV groups showed statistically significant higher values for neutrophil, lymphocyte and monocyte CD64 expressions than control group. The significant value for neutrophil CD64 expression in both patients groups was (P value = 0.01 and 0.01 respectively), lymphocyte CD64 expression (P value = 0.01 and 0.00 respectively) and for monocyte CD64 expression (P value = 0.00 and 0.00 respectively), also neutrophil CD64 index showed statistically significant higher values in both patients groups than control group (P value = 0.000 and 0.000 respectively) Table 4.

4.5. Peripheral Blood CD64 Expressions and Neutrophil CD64 Index in Patients Presented with Different Stages of HBV Infection

A statistically significant difference was detected between different stages of HBV infection as regard neutrophil, lymphocyte and monocyte CD64 expressions (P value = 0.000 between groups). Significantly higher values were detected

Table 3. Laboratory data for patients with HCV before and after treatment.

Parameter	Patients with chronic HCV		
	Before treatment	After treatment	P value
Bilirubin (mg/dl)	1.21 ± 0.72	1.01 ± 1.17	0.00
Albumin (g/dl)	3.5 ± 0.80	3.62 ± 0.66	NS
ALT (U/L)	48.3 ± 31.5	33.7 ± 39	0.02
AST (U/L)	45.9 ± 33	34.8 ± 27	0.02
PT (seconds)	13.6 ± 1.15	13.2 ± 1.36	NS
PC (%)	71.2 ± 8.38	77.2 ± 8.6	0.00
INR	1.12 ± 0.32	1.09 ± 0.47	0.00
WBC (10^3/μl)	7.23 ± 3.96	7.08 ± 4.32	NS
PLT (10^3/μl)	180.2 ± 38.7	190 ± 56.4	NS
HGB (g/dl)	10.4 ± 2.46	12.2 ± 1.18	0.00
Creatinine (mg/dl)	1.04 ± 0.28	1.03 ± 0.59	NS

Data expressed as mean ± SD, paired student-*t*-test was used.

Table 4. Mean values of peripheral blood CD64 expressions and neutrophil CD index in patients and control.

Parameter	Patients and control				
	control	HBV	P value	HCV	P value
Neutophil CD expression	45.2 ± 0.627	47.6 ± 2.32	0.01	47.1 ± 2.54	0.01
Lymphocyte CD expression	5.90 ± 0.286	7.90 ± 0.268	0.01	9.17 ± 0.382	0.00
Monocyte CD expression	51.4 ± 1.67	85.4 ± 4.57	0.001	74.6 ± 2.42	0.00
Neutrophil CD index	0.536 ± 0.448	2.32 ± 3.53	0.000	0.82 ± 0.43	0.000

P value < 0.05 = significant, NS = non-significant. Un-paired student-*t*-test was used.

inpatients with acute infection than other stages. Their values were significantly increasing from patients with chronic hepatitis to LC to HCC. Also, there was statistically significant difference in neutrophil CD64 index between patients with different stages of HBV infection (P value = 0.000 between groups). Patients with acute infection showed higher values than other stages, and its value was significantly increasing from patients with chronic hepatitis to LC to HCC. This is illustrated in Table 5 and Figures 2-5. Also significant higher values; for both expressions and neutrophil CD64 index, were detected in every stage of infection in comparison with control group (P value = 0.000).

4.6. Peripheral Blood CD64 Expressions and Neutrophil CD64 Index in Patients with HCV Infection before and after Treatment

The level of CD64 expressions for neutrophil, lymphocyte and monocyte decreased significantly after direct acting antiviral therapy (P value = 0.000), also neutrophil CD64 index decreased significantly after therapy (P value = 0.000),

Table 5. Mean values of peripheral blood CD64 expressions and neutrophil CD index in different stages of HBV infection.

Parameter	Patients HBV				
	Acute	Chronic	Cirrhosis	HCC	P value
Neutophil CD expression	65.9 ± 1.84	49.74 ± 1.95	51.6 ± 2.84	57.8 ± 1.92	0.000
Lymphocyte CD expression	10.10 ± 0.161	8.62 ± 0.32	8.83 ± 0.09	8.98 ± 0.308	0.000
Monocyte CD expression	85.30 ± 1.25	53.1 ± 2.79	59.19 ± 3.08	62.2 ± 1.07	0.000
Neutrophil CD index	8.06 ± 4.56	0.726 ± 0.57	0.961 ± 0.562	1.04 ± 0.645	0.000

P value < 0.05 = significant. One Way ANOVA test was used between groups.
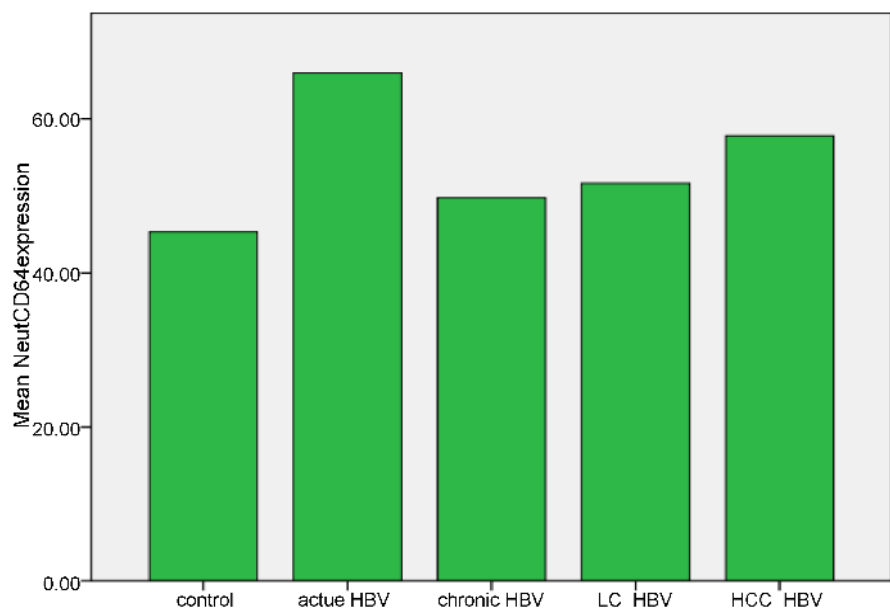

Figure 2. Mean neutrophil CD64 expression in stages of HBV infection and in control group. All stages of HBV was higher than control group. Patients with acute stage showed the highest values and progressive increase from chronic stage to LC to HCC.
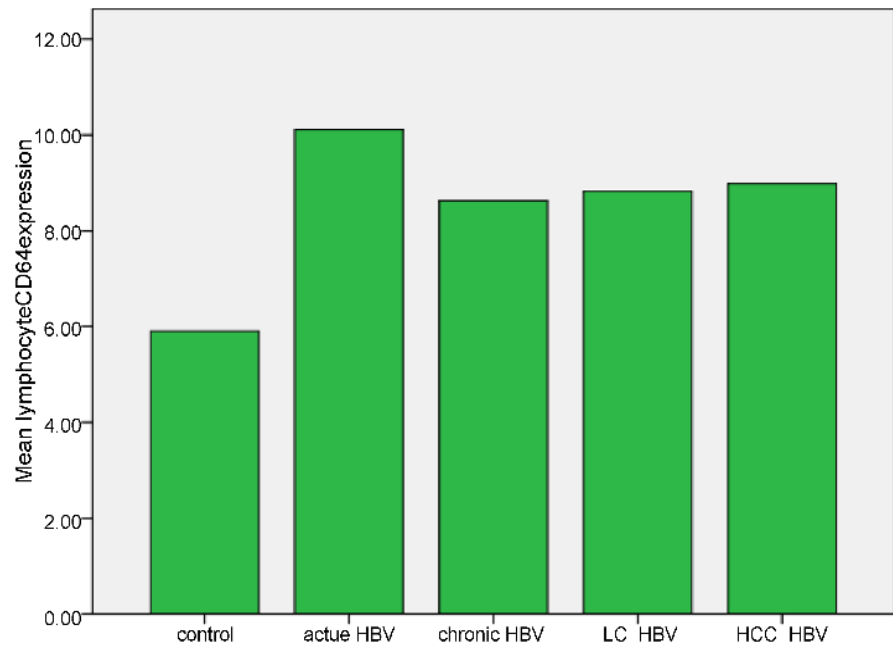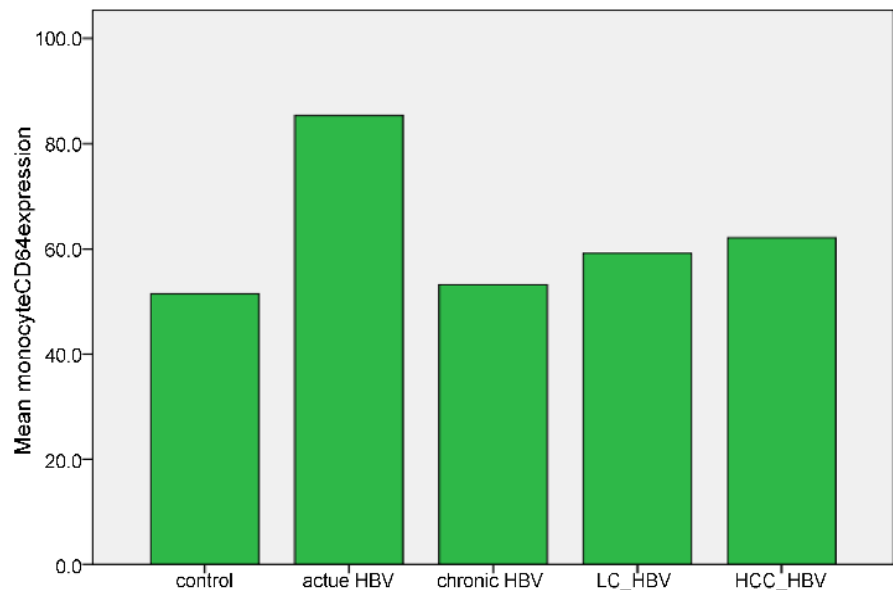
Figure 3. Mean lymphocyte CD64 expression in stages of HBV infection and in control group. All stages of HBV was higher than control group. Patients with acute stage showed the highest values and progressive increase from chronic stage to LC to HCC.

Figure 4. Mean monocyte CD64 expression in stages of HBV infection and in control group. All stages of HBV was higher than control group. Patients with acute stage showed the highest values and progressive increase from chronic stage to LC to HCC.

Table 6 and Figure 6.

4.7. Correlations

No significant correlation could be detected between, CD64 expressions and neutrophil CD64 index, and ALT, AST, PCR viraemia, Child-Pugh score in either group of patients.
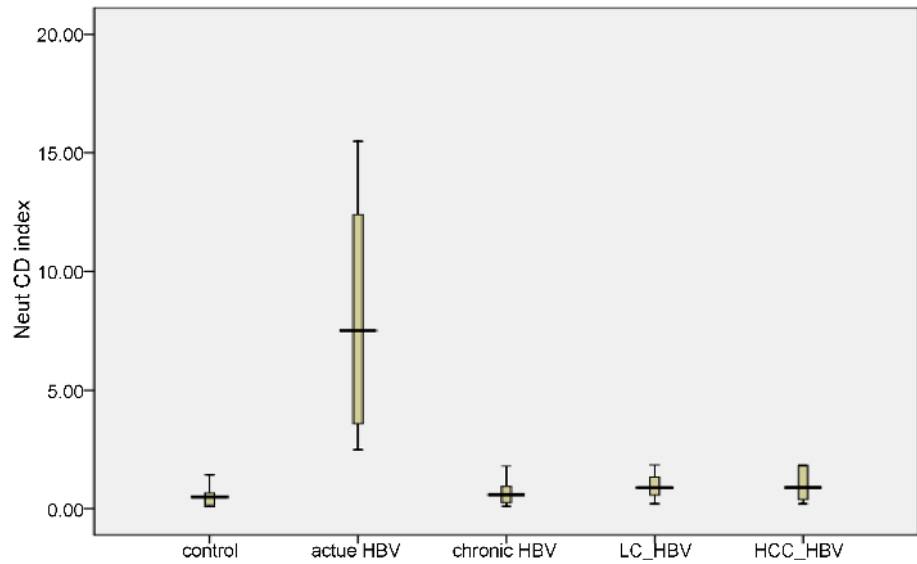
Figure 5. Mean value of neutrophil CD64 index in different stages of HBV infection and in control group. All stages of HBV were higher than control group. Patients with acute stage showed the highest values and progressive increase from chronic stage to LC to HCC.
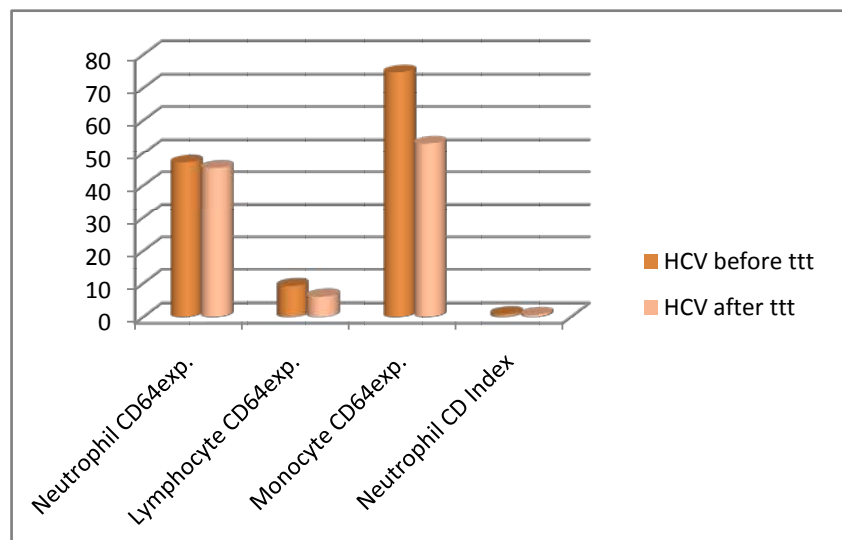

Figure 6. Mean values of neutrophil, lymphocyte and monocyte CD64 expression and neutrophil CD index in patients with HCV before and after direct acting antiviral therapy. Significant reduction in all values after treatment (P value= 0.000).

Table 6. Peripheral blood CD 64 expressions and neutrophil CD index in patients with HCV before and after treatment.

Parameter	Patients with chronic HCV		
	Before treatment	After treatment	P value
Neutophil CD expression	47.1 ± 2.54	45.36 ± 0.98	0.000
Lymphocyte CD expression	9.17 ± 0.382	6.02 ± 0.44	0.000
Monocyte CD expression	74.6 ± 2.42	52.9 ± 3.5	0.000
Neutrophil CD index	0.82 ± 0.43	0.61 ± 0.32	0.000

Data expressed as mean ± SD, paired student-*t*-test was used.

5. Discussion

Infection with HBV induces both cellular and humoral immune response that cause liver cell damage either directly or through antibody-dependent cytotoxicity [17]. One of these antibodies is CD64, which is a receptor of IgG Fc fragment I of immunoglobulin G (IgG) that can be changed in HBV infected patients [10].

In recent years, several studies on Fcg receptor I (FcgRI) have confirmed that it could be a marker of infection. In resting neutrophils, CD64 is expressed at a very low level (approximately 1400 receptors per cell on average). However, the up-regulation of CD64 on the surface of neutrophils is induced by inflammatory cytokines such as interferon gamma [18] and granulocyte colony-stimulating factor [19].

In the current study we evaluated the possible significant role of peripheral blood CD64 expressions and neutrophil CD64 index in patients with different stages of HBV infection and in patients with chronic hepatitis C virus infection before and after treatment as a possible inflammatory markers for rapid and early detection of infection and as a prognostic, reliable and simple marker for detection of improvement or deterioration.

In this study, we found that the levels of neutrophil, lymphocyte and monocyte CD64 expressions were significantly higher in patients with acute hepatitis B virus infection compared with healthy control group and with patients in different stages of chronic hepatitis B virus (chronic HBV infection without LC, chronic HBV related LC and chronic HBV related HCC), this may be related to severe inflammatory and immunological reaction in acute HBV infection. In other stages of chronic HBV infection, HCC patients showed the highest values than earlier stages including chronic infection and LC representing more progression of the disease and maximum intensity of the inflammatory and immunological reactions in those patients.

This was in agreement with the results of (Feng *et al.*, 2015) whom recorded high level of peripheral blood neutrophil, monocyte and lymphocytes CD64 index at different stages of chronic hepatitis B virus infection and the level of CD64 index increase with increasing the duration of chronic HBV infection and reported that the level of lymphocyte CD64 index increased significantly than other indices [10].

Also, we found that neutrophil CD64 index was significantly higher in patients with acute HBV infection and in different stages of chronic HBV infection in comparison to control group indicating the continuity of inflammation in all these stages. Also, (Feng *et al.*, 2015) reported significant increase in level of neutrophil CD64 index in all stages of chronic hepatitis B virus infection in comparison to control group [10].

Hepatitis C virus has been identified decades ago as a cause of chronic viral hepatitis that progress to cirrhosis and HCC. Only a small number of patients can clear the virus spontaneously during acute infection due to rapid induction of innate immune response, especially interferon (IFN) induced genes, and adaptive immune responses [20]. In contrary to other flaviviruses related to

HCV, and against hepatitis B virus (HBV), HCV causes chronic infection in most adults who are exposed to infection as the virus overcome innate and adaptive immune response, causing progression to fibrotic liver injury that finally lead to cirrhosis, liver failure and an increased risk of HCC [21].

The current study is the first study conducted to measure the dynamic changes of peripheral blood CD64 expressions and index in patients with chronic hepatitis C virus infection before and after treatment with DAA as a marker for detection of hepatic inflammation. In the current study, the level of peripheral blood CD64 expression for neutrophil, lymphocyte and monocyte and neutrophil CD64 index decreased significantly after treatment with direct acting antiviral therapy, which attributed to drug induced inhibition of inflammation and regulated immune response with subsequent down-regulation of CD64 index.

So the level of peripheral blood CD64 expression may be used as a marker to follow up the degree of hepatic inflammation in patients during treatment and early predict failure to response to treatment also can be used as a marker to detect progression of liver disease.

We can conclude that CD64 expressions and CD64 index were increased in HBV infected patients especially in the acute condition and changed with the course of the disease. And their levels significantly decreased after eradication of HCV infection with direct acting antiviral drugs, so their levels could be helpful for detecting the stage of liver damage and monitoring therapy.

The limitation of the current study was the relative small numbers of patients for each group, so further studies on large scales of population are needed for further evaluation.

Conflict of Interest

Authors declared that no conflict of interest and no financial support from any agency.

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