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Role of Soluble CD163 and Monocyte Surface Markers in Hepatitis C Virus Infected Patients.

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ABSTRACT:

Background: Chronic stimulation of circulating monocytes and macrophages in hepatitis C virus infection can cause CD163 to be realesed into the circulation from their cell surface. Soluble CD163 (sCD163) can be used as a biomarker of macrophage activation, severity of HCV infection and level of inflammation.

Objective: In this study we determined the levels of soluble CD163 in HCV infected patients with different clinical outcomes and we correlated them to different biochemical indicators for disease progression and monocytic cell surface markers (CD14, CD16).

Methods: Our study was conducted on 60 HCV infected patients:20 patients with chronic HCV infection without cirrhosis; 20 patients with cirrhosis,20 patients with hepatocellular carcinoma and 20 individuals who were healthy controls. Peripheral blood mononuclear cells (PBMCs) were isolated from all subjects and were used in flow cytometric determination of CD14 and CD16 monocytic surface markers . Levels of sCD163 in serum and culture supernatents were determined by enzyme linked immunosorbent assay.

Results: Our results demonstrated a significant increase in double positive monocytic cells in patients groups especially those with cirrhosis compared to the control group. Moreover soluble CD163 levels were significantly increased in HCV patients with different clinical outcomes. The relation between serum CD163 and double positive monocytes was shown to be significantly negative. However, there was a substantial positive connection between these monocytic surface markers and mitogen-induced CD163.

Conclusion: Our findings suggest that HCV infection tends to upregulate CD163 shedding into circulation with a variety of clinical outcomes, allowing sCD163 to be employed as a biomarker for disease severity in HCV infection.

Keywords: soluble CD163, HCV infection, mitogen induced sCD163, CD14+CD16+ monocytes

1. INTRODUCTION

Hepatitis C virus (HCV) is a hepatotropic RNA virus of the genus hepacivirus in the flaviviridae family[1] According to a 2018 meta-analysis, Egypt has the highest rate of HCV infection in the world, with an antibody prevalence of 11.9% [2]. More than 85% of all HCV cases in Egypt have genotype 4, making it the most prevalent genotype. Hepatocellular carcinoma, cirrhosis, and liver fibrosis are all significantly preceded by HCV [2]. Innate immune cells, such as natural killer (NK) cells, dendritic cells (DC), and liver-resident macrophages (Kupffer cells) interact with hepatocytes causing the progression of liver inflammation during HCV infection[3]

Macrophages (MΦ) are innate phagocytic cells that present in almost all tissues. Peripheral blood mononuclear cells (PBMCs) circulate into tissues either continuously or in response to injury [4]. There are two phenotypes: M1 macrophages, which produce high quantities of pro-inflammatory cytokines and mediate resistance to infections; and M2 macrophages, which participate in immunological modulation, tissue remodelling, and parasite control.

HCV is associated with liver damage and chronic monocyte and macrophage activation [6]. Increased macrophage number in HCV can be caused by the development of Kupffer cells which are known as liver resident macrophages and the invasion of circulating blood monocytes

It was found that kupffer cells are similar to either M1 (CD163lo, IL-12, TNF-α) or M2 (CD163hiCD206hi) according to the liver condition and characteristic surface markers [7].Only circulating monocytes and tissue macrophages express CD163, a lineage-specific hemoglobin-haptoglobin scavenger receptor that is upregulated in circumstances that can cause macrophage activation [8] CD163 is released from the surface of macrophages and enters the bloodstream as soluble CD163 (sCD163) when Toll-like receptors

(TLRs) are activated [9].In patients with liver cirrhosis and hepatocellular carcinoma, which are linked to macrophage activation, sCD163 has been shown to act as predictive factor for variceal haemorrhage and overall survival[10].The rate of sCD163 shedding increases in case of portal hypertension, cirrhosis, and the necro-inflammation associated with acute hepatitis [11].

Therefore, sCD163 might represent as a pivotal biomarker reflecting macrophage involvement in different liver associated diseases[12].

This study aimed at assessing the levels of both serum and mitogen induced CD163 in patients with chronic HCV infection and presented with different clinical outcomes and we correlated them to different biochemical indicators for disease progression and essential cell surface markers of monocytes (CD14, CD16).

Subjects &Methods

I. Subjects

The present study was carried out on 80 individuals ;20 patients with chronic active HCV infection,20 patients with liver cirrhosis,20 patients with hepatocellular carcinoma(HCC) and 20 age and sex-matched healthy individuals with absent serological or molecular evidence of HCV infection.

All HCV infected individuals were recruited from outpatient clinics ;Internal Medicine department, Medical Research Institute, Alexandria University. They were all positive for HCV antibody screening test by commercially available IgG3d generation ELISA kits.

Patients with concurrent bilharzial or HBV infections, as well as those with immunological or immune deficiency problems were not allowed to participate in the study. All subjects gave their informed consent. The Medical Research Institute's Ethical Committee gave its approval to the protocol in July 2018

Blood sampling

Seven ml of venous blood were withdrawn from all subjects under strictly aseptic techniques and collected into the following vacationers:; 3 ml of blood was collected on lithium heparin to obtain peripheral blood mononuclear cells(PBMCs) and 2ml was collected on ethylene diamine tetra-acetic acid (EDTA) vacationers to be employed in hematological investigations (CBC). The remaining 2 ml of blood was used for serum separation for biochemical investigations including liver function tests.

Isolation of PBMCs

Density gradient centrifugation on Ficoll-histopaque (1077) was used to separate PBMCs from heparinized blood samples (Biochrom KG Berlin). This was accomplished using the technique outlined by Dagur and McCoy (2015) [13]. The yield was utilised for the flow cytometric counting of CD14 and CD16 surface markers.

Enumeration of monocytic surface markers (CD14, CD16) by flow cytometry

First cell counts were adjusted to 10 x 106/ml. Briefly, 10 µl of fluoresceneisothiocyanate (FITC) labelled monoclonal antihuman CD14 and phycoerythrin (PE) labelled monoclonal anti-human CD16 human were separately added to 100 µl of PBMCs and mixed well. The mixture was left for 10 minutes at room temperature followed by washing and centrifugation .Cell pellets were prepared for analysis and gating by being re-suspended in 2 ml of lysis solution, incubated at room temperature for 10 minutes, and then rinsed with phosphate buffer saline (PBS) twice

Cell culture assays

The cell culture assay was done for isolated PBMCs in order to determine the rate of detachment of CD163 either spontaneously or following polyclonal stimulation with phytohemagglutinin (PHA). Cell culture was performed under strictly aseptic conditions following the protocol of Baust et al. (2017) [14].

1 ml of suspended cells (2×106cells/ml) was added in duplicates into wells of a 96 tissue culture plate. The first well left without mitogen while the other was pulsed with 5 ug/ml of PHA . The tissue culture plate was then subjected to incubation for 48 hours in a humidified CO2 incubator at 37º C. At the end of the culture period, the contents of all wells were aspirated out and centrifuged at 2500 rpm for 10 min. Finally, the obtained culture supernatants were properly aspirated and stored in aliquots at -80°C until they were utilised to evaluate the release of sCD163 both spontaneously and in response to mitogens, as well as to analyse monocyte surface markers CD14 and CD16 using flow cytometry

Assessment of soluble CD163

Soluble CD163 was determined in culture supernatants of purified PBMCs as well as in the sera belonging to all subjects under the study. This was done by standardized sandwich ELISA technique. Briefly, 50 µL of each sample (sera and culture supernatants) were added to each well that was previously coated with sCD163 antibody, incubated for two hours at 37°C then washed with PBS followed by the addition of 50 µL of streptavidin horseradish peroxidase-(HRPNovus, biotechne brand, Canada), incubation at 37 °C and washing with PBS. 50 ul of substrate components A and B(KPL, Gaithersburg, Germany) were added to each well until color development. Finally, 50 ul of stop solution (KPL, Gaithersburg, Germany) was added to end the reaction. The optical density at 450 nm was determined using an ELISA reader.

Statistical analysis

Our data were analysed using the IBM SPSS software programme, version 20.0 (Armonk, NY: IBM Corp.). To assess the data's normality, the Shapiro-Wilk test was performed.. Range, mean and standard deviation, were used to express data distribution. For quantitative variables with abnormal distribution Kruskal Wallis test was used ,Post Hoc test was utilized to compare two groups .Significance of the obtained results was determined at a level of 5% [15].

Results

A.**Demographic data Age & Sex**

Statistical analysis of our results revealed no statistically significant difference regarding age and sex between all studied groups (p=0.066, 0.267).

B.**Laboratory findings**

Liver function tests. AFP and HCV load

Our results are demonstrated in (Table 1)

Table(1): Liver function tests, AFP and HCV load.

	Control group (n = 20)	HCV without complications (n = 20)	HCV with cirrhosis (n = 20)	Hepatocellular carcinoma (n = 20)	Test of significance.
Albumin					
Mean ± SD.	4.52 ± 0.17	3.81 ± 0.81	2.62[c] ± 0.44	2.52 ± 0.52	F= 65.307*
p_0		<0.001*	<0.001*	<0.001*	
Significance between groups		p_1<0.001*,p_2<0.001*,p_3=0.934			
ALT					
Mean ± SD.	11.0 ± 4.69	42.30 ± 29.68	26.60 ± 18.29	61.40 ± 87.50	H= 34.529*
p_0		<0.001*	0.002*	<0.001*	
Significance between groups		p_1=0.044*,p_2=0.957,p_3=0.050*			
AST					
Mean ± SD.	14.50 ± 2.40	32.25 ± 14.17	58.90 ± 32.63	106.65 ± 86.33	H= 55.933*
p_0		0.001*	<0.001*	<0.001*	
Significance of groups		p_1=0.017*,p_2=0.001*,p_3=0.320			
Total Bilirubin					
Mean ± SD.	0.34 ± 0.08	0.68 ± 0.56	4.25 ± 6.12	7.10 ± 8.01	H= 49.569*
p_0		0.029*	<0.001*	<0.001*	
Significance between groups		p_1=0.006*,p_2<0.001*,p_3=0.122			
Direct Bilirubin					
Mean ± SD.	0.14 ± 0.03	0.32 ± 0.37	2.28 ± 3.89	4.83 ± 6.77	H= 52.523*
p_0		0.081	<0.001*	<0.001*	
Significance between groups		p_1=0.002*,p_2<0.001*,p_3=0.097			
Prothrombin activity					
Mean ± SD.	103.39 ± 9.42	81.89 ± 4.98	48.48 ± 16.51	46.14 ± 16.41	H=59.234*
p_0		0.006*	<0.001*	<0.001*	
Significance betweengroups.		p_1<0.001*,p_2<0.001*,p_3=0.957			
AFP					
Mean ± SD.	1.87 ± 0.34	2.83 ± 1.25	2.49 ± 1.70	491.08 ± 602.22	H=23.581*
p_0		0.138	0.870	<0.001*	
Significance between groups		p_1=0.187,p_2=0.005*,p_3<0.001*			
HCV PCR ($\times 10^5$)					
Mean ± SD.	–	14.69 ± 1.82	139.58 ± 72.28	181.25 ± 23.18	H= 31.688*
Significance between groups		p_1<0.001*,p_2<0.001*,p_3=0.072			

SD**: Standard deviation**

H: H for **Kruskal Wallis test**, Pairwise comparison bet. each 2 groups was done using **Post Hoc Test (Dunn's for multiple comparisons test)**

F: F for ANOVA test, Pairwise comparison bet. each 2 groups was done using Post Hoc Test (Tukey)

p0: p value for comparing between control group and each other group

p1: p value for comparing between **HCV infected patients without complications** and HCV infected patients with cirrhosis

p2: p value for comparing between **HCV infected patients without complications and hepatocellular carcinoma**

p3: p value for comparing between HCV infected patients with **cirrhosis** and **hepatocellular carcinoma**

*: Statistically significant at $p \leq 0.05$

C. Enumeration of CD14+/CD16+ monocytes

A lower proportion of CD14-expressing monocytes.was detected in HCV patients with different comorbidities when compared to control group . However a higher proportion of CD16 expressing monocytes as well as double positive monocytic cells (CD14+ CD16+) were noticed in all patients groups when compared to control group. Table 2 (Figures 1&2)

Table (2): Comparison between the studied groups according to monocytes markers.

	Control group (n = 20)	HCV without complications (n = 20)	HCV with cirrhosis (n = 20)	Hepatocellular carcinoma (n = 20)	Test of significance H
CD14%					
Mean ± SD.	79.45 ± 6.07	40.01 ± 13.90	50.15 ± 16.45	59.30 ± 14.18	48.423*
p_0		<0.001*	<0.001*	<0.001*	
Significance between groups		p_1=0.089,p_2=0.003*,p_3=0.210			
CD16%					
Mean ± SD.	2.29 ± 1.44	10.55 ± 4.72	8.95 ± 4.97	7.09 ± 6.45	35.040*
p_0		<0.001*	<0.001*	0.002*	
Significance between groups		p_1=0.396,p_2=0.015*,p_3=0.116			
(CD14+& CD16+%)					
Mean ± SD.	13.80 ± 5.0	33.25 ± 20.14	33.10 ± 16.35	24.65 ± 15.91	17.473*
p_0		0.001*	<0.001*	0.022*	
Significance between groups		p_1=0.542,p_2=0.325,p_3=0.111			

SD: **Standard deviation**

H: H for **Kruskal Wallis test,** Pairwise comparison bet. each 2 groups was done using **Post Hoc Test (Dunn's for multiplecomparisons test)**

p_0: p value for comparing between **Control group** and each other group

p_1: p value for comparing between **HCV infection without complications** and **HCV patients with cirrhosis**

p_2: p value for comparing between **HCV infection without complications** and **hepatocellular carcinoma patients**

p_3: p value for comparing between **HCV patients with cirrhosis** and **hepatocellular carcinoma patients**

*: Statistically significant at $p \leq 0.05$

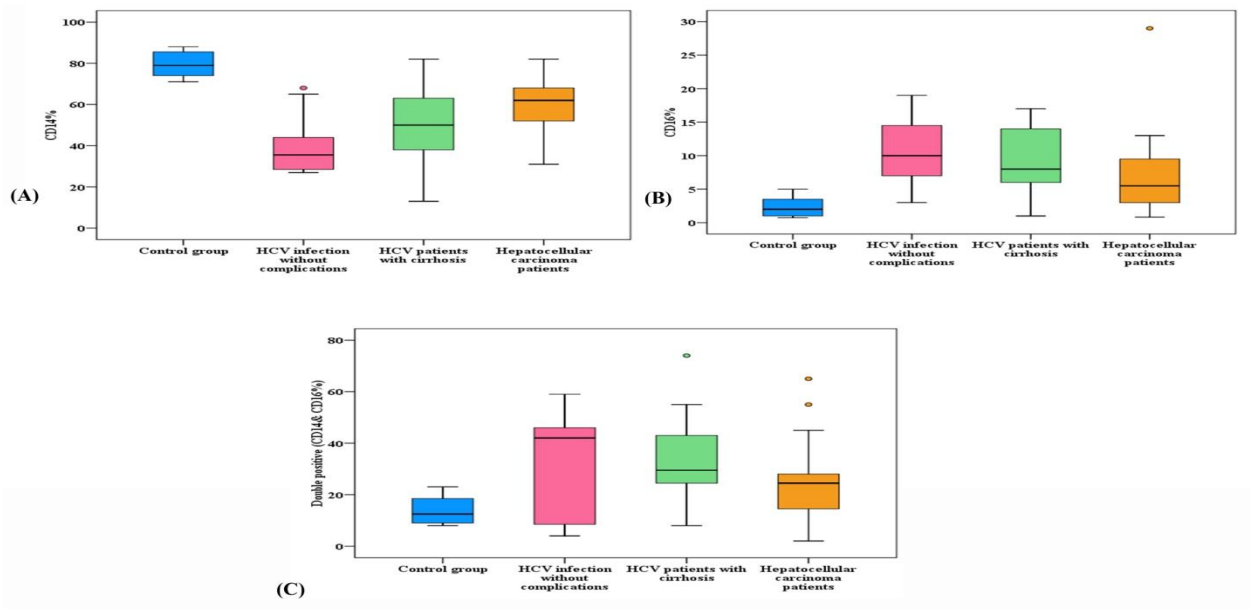

Figure 1:Distribution of monocyte surface markers (CD14,CD16) in different studied groups

A. Percentage of CD14+ monocytic cells in different studied groups

B. Percentage of CD16+ monocytic cells in different studied groups

C. Percentage of double positive monocytic cells (CD14+,CD16+) in different studied groups

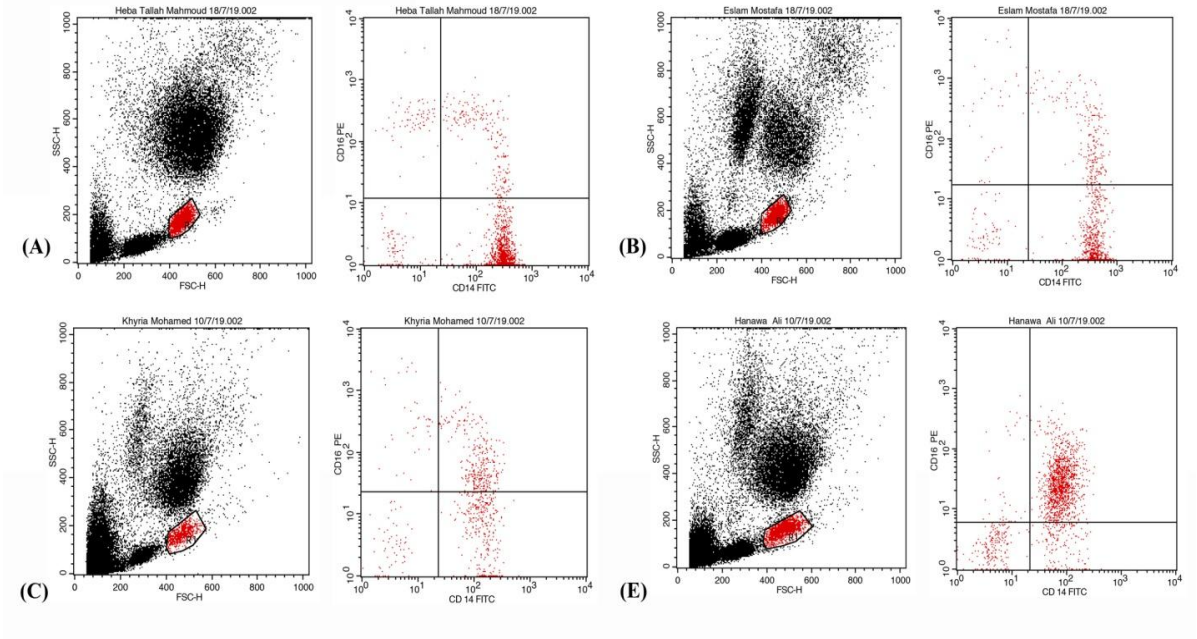

Figure 2:Gating of monocyte surface markers (CD14 ,CD16) by flow cytometry

D. **Soluble CD163 (serum and culture supernatents)**

Our results showed a significantly elevated levels of sCD163 in all patients groups compared to normal subjects most pronounced in those patients with HCC.(Table 3) (Figure 3)

Table 3: Comparison between the four studied groups according to soluble CD163.

	Control group (n = 20)	HCV without complications (n = 20)	HCV with cirrhosis (n = 20)	Hepatocellular carcinoma (n = 20)	Test of significance H
Serum sCD163					
Mean ± SD.	17.16 ± 5.87	32.83 ± 20.39	37.74 ± 23.32	82.19 ± 45.84	34.650*
p_0		0.034*	0.007*	<0.001*	
Significance between groups			p_1=0.561,p_2<0.001*,p_3=0.002*		
Basal sCD163 (culture supernatent)					
Mean ± SD.	0.25 ± 0.15	2.14 ± 3.43	1.65 ± 1.09	2.77 ± 2.43	47.356*
p_0		<0.001*	<0.001*	<0.001*	
Significance between groups			p_1=0.249,p_2=0.037*,p_3=0.351		
Mitogen induced sCD163 (culture supernatent)					
Mean ± SD.	19.62 ± 11.58	80.65 ± 52.53	122.7 ± 143.8	201.9 ± 236.8	32.805*
p_0		<0.001*	<0.001*	<0.001*	
Significance between groups			p_1=0.976,p_2=0.446,p_3=0.464		

SD: **Standard deviation**

H: H for **Kruskal Wallis test,** Pairwise comparison bet. each 2 groups was done using **Post Hoc Test (Dunn's for multiplecomparisons test)**

p: p value for comparing between the studied groups

p_0: p value for comparing between **Control group** and each other group

p_1: p value for comparing between **HCV infection without complications** and **HCV patients with cirrhosis**

p_2: p value for comparing between **HCV infection without complications** and **hepatocellular carcinoma patients**

p_3: p value for comparing between **HCV patients with cirrhosis** and **hepatocellular carcinoma patients**

*: Statistically significant at $p \leq 0.05$

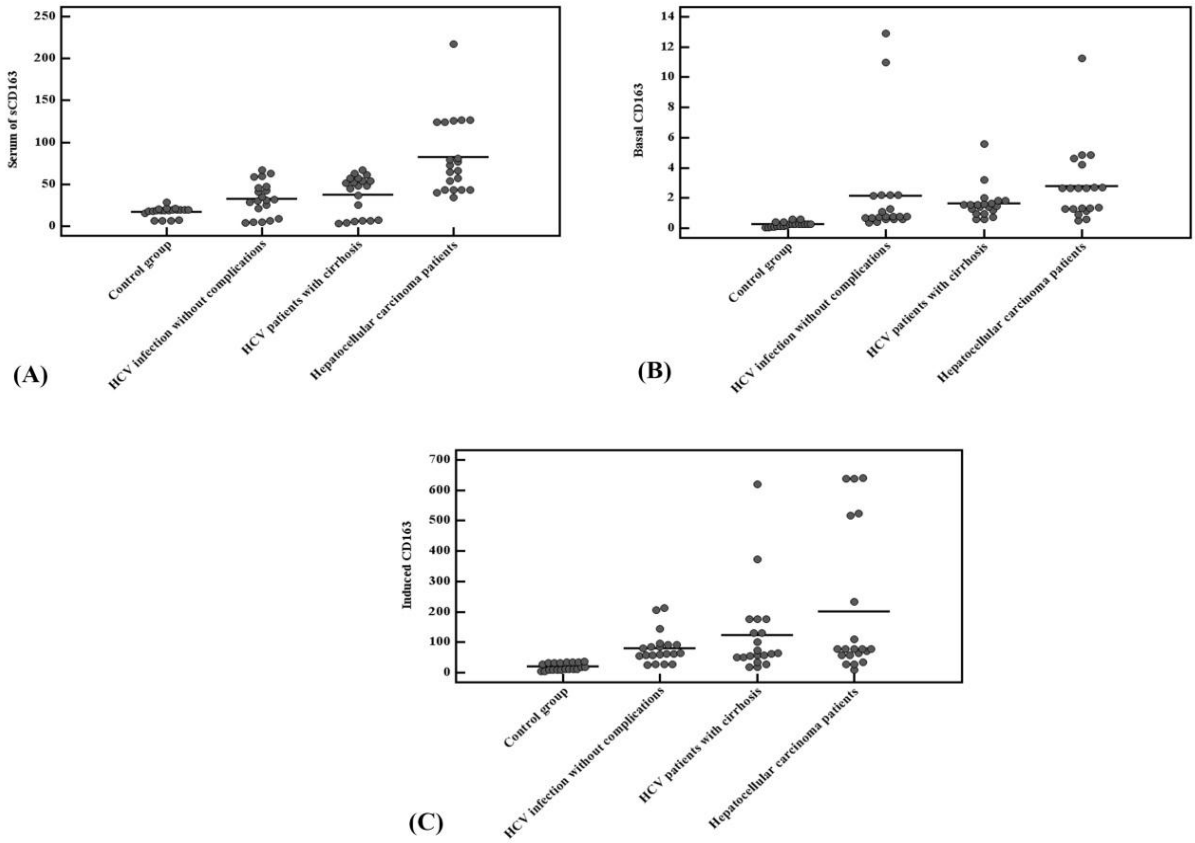

Figure 3: Levels of soluble sCD163 in different studied groups
A) in serum B) Basal CD163 in culture supernatent C) Mitogen induced CD163 in culture supernatent

E. **Correlation analysis**

There was a significant negative correlation between serum CD163 and albumin

There was a significant positive correlation between serum CD163 levels ,AST, total ,direct bilirubin levels and viral load . Otherwise no significant correlations were found. (Table 4)

Table 4: Correlation between sCD163 with different parameters in patients groups (n = 60)

	Serum of sCD163		Induced CD163	
	r_s	p	r_s	p
Albumin	-0.261	0.044*	-0.119	0.364
ALT	0.166	0.205	0.213	0.102
AST	0.338	0.008*	0.146	0.264
Total Bilirubin	0.416	0.001*	0.027	0.840
Direct Bilirubin	0.416	0.001*	0.084	0.523
CD14%	0.203	0.119	-0.074	0.572
CD16%	-0.130	0.322	0.102	0.438
Ratio (CD14& CD16%)	-0.131	0.317	0.133	0.310
Viral load	0.341	0.008*	0.114	0.386

r_s: Spearman coefficient

*: Statistically significant at $p \leq 0.05$

Discussion

The objective of the current study was to determine soluble CD163 levels in both sera and culture supernatant of PBMCs in HCV patients with different clinical outcomes and correlated it with viral load, essential cell surface markers of monocytes (CD14, CD16), conventional biochemical and hematological indicators for disease progression.

Host and viral interactions are characterized by their diverse role in the immunopathogenesis of HCV infection [16]. In all phases of liver inflammation and fibrosis, both tissue-resident kuppfer cells and invading macrophages are crucial[17].

The anti-inflammatory activity of CD163 is mostly associated with circulating monocytes and M2 macrophages [18]. Activated macrophages can release CD163 in its soluble form into the circulation..Overexpression of sCD163 is considered as an important marker reflecting progressive inflammation and predict mortality among patients with fulminant hepatic failure [19]. In light of this, the clinical importance of expression of both hepatic CD163 and its corresponding soluble form are of particular interest.

Our results revealed that HCV infected patients have elevated levels of liver enzymes , total and direct bilirubin levels with decreased albumin levels and prothrombin activity in comparison to control group. These results were expected due to liver injury and ongoing inflammation caused by HCV infection.

Supporting our results Mahaboob et al. (2013) reported elevated levels of liver enzymes and bilirubin in HCV infected patients with or without comorbidities [20]. Hypoalbuminemia reported in our study was in agreement with Thapa and Walia (2007) who found that albumin levels showed significant decrease in cirrhotic patients and those with ascites [21]. In addition, the actual relationship between prothrombin activity and liver disease was investigated by Zermatten et al (2020).who speculated that cirrhotic patients have decreased prothrombin activity with prolonged PT and PTT [22].

Moreover, the highest levels of AFP were noticed in HCC patients of the current study in comparison to other groups and this can be devoted to its role as a tumor marker for predicting HCC occurrence. However,Rojaz et al (2016) stated that increased serum levels of AFP can occur in other disease conditions as pregnancy, cirrhosis or acute hepatitis [23].

As the pivotal role played by macrophages in HCV infection, flow cytometric analysis for monocyte surface markers was done. Compared to control group the current study showed decreased percentage of CD14+ monocytes in all chronically infected HCV patients with or without complications. The above results were in contrast to Zhao et al. (2020) who stated that the levels of monocytes expressing CD14 were increased in patients with chronic HCV infection mainly those with severe fibrosis [24]. A study carried out by Seha et al (2016) reported that early stages of HCV were associated with higher levels of proinflammatory cytokines, but late cytokine response was characterized by the generation of anti-inflammatory cytokines that may result in decreased expression of CD14+ monocytes [25].

Additionally, compared to the control group, all HCV patients had a considerably increased proportion of CD16 expressing monocytes.In line with our results Coquillard et al (2009) revealed the ability of HCV to infect only monocytes subsets that express CD16 [26].

Contradictory CD14 and CD16 expression results might be due to the variability in the expression of both CD14 and CD16 surface markers in different monocytes subsets.Our data revealed that double positive CD14+/CD16+ monocytes were significantly increased in all HCV infected populations with or without cirrhosis in comparison to control group.

These findings were confirmed by the work of Zheng et al (2014) who claimed increased proportion of double positive monocytes in patients with CHC as well as in cirrhotic patients.This elevation was concomitant with increased production of pro-inflammatory cytokines,such as TNF-α and IL-1β, [27] Moreover, a study carried out by Saha et al (2016) employed co-cultures of healthy human monocytes and HCV-infected hepatoma cells and showed that HCV caused monocytes to differentiate into macrophages and this was accompanied by higher expression of the characteristic surface markers of monocytes ;CD14, CD16, and CD68 [25].

The results of soluble form of CD163 were assayed in both sera and culture supernatants of PBMCs. It was noticed that the results of serum CD163 were in the same line with those of culture supernatants. In contrast to other patient groups and healthy controls, individuals with HCC had increased levels of both serum and CD163 within culture supernatents. This can be expected as CD163 can function as a marker of macrophage activation which is known to be involved in inflammatory and fibrotic changes in chronic liver diseases.

In line with our results, Melino et al (2012) noticed an increased levels of circulating CD163 which was concomitant with increased hepatic CD163 expression, indicating that serum levels of CD163 can reflect the changes of CD163 in inflamed liver tissue. These findings supported that soluble CD163 might function as a non-invasive biomarker to predict hepatic inflammatory disorders and their prognosis.[28].

The importance of sCD163 as a non invasive biomarker was supported by Tacke et al (2017) who explained that circulating sCD163 levels can be taken as an independent non-invasive risk factor for variceal bleeding, fibrosis and death in cirrhotic patients [16].

In accordance to our results, Zhao et al. (2020) concluded that patients with chronic HCV infection had higher levels of

soluble CD163, which indicated hepatic macrophage activation with subsequent fibrotic changes .Additionally, they observed that the proportions of CD14+ monocytes and CD163+ macrophages in individuals with chronic HVC may serve as markers of the progression of the diseses course [24]. sCD163 expression is not only a characteristic of virally induced hepatic cirrhosis and HCC. This finding was reaveled by Kazankov et al. (2015) who reported that patients with fatty liver disease exhibited considerably higher sCD163 levels, which may be related to early hepatic complications, macrophage activation and inflammatiory changes, which can occur prior to detection of histological changes. They also claimed that the level of sCD163 was correlated with advanced fibrosis, suggesting continous macrophage activation throughout all disease stages. In addition, patients suffering from acute liver failure showed increased levels of sCD163 [29].

It was noticed that both liver enzymes and sCD163 in both sera and culture supernatents are positively correlated and these findings can be expected as both parameters are associated with inflammatory changes with subsequent liver injury and sCD163 is known as a marker of macrophage activation associated with different stages of HCV. Supporting these results Glavind et al (2020) revealed that cirrhotic patients showed greater levels of sCD163 than those without cirrhosis and it was linked to biochemical indicators of liver damage and hepatocellular function. [30].

Additionally, a negative correlation between the proportion of CD16+ monocytes, double-positive monocytes for CD14 and CD16, and serum CD163 was detected. Concomitant with our results, Davis et al (2004) stated an inverse relation between membrane and sCD163 levels in human blood suggesting that plasma sCD163 is derived from circulating monocytes as well as another component which can be derived from tissue macrophages. Moreover, they stated monocyte functional alterations rather than circulating cell counts [31]. However a positive correlation was detected between CD163 within mitogen induced culture supernatant , proportion of CD16+ monocytes, double-positive monocytes for CD14 and CD16 . The previous finding can be explained by the fact that the assessment of CD163 levels in cultured cells may be more precise than serum CD163 in reflecting its hepatic expression. According to our knowledge, this work is the first to evaluate the amount of sCD163 in the PBMCs culture supernatant.

Another positive correlations were found between soluble CD163 (serum and mitogen induced) and viral load in different patients groups. In line with our findings, Kazankov et al. (2014) reported elevated sCD163 levels in individuals with persistent HCV and HBV infections with fibrotic changes [32]. In addition ,Isaac et al (2019) reported that HCV infected patients receiving successful therapy had lower levels of sCD163. Decreased levels of sCD163were associated with regression of fibrosis and disease severity [33].

From our results we can conclude that peripheral blood monocytes expressing the cognate marker CD14 was reduced in HCV patients mainly those without complications. On the other hand CD16+ monocytes and double positive monocytes (CD14+,CD16+) were remarkably elevated in HCV patients with different outcomes. In addition, CD163 shedding into the circulation tends to be upregulated due to HCV infection and complications manifested by increased levels of both serum and mitogen induced CD163 in all HCV clinical outcomes.

We recommend the use of sCD163 as a non-invasive prognostic index of HCV complications and as a valuable indicator of progression towards malignancy in HCV infected patients. In addition determination of CD14 and CD16 surface markers expression on different phenotypes of circulating monocytic cells in HCV patients with different clinical outcomes is needed.

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Conflict of Interest: No conflict of interest to be declared by the authors

Ethics approval: This study was approved by the Ethics Committee of the Medical Research Institute. Informed written consent for patient's participation in a clinical Research was obtained from all participants before enrollment into the study

Data availability: The data of the current study can be provided by the corresponding author upon reasonable request

Author Contributions: All authors contributed equally in the current study

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