Assessment of Gene Expression of Some Human Cluster of Differentiation in Chronic HCV Patients

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HCV infection, gene expression, CD25, CD39 and CD127.

ABSTRACT:
Background: Hepatitis C virus (HCV) is one of the most potential pathogens all over the world. It is one of the main causes of chronic liver diseases. The host immune response against HCV sets off a chain reaction that results in liver damage after HCV infection in addition to necrosis and cellular inflammation, fibrosis, and hepatocellular carcinoma. Objective: This study aimed at evaluation of gene expression of human cluster of differentiation (CD25, CD39 and CD127) in patients with chronic HCV infection. Materials and Methods: The study involved 70 HCV patients who were diagnosed in Hepatology and Gastroenterology Center at Azadi Teaching Hospital and 50 control subjects during October 2023 until April 2024. HCV patients were confirmed with real time PCR. In addition RNA extraction was conducted on patients and control whole blood to assess the gene expression of CD25, CD39 and CD127 in both groups. Result: Regarding gene expression of CD25, CD39 and CD127, our data revealed significant overexpression of those marker in HCV patients in comparison to control group with P value (0.0001, <0.0001 and <0.0001) respectively. Conclusion: The gene expression of CD25, CD39 and CD127 were significantly up-regulated in HCV patients compared to healthy control indicating the role played by these markers.

1. Introduction

HCV was recognized in 1989 as one of the etiological causes of viral hepatitis [1]. HCV is an enveloped small RNA virus belonging to family Flaviviridae and genus Hepacivirus. The single-stranded positive-sense RNA genome of HCV is around 9.6 kb in size, which is flanked by untranslated regions (UTRs) on both ends. The single open reading frame that present in this genome encodes for a nonfunctional poly protein precursor which is cleaved by viral and cellular proteases into seven nonstructural and three structural proteins [2,3]. HCV is classified into eight genotypes and several subtypes that expand the diversity of the genomic sequence [4]. Approximately 177.5 million people around the globe are infected by HCV, and annually, 1.3–3.7 million new cases of HCV infection are recorded [5,6]. HCV is spread parenterally, as well as via blood transfusion, mother to fetus, sexual, and organ transplantation. The clinical picture of HCV infection is initiated as acute stage, followed chronic infection in about 80% of patients [7,8]. The acute stage of infection might show mild flu-like symptoms, but is frequently asymptomatic. Chronic HCV infection tend to progress to cirrhosis and hepatocellular carcinoma (HCC) [9]. The human immune system has established two supports innate and adaptive immunity that act cooperatively, protecting from infections and limiting the damages caused by the invading pathogens [10]. Innate immunity acts directly following the infection, directs the production of proinflammatory cytokines as well as orchestrating adaptive immunity. HCV has been evolved mechanisms to evade the host innate immune responses that leads to viral persistence. The HCV persistence leads to chronic infection then eventually to advances liver disease [10]. The populations of CD4+ T cells and CD8+ T cells are no longer encouraged by continuous antigens once HCV is eliminated by efficient immune responses. Instead, they show a high levels of the memory marker (CD127), which is required for the homeostatic proliferations, and their frequencies decreases after infection [11]. CD127, encoded by the IL7R gene, assembles with the IL2RGencoded common γ-chain to form the heterodimeric IL-7 receptor. It is predominantly expressed in various stages of lymphoid progenitor cells and in mature lymphocytes, including T cells and innate lymphoid cells. Early specific HCV- CD8+ T cell expression of interlukin-7 receptor(IL-7) alpha (CD127) is associated with effective immunological response that leads to viral elimination [12]. CD25 is the alpha chain of the trimeric IL-2 receptor and considered to be the most prominent cellular activation marker. It plays a key role in responsiveness to IL-2 resulting in lymphocyte activation and further IL-2 production. It is expressed constitutively on the surface of several subsets of peripheral blood lymphocytes, such as regulatory and resting memory T cells[13]. During the chronic HCV infection, CD4 T- regulatory (Treg) cells express CD25, that is not constitutively expressed on resting CD8 T- cells but it is up-regulated following T cell receptor (TCR) triggering [14]. CD39, the NTPDase (ecto-nucleoside triphosphate

diphosphohydrolase),is transmembrane glycoprotein that is present in activated lymphocytes, endothelial and cancer cells, but not resting cells. It is regulate immune responses balance by hydrolyzing ATP and ADP. It is now again becoming a newly recognized “immune checkpoint mediator” that interferes with antitumor or anti-inflammatory immune response[15,16].The expression of activation-associated markers (PD-1, CD39) is a characteristic of virus-specific CD8+ T cells linked to human HCV clearance [17]. The current study was designated to evaluate the gene expression of CD25, CD39 and CD127 in HCV positive patients and control group in order to understand the role played by these marker in the pathogenesis of the infection.

2. Materials and Methods

Study design

A case-control study design of our study to assess some immunologic markers gene expression, whole blood from chronic HCV patients and the control group were collected then extracted. Patients attending Hepatology and Gastroenterology Centers at Azadi Teaching Hospital who were infected with HCV were involved, in addition to control group who were apparently healthy and have no history of clinical disorders.

Ethical approval

The study protocol and the subject information and consent form were reviewed and approved by a local ethics committee according to document number 598 in September 19,2023 to get this approval.

Study samples and methods

In this investigation, 120 samples including 70 HCV patients and 50 healthy controls were used. Blood samples were taken from each group under study and one part incubated in

the gel tubes that were centrifuged to obtain serum tat used for confirmation of HCV

infection via polymerase chain reaction(PCR) test (Real Time PCR (RT PCR)). The second part of blood were placed into EDTA tubes that submitted for total RNA extraction and assessment of CD25, CD39, CD127 gene expression via RT PCR.

HCV RNA extraction

The extraction of HCV nucleic acid was performed according to the manufacturer instruction (Viral Nuclic Acid Extraction, Zybio Ltd, China)

Total RNA extraction form whole blood

Total RNA was extracted from patients and control following the provider instruction (FavorPrepTM Blood/Cultured Cell Total RNA Mini Kit, Favogene Ltd, Korea).

HCV RNA detection

HCV was amplified in all suspected patients according to the direction of the manufacturer company (Bosphore HCV detection kit, Turkey). 24µl of the master was pipetted into the PCR tubes, and 16µl of RNA (sample/ /positive or negative control) were added. The amplification protocol was set as follow: Reverse Transcription at 50 ºC for 30min, Initial Denaturation at 95 ºC for 14:30min, Denaturation at 97 ºC for 30sec, Annealing at 55 ºC for 90 seconds and finally extension at 72ºC for 15sec. The last three steps(Denaturation, Annealing and Extension) were set for 50 cycle.

CD25, CD39, and CD127 gene expression.

The gene expression of CD25, CD39 and CD127 were performed by admixing the following ingredients; 10µl reaction mix with 0.4µl GoScript™, 1µl of forward and revers primers for each gene and 5µl extracted RNA in addition to 4.6µl of nuclease free water. The forward primer utilized for CD25 (5'- CTTAAGTCAGGATCGACTTC- 3') and revers primer (3'- GAGAGGGGAGATTCCGTCCC - 5'), for CD39 the forward primer (5'- ACCTCGAGAATATCCGATCC - 3') and the reverse (3'- CCTCGGTCTCCACCCCTACA -5') while for CD127 the forward primer (5'- TTGTACTGTTCCTCGGACCC - 3') and the reverse primer (3'- TTCCGGTGGGAATTGATTCC - 5'). For each gene two separated tubes were prepared one dedicated for the test and control and the other one for the House Keeping Gene. The three genes were run separately yet the same amplification protocol was applied

with the exception of annealing temperature; Reverse transcription at 38°C for 15min, 95°C for 10min, Denaturation at 95°C for 10sec, Annealing at 58°C, 59°C and 54°C for CD25, CD39 and CD127 respectively for 30sec and finally Extension at 72°C for 30sec the last three steps(Denaturation, Annealing and Extension) were set for 40 cycles.

Statistical analysis

Statistical analysis was performed using Graph Pad Prisim version 10.1 statistic software, Qi square and tTest were applied in the comparison between the groups. Significant data is considered were $P<0.05$ and non significant differences represented by $P>0.05$.

3. Results

This study involved the assessment of gene expression of some immunological markers in patients with chronic HCV and control group. HCV- RNA was detected in in 47(67.14%) of the 70 patients who were HCV ELISA antibody positive, while all control group were negative for HCV antibodies. Patient biometric data are illustrated in Table 1 which demonstrates the distribution of gender, age and residency of the patients. The percentage of males was 62.2%, while in females 37.14%. According to age, divided into three categories: 30 years or below (32.86%), 31-50 years(44.28%) and 51 years and above (22.86%). About residency, the percentage of patients from urban area (68.57%) were more than those from rural area(31.43%) .

Table 1: Common characteristics of HCV patients

Demographic variable		%
Gender	Female	37.14
	Male	62.86
Age	≤20	10
	21-30	22.86
	31-40	31.43
	41-50	12.86
	>50	22.86
Residency	Rural	31.43
	Urban	68.57

When it came to chronic diseases associated with HCV infection, Our data demonstrated that the most common disease in HCV infected patients is hypertension(22.86%) followed by diabetes mellitus(17.14%) and cardiovascular diseases(15.17%) as shown in Table 2.

Comorbidity diseases	HCV patients No.(%)	Controls No.(%)
Hypertension	11(15.71)	0(0.0)
Diabetes mellitus	12(17.14)	0(0.0)
Cardiovascular disease	16(22.86)	0(0.0)
Liver fibrosis	7(10)	0(0.0)
Total	46(65.71)	0(0.0)

Regarding viral load, our data illustrated that HCV viral load was slightly higher in males than female patients with mean (2470252 and 1058836) respectively. Significant difference was recorded between them with $P<0.05$ utilizing Mann Whitney tTest as shown in Table 3.

Table 3: Viral load IU/ml

HCV viral load	Mean±SD	Total (n)
Male	2470252±1682607.00	44
Female	1058836±879393.00	26
P- value	0.037	

Our data demonstrated significant up regulation of CD25 in HCV positive patients compared to control group with $P<0.05$ as shown in Table 4.

Table 4: CD25 gene expression in patient and control group.		
CD25 gene expression	**Mean± SD**	**Total (n)**
HCV patients	6.11±1.82	70
Control	1.09±0.33	50
P- value	0.0001	

In the same way, the gene expression of CD39 expressed significant elevation in HCV positive cases in comparison to control group with mean fold change 7 times greater than the control, P<0.05 as displayed in Table 5.

Table 5: CD39 gene expression in patient and control group.		
CD39 gene expression	**Mean± SD**	**Total (n)**
HCV patients	7.23±1.926	70
Control	1.086±0.3295	50
P- value	<0.0001	

The data of our research recorded significant up regulation of CD127 gene expression in patient group with mean fold change 4 times greater than control group (P<0.05) as displayed in Table 6.

Table 6: 127 gene expression in patient and control group.		
CD127 gene expression	**Mean±SD**	**Total (n)**
HCV patients	4.07±0.96	70
Control	1.09±0.33	50
P- value	<0.0001	

4. Discussion

In order to investigate their relationship with HCV infection in groups under study (patients with chronic HCV infection and controls), it was attempted in this study to assess Cluster Differentiation of Human (CD25, CD39, and CD127).

The current study revealed that the frequency percent of HCV is higher in males (62.86%) rather than females (37.14%). This frequencies are consistent with another findings that reported by others authors[18][19] as the prevalence was higher in males than females. While, our data conflicts [20] which reported that the ratio of female to male were(1.4:1). According to Chinese scientists, male-specific liver proteins called apolipoprotein A-I aid in the infection of males more frequently than females [21].

Regarding age group the age(31-40) years old was the most prevalent range among HCV patients (44.28%) this data are in line with another study [22] which indicated that the largest age group among HCV patients was in those between 30-39 years in also in agreement with [23] who reported that HCV patient within age group (31-40)years. While, our data conflicts (Hasan) who showed that the age group(41-60) years old was the most prevalent range among HCV patients [24]. The differences between our data and other report could be attributed to several factors including the size of the sample included in the study , the bias selective of patient according to HCV positivity in addition to other geographical influences on the study design.

In our study most of HCV patients were residing city center, these result agrees with [25] report also it is in parallel with another report (Hassan etal) who reported increase HCV cases in urban population [26]. However, our data in conflicts with [27] investigation who noted increased HCV positive cases in rural population. The discrepancies in these data could reflect the influence of geographical area where HCV infection is endemic especially in crowded population, also the site where the study was conducted and to the health protocol imposed in the study area.

Our study revealed that nearly half of HCV patients had one or more HCV-related comorbidities; the most frequent comorbidity was hypertension followed by diabetes, this data is in agreement with [28] who reported that people with HCV have a higher prevalence of comorbidity and multi morbidity compared to the general population. These results obtained are influenced by several factors such as patients age, personal life style such as smoking and alcohol consumption , stage of disease and use of medication.

Our research illustrated that the viral load of HCV in males was significantly higher than female(P<0.05).this result is in agreement with [29] and [30] who reported significant elevation of HCV viral load in males than in females this may be due to spontaneous clearance of acute infection in females [31]. The reason to this

clearance has been attributed occurrence of certain genetic factors such as IL28B genetic variants in females [32 ,33].

The results obtained indicated that CD25 illustrated significant elevation in HCV cases in comparison to control group. Our result is in line with Ouaguia et al [34] investigation that reported increase CD25 expression in HCV positive patients. Moreover, the data displayed here is close to Abdelfattah et al [35] who noted that CD25 is significantly elevated in HCV patients with increased in cirrhotic and liver carcinoma. The reason behind the increase expression of CD25 could be attributed to the role played by the T reg cells which help in controlling the immune response following the activation against HCV infection.

HCV-specific T cells are impaired in chronic HCV infection, as evidenced by decreased proliferation, cytokine production, and cytolytic activity. This impaired phenotype has been attributed to a variety of inhibitory mechanisms, one of them is an increased levels of regulatory T cells (Tregs) [36]. An increased frequency of Treg cells and its suppressive activity associated with chronic disease, they attenuate HCV-specific T cell responses in the liver and reduce the risks of hepatic injury caused by the presence of a sustained CTL response [37]. So, in HCV infection, Tregs are function to down regulate tissue damaging response to infection in liver as well as promote the maintenance of HCV persistence [38]. Treg cells express high levels of CD25 on their surface and this marker has been widely used to detect, sort and target Treg cells in vitro and in vivo [39]. Interleukin-2 is driving T-cell proliferation and is important to sustain the Treg-cell population as well. Treg cells may use CD25 to capture IL-2 from its environment, thereby depriving effector T cells of this stimulatory cytokine [40].

On the other hand, CD39 was markedly upregulated in response to HCV infection, our data are in parallel with other reports that found an upregulation in CD39 expression in patients with HCV active infection [41,42]. The similarities between our data and other reports could be due to the role of this marker in activating lymphocytes (T reg) which allow the immune response to be regulated after HCV infection. CD39 is an ectoenzyme that can hydrolyze adenosine triphosphate (ATP) and adenosine diphosphate to adenosine monophosphate (AMP). The hydrolyzed AMPs are then converted by CD73 into anti-inflammatory adenosine (ADO), which can bind to adenosine receptors on T cells and antigen‐presenting cells [43]. ADO, which occurs particularly as a result of changes in ATP metabolism, plays an important role in the suppression of the immunity also it acts as an anti‐inflammatory effector to Tregs [44].

Regarding CD127, our data revealed that the expression of CD127 was upregulated in HCV infected cases compared to control subjects. Our results are in line with (Smits et al) who observed changes in the HCV-specific CD4+ T cell compartment that indicate a trend toward memory development, most prominently characterized by upregulation of CD127 [45], also it agrees with (Wieland et al) who demonstrated that the appearance of memory HCV-specific CD8+ T cells at the chronic stage of hepatitis C infection is defined by upregulation of CD127 [46]. On other hand these data are in conflict with (Hou et al) who reported a down regulation of CD127 in chronic HCV patients [47]. Also it oppose the result of (Maretti-Mira etal) who noted a down regulation in CD127 expression in HCV chronic patients [48]. The discrepancies in the data describe above could be explained by the administration of antiviral therapy by the patients that leads to significantly increase the frequencies of HCV-specific CD4+ T cells early after initiation of antiviral therapy [45] due to the short lifespan of hepatitis C virions (approximately 3 hours) [49], inhibition of their replication by antiviral therapies result in rapid decline of viral loads in treated patients. Consequently, HCV-specific T cells are no longer required in the liver parenchyma to suppress viral replication. Based on the presence of liver-infiltrating HCV-specific T cells targeting the virus in chronic HCV infection [50]. Also the discrepancies in the data could be explained by the fact that CD127 is receptor for IL-7 and has abundant expression of various immune cells like immature B cells as well as stimulated T cells as well as endothelial cells of the vessels [51]. Hence, this markers play an important role in the formation T memory cells that allow the immune system to generate memory T cells that promote the immune system to activate rapidly following viral activation or on second exposure to the disease [52]. In addition, due to the expression of this marker on

Various cell type, its over expression may involve

or mediate the migration process of the activated T cells to the active sites where the virus engage cellular damage or in the process of active infection of new hepatocyte[53].

5. Conclusion

In contrast to patients with HCV and the control group, this study found that the gene expression of immunological marker (CD25, CD39 and CD127) were up regulated in HCV patients several folds more than control groups. This upregulation may be occur after immune system activation against HCV infection and resulting in controlling of HCV infection and in protection from cellular damage.

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Conflicts of interest

There are no conflicts of interest.

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