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RESEARCH ARTICLE

CD127 as a Novel Potential Marker for Clearance in Chronic Hepatitis C Virus Patients

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Abstract

Introduction; Chronic HCV infection is considered a major health problem, especially in developing countries. CD127 may be a novel marker to assess viral clearance and response to therapy in chronically infected patients.

Objective; A case control study to assess CD127 in different $CD8^+$ T cell subsets as a new marker to detect viral clearance and response to therapy in hepatitis C. We also determined the correlation between CD127 level and plasma HCV RNA load.

Methods; Fifty three chronically infected HCV patients who presented to the Tropical Medicine and Internal Medicine Department of Ain Shams and Al Zahra university hospital respectively (in the period between October 2013 and September 2014) and twenty healthy volunteers were included in this study. Clinical laboratory data and plasma RNA viral load were measured. Blood sample was obtained and target cells were stained using fluorescent labeled monoclonal against CD8, CD27, CD45RA and CD127. Analysis of CD127 expression on different $CD8^+$ T cells subsets and correlation of the level of expression of CD127 with hepatitis C viral load and liver enzyme.

Results; The percentage of expression CD127 on different $CD8^+$ T cell subsets was measured in HCV-infected patients. A significant difference was found between patients and control ($p < 0.001$) regarding $CD8^+$T cells (31.61$\pm$ .23vs 43.2$\pm$2.3) and their subsets. Negative correlation was found between viral loads and %of CD127 expression on $CD8^+$T cells (r=-0.437).

Conclusions; These findings suggest that CD127 could be used as a novel therapeutic and/or prognostic marker for assessment of interferon response in chronic hepatitis C patients.

INTRODUCTION

Hepatitis C virus (HCV) infections are a major health problem that ends in either in complete recovery (only in a minority of cases), or develop into persistent and chronic hepatitis over a number of years (in the majority of cases) **(Thimme et al.,2001 and Varshney 2012)** . Chronic HCV infection is considered as a major universal health problem; all over the world, with about 170 million affected individuals **(Saravanan et al.,2008).** Subsequently to chronic HCV disease, approximately one-third develop liver cirrhosis and one-fourth were

terminated to hepatocellular carcinoma (HCC) **(Barathan et al., 2015).**. The highest prevalence of HCV infections were found in Egypt, this, in turn, results in massive numbers of HCV related HCC **(Omran et al.,2015).**

Following viral infections naive $CD8^+$ T (NT) cells that conflict their cognate antigen (Ag) subordinates to a complex process of maturation and differentiation. The generated plentiful reservoir of long-lived memory $CD8^+$ T (MT) cells will mediate immune defense from another subsequent attack with the same Age **(Sallusto et al.,2004**).

MT cells are classified according to their expression of the chemokine receptor CCR7 and CD45RA into three main groups: effector memory (T_{EM}); described as $CCR7^-$ $CD45RA^-$ with lower proliferative capacity and higher gamma interferon production, central memory (TCM); described as CCR7+ CD45RA− with a high proliferative capacity and lower gamma interferon production, and finally terminally differentiated effectors; described as $CCR7^-$ $CD45RA^+$ and having the lowest proliferative capacity and highest gamma interferon production **(Omran et al.,2015)** . Other markers have been used to identify different subsets or the maturation status of memory T cells **(Kaech et al., 2003 and Van Leeuwen et al.,2005)** . HCV specific immune tolerance and viral persistence has been connected with unbalanced distribution of circulating $CD8^+$T cell subsets and impairment of homing capacity and effector function **(Joshi and Kaech, 2008)**. Persistent exposure to microbial antigens and chronic immune activation (CIA) appears to direct the begging of immunosenescence, which is the premature ageing of immune cells **(Larsson et al.,2013 and Dolfi et al.,2013).**

Viral load and alanine aminotransferase/aspartate aminotransfearse (ALT/AST) were used as clinical markers to evaluate HCV infection progression and the effect of antiviral therapies. Virus-specific CD8 T-cell responses play a major role in the outcome and pathogenesis of several viral infections, several mechanisms may contribute to the dysfunctions of viral specific CD8+ T cell immune responses such as; high viral load, lack of CD4 (T-cell help), action of regulatory T cells, and defects in T-cell differentiation. Easy and uncomplicated $CD8^+$T cell maturation/activation markers required to assess viral replication and/or disease progression are desired **(Shenet al.,2010)**.

Heterodimer IL-7 receptor (IL-7R) is composed of a unique α chain (CD127) and a common γ chain (CD132), signaling via this receptor is required for CD8 T-cell proliferation and function. During any viral infection, CD127 expression on $CD8^+$ T cells occurs only when the viral load is contained and sufficient CD4 T-cell help is available . **(Kaech and Wherry, 2007)**. Previous studies on patients with acute HBV infections showed that CD127 expression on CD8 T cells increased significantly after viral clearance. CD127/IL-7 system plays a significant role in maintaining homeostasis of circulating T cells **(Badr et al.,2008)** . Studies showed that increased expression of the IL-7Ralpha identifies the effector $CD8^+$T cells that will differentiate into memory cells from the majority of activated $CD8^+$T cells that will die after an acute viral infection**(Barathan et al., 2015)**. The role and regulation of molecules associated with immunosenescence on HCV infected T cells is poorly identified and numerous studies showed that CD127 might be a potential marker of clinical status patients infected by HCV **(Boni et al., 2007 and Fülöp et al., 2013)** .

Methods:

Blood samples collected from HCV-infected patients attending Tropical Medicine department, Ain Shams University Hospital and internal medicine department, Al Zahra university hospital. During the period between October 2013 and September 2014,Fifty three HCV-infected patients were included in this study. Informed written consent from each patient was obtained before starting the data collection. Twenty healthy volunteers as controls were selected from blood donation center. They were defined as negative for HCV, HBV and HIV. Patients were negative for HBV and HIV infection. Chronic HCV infection was diagnosed by elevated liver enzymes ALT and AST and by the presence of anti HCV antibodies using ELISA (Ortho Diagnostic System, Raritan, NJ, USA). None of the HCV-infected patients had received any HCV-specific antiviral therapy nor complicated by cirrhosis as indicated by liver biopsy. Peripheral venous blood sample was obtained from both patients and control group (5ml) after taking their consent. The blood sample was divided into three parts; one part to obtain serum after centrifugation of clotted blood, the second part added to citrate and the third part was added to EDTA containing tube.

HCV RNA testing and quantification; plasma Qualitative HCV RNA tests EDTA containing tubes were used. The assay performed using an automated COBAS AmpliPrep/COBAS Amplicor HCV test, version 2.0 (Roche Molecular Systems, Inc., New Jersey).

Clinical Biochemical Tests; Liver associated enzymes including ALT, AST, ALP (alkaline phosphatase), albumin, globulin and bilirubin was assessed using serum. Citrated blood was used to determine prothrombin time and whole blood was used for complete blood count.

Flow Cytometer Analysis (FCM); 50µl blood was studied immediately for CD8, CD27, CD45RA and CD127 expression on the peripheral blood lymphocytes. The following monoclonal antibodies were used for direct

staining of the target cells: antiCD8PerCP, antiCD27APC, antiCD45RAFITC and CD127PE (BD Pharmigen, San Diego, CA). Appropriate isotope control was used. FCM quality control including calibration, alignment and color compensation was performed before sample acquisition according to manufacturer's instructional manual **(Santos et al.,2007)** .

Statistical analysis; Data was analyzed using Statistical Package for Social Science (SPSS) program, (version 13). t-test and Mann Whitney test were used. Pearson's correlation was used to study correlation between two variables having normally distributed data. P-value < 0.05 was considered statistically significant.

Results:

Two groups of participants were studied in this study: chronic HCV-infected patients and healthy controls. Both groups were matched according to ages (44.97± 9.27 vs 45.45± 9.36).

Table (1): Laboratory characteristics of participants enrolled in the study

Studied Parameters	Patients (n=52)(M/F=41/1) Mean ± SD	Controls (n=23)(M/F=1) Mean ± SD	P. value
*ALT IU/ml	79.44±32.56	23.00±3.94	0.00
**AST IU/ml	61.50±29.10	20.17±5.581	0.00
WBCs /mm3	6852.37±1650.477	6017.26±1406.48	0.038
Lymphocytic count/mm3	2917.29±901.830	2806.61±828.118	0.617
CD8T lymphocyte count /mm3	830.451±354	794.834±202.843	0.672
Alkaline phosphatase IU/ml	73.83±48.53	82.85±42.85	0.456
Albumin mg/dl	4.3±.3	4.1±1.5	0.07
INR	0.7±0.3	0.8±0.2	0.25
Hemoglobin mg/dl	12.8±1.3	13.8±.4	0 .23
Total Bilirubin mg/dl	1.26±.47	1.1±.3	0.4

*ALT (alanine aminotransferase),**AST (aspartate aminotransferase),
In Table (1) Significant increased AST, ALT was observed in patients compared to controls (P<0.05).

CD8+T cell subsets distribution in peripheral blood of HCV patients: $CD8^+$ T cells can be divided into four subsets according to CD45RA and CD27 expression: naïve NT ($CD45RA^+CD27^+$), memory MT ($CD45RA^-$ $CD27^+$), effector memory ET ($CD45RA^-$ $CD27^-$) and terminally differentiated effector memory TEM ($CD45RA^+$ $CD27^-$). Gating strategy and dot plot analysis of $CD8^+$T cell subsets was done (Figure1). The percentage of naïve $CD8^+$Tcells (NT cells) in HCV infection was significantly decreased in patients compared with healthy controls (36.90+11.260 vs 56.65+7.935).While percentage of terminally differentiated effector memory (TEM) showed an opposite trend, which was increased in HCV infection (36.71±7.421 vs 25±4.30) (p <0.05). No significant difference between patients and control group regarding the percentage of total CD8T cells (28.36±7.676vs28.60±6.49), ET (11.77±5.33 vs 10.22±3.16) and MT cell (14.48±3.79vs15.78±5.26) (p>0.05).

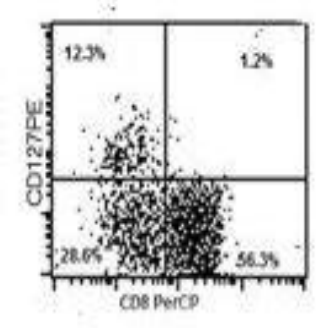


Figure (1A): Representative dot plots showing the expression of CD127 by $CD8^+$ T cells.

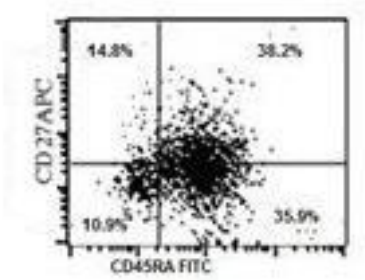


Figure (1B): Representative dot plots showing different $CD8^+$Tcells subsets**.**

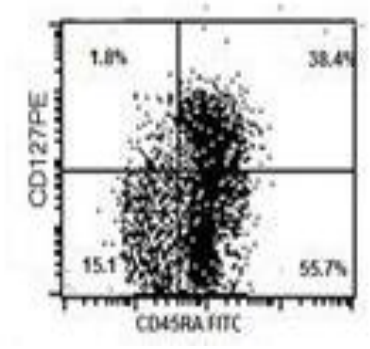


Figure (1C): Representative dot plots showing the expression of CD127 by memory T cells.

Expression of CD127 on CD8T cells and their subsets:

The percentage of expression CD127 on different CD8+ T cell subsets was measured in HCV-infected patients. A significant difference between patients and control in percentage of CD127 expression on total CD8+ T cells (31.61± .23vs 43.2±2.3) and different CD8+ T cell subsets as follow: ET(29.50±6.32 vs 48+5.38), MT(38.94±6.59 vs 48.71±6.24), TEM (41.63±2.97 vs 46.26±6.29), NT cells (7.37±2.57 vs 5.78±1.99) (figure 2).

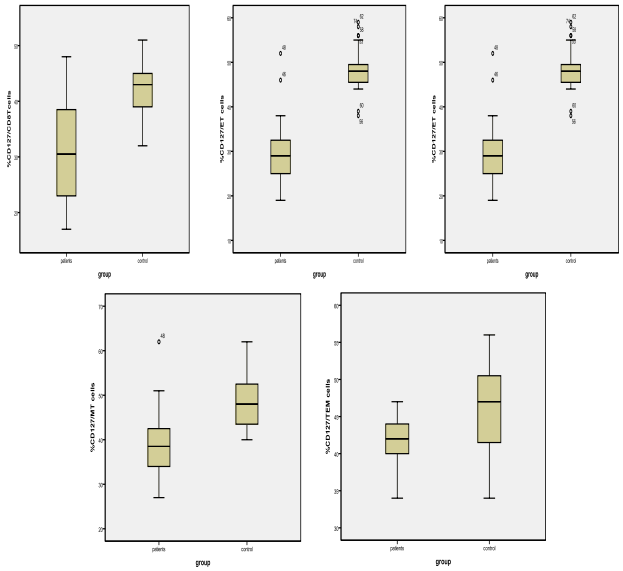
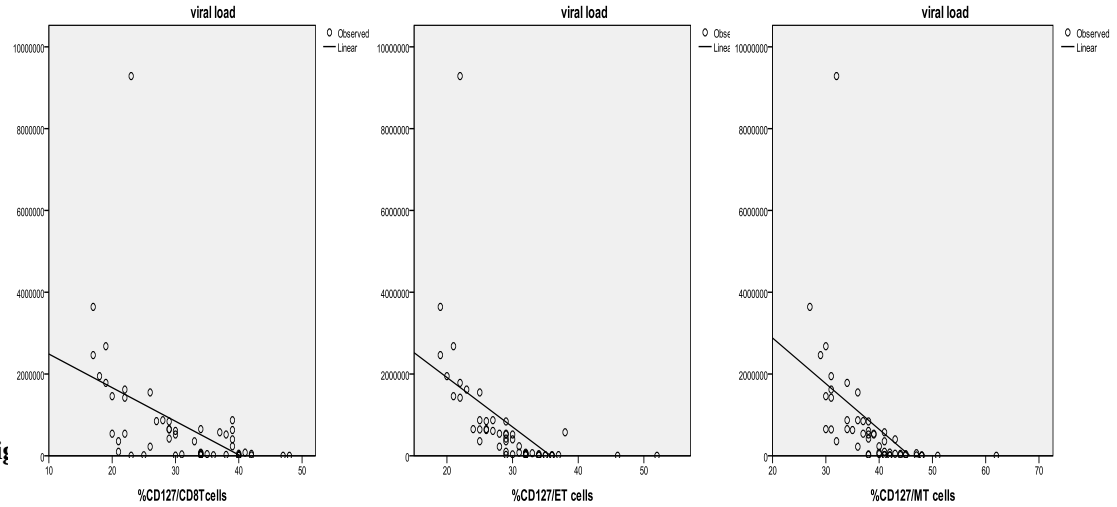


Figure (2): The percentage of CD127 expression on CD8 T cells in peripheral blood of HCV patients in comparison to control.

Correlation between the percentage of CD127expression on different CD8T cells and viral load:

No correlation was found between the percent of effector T cells or memory T cells and viral load (r=0.178 and r=0.212). Also there was negative correlation was found between viral loads and %of CD127 expression on $CD8^{+}$T cells (r=-0.437), ET(r=-0.533) and MT (r=-0 .521) and TEM cells(r=-0.344) (figure 3).



Fig

Discussion:

The response of T cells to an acute viral infection can be characterized by three phases: expansion and differentiation phase, which involves profound clonal expansion and acquisition of effector functions, contraction phase, which involves death via apoptosis of the majority of activated effectors T cells, and finally a stable memory phase, which involves the formation of a numerically stable long-lived population of memory T cells **(Williams and Bevan, 2007)**. Following priming, naïve cells can clonally expand and later differentiate into TEM cells **(Steel and Nutman, 2011)**. Now, it is known that T cells are not necessarily physically deleted under conditions of antigen persistence but can become functionally inept and incapable of elaborating the common array of effector activities typically associated with protective, effector and memory T-cell populations **(Boni et al., 2007)** .

Interleukin 7 receptor alpha (CD127) plays an important role in lymphocyte differentiation, proliferation, and survival. Signaling through this receptor is essential for T-cell development and regulation of naive and memory T-cell. Studies from both pathogenic and controlled human immunodeficiency virus infections indicate that the containment of immune activation and preservation of CD127 expression is critical for the stability of $CD4^{+}$ T cells in infection **(Kiazyk et al., 2008)**.. It's now suggested that persistent viral antigen load suppresses CD127 expression on T cells and causes exhaustion of a previously stable primed T-cell population **(Guocai et al.,2010 and Kared et al., 2014)**.

In this study no difference was found between patients and control regarding the percentage of CD8 T cells among lymphocytes. However the percentage of naïve CD8 T cells (NT cells) in HCV infection was significantly decreased in patients when compared with health control. These finding might be due to continuous antigen persistence with the resulting deletion of these cells **(Simpson, 2011)**. Percentage of terminally differentiated effector memory (TEM) and effector memory (ET) cells showed an opposite trend being increased in patients; these results may be explained by the significant decrease in the percentage of naïve CD8 T cells **(Bekker et al.,2005)**. Nevertheless it has been reported that a 5-fold increase in absolute numbers of terminally differentiated effector memory CD8 T cells in patients with chronic HCV that could be explained by the impaired function of these cells **(Freeman et al.,2006)**. During chronic infection virus-specific T cells remain $CD62L^{LOW}$ $CCR7^{LOW\ 5}$ .These T cells also have a low proliferative ability, express low amounts of IL-7 and IL-15 receptors, and fail to respond probably to these cytokines**(Shin et al., 2007)**.

All subsets of CD8T cells showed decreased expression of CD127 in particular memory and effector T cells compared with healthy controls. Also it has been reported that a decrease of CD127 expression on T cells

subsets in HCV patients due to down regulation of this surface marker by the persistent viral antigen that was restored upon successful therapy with interferon **(Larrubia et al., 2011)**.

No correlation was found between the percentage of effector T cells, memory T cells and viral load suggesting that chronic HCV might affect the function of activated CD8T cells rather than the deleting of cells . This may be clarified by the negative correlation between viral loads and % of CD127 expression on all T cell subsets **(Simpson, 2011)**.

It was noticed that antiviral therapy increased CD127 expression on CD8 memory T cells in well responder chronic hepatitis B infected patients **(Bekker et al., 2005)**. Also **Sharma et al., 2008** reported that the resolving acute viral infection with antigen withdrawal was accompanied by up-regulation of CD127 on CD8T cells . Recent studies have associated spontaneous resolution of acute HCV infection with up-regulation of CD127 **(Barathan et al., 2014)**. In contrast, other studies have demonstrated that $CD127^+$ HCV-specific $CD8^+$ T cells are detectable in persistently infected **(Radziewicz et al., 2007)**. Further studies are advised to determine if increases CD127 expression on T cells could enhance viral clearance and development of long lived central memory cells. Also more studies are required to assess if CD127 expression on T cells could be a novel marker for assessment of response to interferon therapy in HCV infected patients.

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