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T Cell Immunoglobulin Mucin (TIM)-3 Expressions on Peripheral Blood Lymphocytes from Patients with Chronic Hepatitis Virus C Infection: A Possible Role in the Pathogenesis of the Disease

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ABSTRACT

Background: T cell immunoglobulin mucin (TIM)-3 acts as a negative regulator of Th1/Tc1 cell function by triggering cell death upon interaction with its ligand, galectin-9. This negative regulatory function of TIM-3 is involved in establishing and/or maintaining a state of T cell dysfunction or "exhaustion" observed in chronic viral diseases. 25-hydroxyvitamin D (vitamin D) plays a role in decreasing the risk of many chronic illnesses, including common cancers, autoimmune diseases, infectious diseases, and cardio-vascular diseases.

Objectives: To evaluate the level of expression of TIM-3 on Peripheral Blood Mononuclear cells (PBMs) in cases of chronic hepatitis C (HCV), and to evaluate its association with vitamin D level.

Patients and Methods: In this study flow cytometric detection of expression TIM-3 was performed on PBMs of 70 chronic HCV cases. Patients were divided into compensated and decompensated groups (35 each) and 20 apparently healthy persons were included in the study as a control group. Quantitative detection of HCV RNA was performed by real time PCR and assay of serum 25-hydroxyvitamin D for all cases and controls was performed using fully automated ARCHITECT.

Results: The absolute number and percentage of lymphocytes were highest in control group followed by decompensated group and minimum in the compensated group (p<0.001). CD4% cells was higher in the compensated and control groups, than in the decompensated group (p=0.221) while CD8% was significantly higher in decompensated group compared to compensated and control groups (p=0.004), CD14%, showed significant elevation in decompensated compared to compensated and control groups (p=0.008). CD56 showed insignificant differences between the three groups (p=0.792). Increase in the percentage of TIM +ve CD4, CD8, CD14 and CD56 cells showed maximum percentage expression in the decompensated group, and least in the control group. The differences were significant regarding CD8 and CD56 (p=0.043 and 0.007 respectively) and highly significant regarding CD4 and CD14 +ve cells (p=<0.001). The mean level of 25-(OH) vitamin D was significantly lower in decompensated group compared to compensated and control group (p<0.001). There was no correlation between vitamin D and TIM-3 percentage expression in compensated and decompensated HCV patients. In all chronic HCV patients, negative correlation was encountered between vitamin D and CD4 TIM +ve% (r=–0.328, p=0.013) and strong negative correlation was obtained between vitamin D level and CD14 TIM +ve% (r=–0.518, p=0.000). No correlation was encountered between TIM-3 expression and ALT except for negative correlation between CD56 Tim-3 +ve% and ALT in decompensated group (r=–0.505, p=0.046). No correlation was found between TIM-3 expression and INR except for positive correlation with CD56 in chronic HCV patients which was good in the compensated (r= 0.560, p=0.005) and strong in the decompensated (r=0.74, p=0.001) group. In multivariate regression analysis, low vitamin D level was a significant risk factor for chronic HCV (p=0.006, OR: 1.81, 95% CI=1.18-2.78).

Conclusions: Our findings demonstrated that expression of TIM-3 + T cells on lymphocytes of chronic HCV patients is associated with sever HCV disease as levels were higher in decompensated patients than the compensated patients. Low vitamin D level may be an independent risk factor for chronic HCV infection.

Key Words: *Flow cytometry – Vitamin D – TIM-3 – Lymphocytes – Hepatitis C.*

INTRODUCTION

Hepatitis C Virus (HCV) is major causative agent of chronic hepatitis, affecting approximately 200 million people throughout the world; the majority of individuals exposed to HCV become persistently infected. A broad array of functional impairments of virus-specific T cells from early to chronic stages of infection, including exhaustion (decreased antiviral cytokine production, cytotoxicity, and proliferative capacity and arrested stages of differentiation **[1,2]**.

T cell Immunoglobulin Mucin-(TIM-3) was first identified as a molecule specifically expressed on IFN-gamma-secreting CD4 (+) T helper 1 (Th1) and CD8 (+) T cytotoxic (Tc1) cells in both mice and human. TIM-3 acts as a negative regulator of Th1/Tc1 cell function by triggering cell death upon interaction with its ligand, galectin-9. This negative regulatory function of TIM-3 has now been expanded to include its involvement in establishing and/or maintaining a state of T cell dysfunction or "exhaustion" observed in chronic viral diseases. In addition, it is now appreciated that TIM-3 has other ligands and is expressed on other cell types, where it may function differently. This supports an important role for TIM-3 in both autoimmune and chronic inflammatory diseases in human **[3]**. TIM-3 expression may play an important pathogenic role in patients with long-standing chronic **[1]**.

HCV infection vitamin D is metabolized by the liver and converted to 1, 25-dihydroxyvitamin D3, which is the active form of the vitamin. Individuals with chronic liver disease may have poor conversion from vitamin D3 or any of its other biologically active metabolites **[4]**.

In this study we aimed to determine the level of expression of TIM-3 on Peripheral Blood Mononuclear cells (PBMs) in cases of HCV during different disease stages and to clarify its possible role in the pathogenesis of the disease. We also aimed to determine the level of vitamin D concentration in different HCV disease status and to correlate it with the level of TIM-3 expression.

PATIENTS AND METHODS

The study included 70 (40 males and 30 females) chronic HCV patients, who were consecutively selected from the Inpatient Unit of the Internal Medicine Department, Sohag University Hospital, from March 2014 to July 2014. Their ages ranged from 40-70 years with a mean of 41.4±9.7 and a median of 40 years. All patients had established HCV infection, previously proved by PCR of HCV-RNA. Twenty age and gender matched apparently healthy subjects were included as a control group (12 males and 8 females); their age ranged from 43-68 with a mean of 49±12 and a median of 46 years. They had no clinical signs of liver disease as assessed by medical history and clinical examination and they were HCV antibody negative. Patients were divided into two groups: Group 1 (35 patients) included HCV antibody positive/HCV RT-PCR positive patients with normal liver functions (compensated), Group 2 (35 patients) comprised HCV antibody positive/HCV RT-PCR positive patients with abnormal liver functions (decompensated). Exclusion criteria included pregnancy and taking interferon therapy and other causes of chronic liver diseases. The study was approved by the Ethics Committee of the Faculty of Medicine, Sohag University and informed consent was obtained from all the participants.

Methods:

All subjects underwent a complete screening panel, including history taking and physical examination. Blood samples were withdrawn for routine laboratory investigations (complete blood picture, AST, ALT, Alkaline Phosphatase (ALP) albumin, Total Bilirubin (TBIL), Direct Bilirubin (DBIL), Total Protein (TP), albumin, prothrombin time and concentration and INR and abdominal ultrasound was performed. The degree of liver decompensation was evaluated using the Model of the End-stage Liver Disease (MELD) score **[5]** and Child-Turcotte-Pugh score (CPTS) **[6]**.

Anti-HCV was tested using commercially available micro particle enzyme immunoassay kits (AXSYM, Abbott Laboratories).

- Detection of HCV RNA was performed on 7500 fast real time PCR system (Applied Biosynthesis) using ready to use PCR kit supplied by artus® HCV RG RTPCR Kit 24, Version 1, catalog no. 52963, QIAGEN GmbH, Germany) **[7]**.
- Serum 25-hydroxyvitamin D test was performed using fully automated ARCHITECT instrument (Abbott Diagnostics Division, Chicago) based on Chemiluminescent Microparticle Immunoassay (CMIA). The level was measured by Abbott Architect i1000 Chemiflex device (kits manufactured by Abbott#3L52-46) **[8]**.

TIM-3 expression on various cell populations: Detection of expression of TIM-3 on different cell populations was performed using

monoclonal antibodies and analysed by flow cytometry. TIM-3 PE, CD4 FITC, CD 14 Per-CP, CD8 FITC and APC CD56 were obtained from BD Biosciences (BD Pharmingen, USA) and from BioLegend, USA. Dual staining was performed to detect TIM-3 expression on each cell population. PBMCs were stained in the dark for 15 minutes at room temperature (20-25ºC). The cells were washed twice with the washing solution and re-suspended in 0.5ml PBS. Stained cells were analysed by flow cytometry within 3 hours of staining using FACS Calibre FCM (Becton Dickinson) flow cytometry and Cell Quest software. Lymphocytes were gated on a dot plot was created for CD4 versus TIM-3, CD8 versus TIM-3, CD56 versus TIM-3 and CD14 versus TIM-3 **[9]**. The percentage of cells of the lineage+/TIM-3 was used for statistical analysis of the data.

Statistical analysis:

SPSS version 19.0 was used for statistical analysis. Data were summarized as mean ± SD, range or median. Student *t*-test was used to compare the means between two groups, and one-way analysis of variance (ANOVA) test was used to compare means of more than two groups. Multivariate and multivariate regression analysis was used to examine the role of TIM-3 positive cells and vitamin D as risk factors. *p*-value <0.05 was considered significant.

RESULTS

Laboratory variables of the studied groups are demonstrated in (Table 1).

The mean level of HCV-RNA in compensated group was insignificantly lower than in the decompensated group (144.906±479.509 vs. 162.59±308.7926, p=0.89) TIM-3 expression on different lymphocytes subsets in chronic HCV patients is presented in (Table 2). The absolute numbers and the percentage of lymphocytes are percentage and absolute lymphocyte count were significantly higher in control group followed by the decompensated group and lowest in the compensated group (p<0.001). CD4% cells was higher in the compensated and control groups, than in the decompensated group, with in significant difference (p=0.221) while CD8% was significantly higher in decompensated group compared to compensated and control groups (p=0.004). CD14% showed significant elevation in decompensated compared to compensated and control groups (p=0.008). CD56 showed insignificant differences between the three groups (p=0.792). TIM-3 CD4, CD8, CD14 and CD 56% were highest among the decompensated group, and least among the control group. The differences were significant regarding CD8 and CD56 (p=0.043 and 0.007 respectively) and highly significant regarding CD4 and CD14 cells (p=<0.001).

The mean level of 25-(OH) Vitamin D was significantly lower in decompensated compared to compensated and control groups (p<0.001), (Table 1).

No correlation was encountered between TIM-3 expression and ALT except for negative correlation with CD56 TIM-3 +ve% in the decompensated group (r=–0.505, p=0.046). Also, no correlation was encountered between TIM-3 expression on all lymphocyte subsets and serum albumin or bilirubin (Table 3).

No correlation was found between TIM-3 expression and INR except for positive correlation with CD56 TIM-3 +ve% in chronic HCV patients group which was good in the compensated (r=0.560, p=0.005) and strong in the decompensated group (r=0.74, p=0.001; Figs. (1,2).

There was no correlation between vitamin D and TIM-3 percentage expression in compensated and decompensated HCV patients (Table 4). In all chronic HCV patients (n=70), negative correlation was encountered between vitamin D level and CD4 TIM-3 +ve% (r=–0.328, p= 0.013) and good negative correlation between Vitamin D and CD14 TIM-3 +ve% (r=–0.518, p=0.000).

In multivariate regression analysis, low vitamin D level in HCV cases was found to be a significant risk factor for development of chronic HCV (p=0.006, OR: 1.81, 95% CI=1.18-2.78).

Univariate regression analysis showed that low vitamin D level is a highly significant risk factor in chronic HCV cases (p<0.001 OR=0.58, 95% CI: 0.425-0.793), followed by CD14 TIM-3 positive cells (p=0.003, OR=1.04, 95% CI: 1.016-1.079), then CD4 TIM-3 positive cells (p=0.022, OR=1.411, 95% CI: 1.051-1.895).

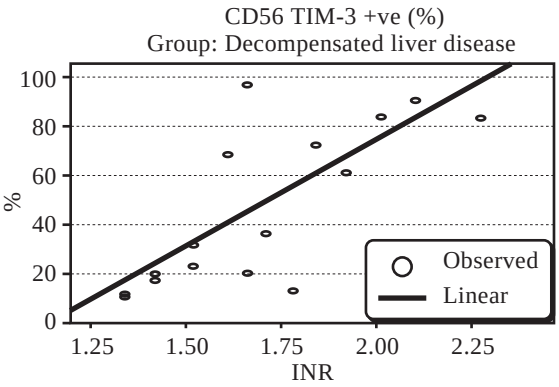

Fig. (1): Correlation between INR and CD56 TIM-3 positive cells in decompensated chronic HCV patients.

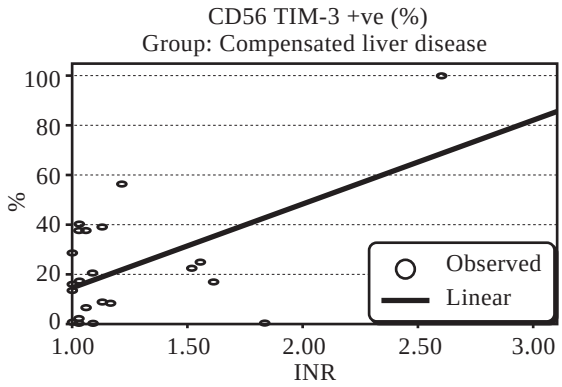

Fig. (2): Correlation between CD56 TIM-3 positive cells and INR in compensated chronic HCV patients.

Table (1): Laboratory variables of the studied groups.

Parameter	Chronic HCV n=70		Control n=20 (Group III)	*p*-value		
	Decompensated n=35 (Group I)	Compensated n=35 (Group II)		I vs. III	II vs. III	I vs. II
PLT: 10^9/L	69.28±17.53	173.9±78.01	276±82.4	<0.001	<0.001	<0.001
HB: gm/dL	8.47±0.96	16.28±5.13	13.33±1.25	<0.001	0.030	<0.001
WBC: 10^9/L	4.94±1.46	7.026±2.43	8.13±1.72	<0.001	0.126	<0.001
INR	1.70±0.27	1.28±0.38	1.02±0.03	<0.001	0.034	<0.001
PC: %	44.28±9.35	79.78±22.95	97.5±5.77	<0.001	0.005	<0.001
PT: Seconds	19.58±2.97	14.30±4.46	11.86±0.33	<0.001	0.036	0.003
IBIL: mg/dL	1.68±0.79	0.85±0.97	0.36±0.12	<0.001	0.037	0.002
DBIL: mg/dL	1.69±0.83	0.70±1.09	0.19±0.08	<0.001	0.068	0.003
TBIL: mg/dL	3.37±1.54	1.54±1.94	0.54±0.17	<0.001	0.049	<0.001
TP: g/Dl	6.56±0.789	7.421±0.915	7.74±0.26	<0.001	0.189	<0.001
ALB: g/dL	2.35±0.504	3.726±0.931	4.86±0.41	<0.001	<0.001	<0.001
SGPT: U/L	70.56±38.89	33.087±27.09	21.06±5.62	<0.001	0.090	<0.001
SGOT: U/L	93.5±40.93	33.347±26.72	17.31±7.47	<0.001	0.025	<0.001
Vit D: ng/mL	10.83±3.79	14.43±3.41	22.84±6.07	<0.001	<0.001	0.003

PLT : Platelets.
HB : Hemoglobin.
WBCs : White Blood Cells.
INR : International Normalized ratio.
PC : Prothrombin Concentration.
PT : Prothrombin Time.
IBIL : Indirect Bilirubin.
DBIL : Direct Bilirubin.
TBIL : Total Bilirubin.
TP : Total Protein.
ALB : Albumin.
SGPT : Serum Glutamic-Pyruvic Transaminase.
SGOT : Serum Glutamic Oxaloacetic Transaminase and Vit D:25 (OH) hydroxyl vitamin D.

Table (2): Lymphocyte subsets in chronic HCV patients and control.

Parameter	Group I n=35	Group II n=35	Group III n=20	*p*-value	I vs. III	II vs. III	I vs. II
TLC X 10^9/L	26.72±93.87	19.19±84.14	29.1±10.65	0.004	0.512	0.001	0.001
Lymphocyte	28.87±8.95*	19.19±8.41	31.49±9.57	<0.001	0.416	<0.001	0.001
CD4%	32.13±14.32	38.56±12.02	37.17±8.21	0.221	0.225	0.691	0.126
CD4/TIM-3 +	16.61±16.82	5.78±5.3	3.12±2.13	<0.001	0.003	0.066	0.016
CD8%	36.67±29.12	17.95±9.07	20.82±5.79	0.004	0.042	0.272	0.02
CD8/TIM-3 +	27.52±30.86	17.82±10.16	11.18±4.18	0.043	0.046	0.008	0.228
CD14%	7.76±3.27	9.51±3.99	5.99±2.17	0.008	0.077	0.001	0.149
CD14/TIM-3 +	79.09±17.26	55.55±16.19	42.09±26.3	<0.001	<0.001	0.08	<0.001
CD56%	0.5±0.34	0.45±0.5	0.54±0.33	0.792	0.732	0.5	0.724
CD56/TIM-3 +	46.26±31.92	22.18±22.94	21.36±18.3	0.007	0.013	0.93	0.016

Group I : Decompensated.
Group II : Compensated.
Group III : Control.
* : Percent.
CD : Cluster of Differentiation.
TIM-3 : T-cell immunoglobulin domain and mucin domain 3.
TLC : Total Lymphocyte Count.
$p<0.05$ is significant.
$p<0.001$ is highly significant.

Table (3): Correlation between TIM-3 +ve expression on lymphocytes subsets and ALT in HCV patients and control.

Parameter	Controls	Chronic HCV patients	
		Com-pensated	Decom-pensated
CD4 TIM3 +ve (%):			
r	0.071	–0.175	–0.245
p	0.794	0.425	0.328
CD8 TIM3 +ve (%):			
r	0.169	–0.065	–0.457
p	0.532	0.767	0.065
CD14 TIM3 +ve (%):			
r	0.203	0.117	–0.281
p	0.451	0.594	0.274
CD56 TIM3 +ve (%):			
r	–0.215	–0.113	–0.505
p	0.442	0.607	0.046

Table (4): Correlation between vitamin D and TIM-3 +ve expression on lymphocytes subsets in chronic HCV patients and control.

Parameter	All subjects	Control	Chronic HCV patients	
			Com-pensated	Decom-pensated
CD4 TIM-3 +ve (%):				
r	–0.328	0.070	–0.259	–0.018
p	0.013	0.797	0.233	0.945
CD8 TIM-3 +ve (%):				
r	–0.168	0.072	–0.094	0.243
p	0.217	0.791	0.668	0.347
CD14 TIM-3 +ve (%):				
r	–0.518	–0.364	–0.027	–0.095
p	0.000	0.165	0.904	0.717
CD56 TIM-3 +ve (%):				
r	–0.212	0.233	–0.101	–0.085
p	0.123	0.403	0.647	0.755

CD : Cluster of Differentiation.
TIM-3 : T-cell immunoglobulin domain and mucin domain 3.
r : Pearson correlation.
p : Probability value.

DISCUSSION

T-cell Immunoglobulin and Mucin domain-containing molecule 3 (TIM-3) plays an important role in regulating T cells in chronic hepatitis C virus infection and Hepatocellular Carcinoma (HCC).

Our study included 90 subjects (70 HCV +ve patients and 20 normal controls) and the HCV infected patients were subdivided into two groups; patients with compensated and patients with decompensated liver functions, and each subgroup involved 35 patients.

TIM-3 is expressed on only a very small percentage of CD4+ or CD8+ T cells, and its over-expression may indicate T cell exhaustion and represent a pathological immune state. However, innate immune cells including monocytes, macrophages and DCs show constitutive and high-level TIM-3 expression that can be further elevated in some diseases. Gerlach et al., **[11]** found that in patients with acute HCV infection if the T-helper (CD4+) immune response was weak, less efficient or not maintained for a sufficient length of time, patients would proceed to persistent infection and chronic hepatitis. Furthermore, Hoffman et al., **[12]** found that HCV-RNA positive individuals without clinical or histopathologic evidences of liver disease had a statistically significantly higher CD4+ proliferative response to the HCV core protein than patients with chronic hepatitis, suggesting a protective role of CD4+ cells against hepatocellular damage. Bowen and Walker **[13]** evidently showed the role of both CD4+ and CD8+ T cells in HCV viral clearance. Strong and sustained CD4+ and CD8+ T cell responses together lead to the resolution of HCV. Also, DCs play a major role by processing and presenting the antigens on MHC-I and MHC-II molecules and providing optimum stimulation to CD8+ and CD4+ T cells, respectively **[14]**.

Tim molecules are expressed on T cells, monocytes, and antigen-presenting cells, including macrophages and dendritic cells **[15]**. TIM-3 has been shown to be involved in the suppression of T-cell effector function in chronic HIV infection, defining a population of exhausted T cells that is distinct from the PD-1-expressing population **[16]**.

Mason et al., **[1]** stated that TIM-3 expression may play an important pathogenic role in patients with long standing chronic HCV infection.

In our study, CD8-TIM-3 positive cells were significantly higher in decompensated group compared to compensated and control groups (p=0.004). Similar results were found by McMahan et al., **[2]** who reported that TIM-3 expression by both CD4 and CD8 increases significantly in HCV infected patients, compared to control (p=0.0047 and (p=0.0002 respectively), regardless of the liver functions. They, also, demonstrated that early accumulation of PD-1+ TIM-3+ T cells is associated with functional impairment, and consequently with development of persistent HCV.

Our results are consistent with Mason et al., **[1]** who examined the expression of Tim-3 by flow cytometry on PBMCs from 42 patients with persistent hepatitis C viremia and 10 normal controls. They found that, chronic HCV infection is associated with elevated frequencies of TIM-3-expressing CD4+ and CD8+ T cells relative to those uninfected with the highest expression on HCV-specific cytotoxic T lymphocytes (CTLs) (p=0.02 and 0.008 respectively).

In the current study, CD14 cells were maximum in the compensated group, followed by decompensated and minimum in the control group, again with a significant difference (p= .0.008).

CD56 showed insignificant differences between the three groups. There was steady increase in the percentage of TIM-3 +ve CD4, CD8, CD14 and CD56 cells, with maximum percentages among the decompensated liver disease group, and least percentage among the control group. This is consistent with a study reported by Vali et al., **[17]** which revealed that HCV-specific T cells in HCV/HIV co-infection show elevated frequencies of dual TIM-3/PD-1 expression that correlate with liver disease progression.

In the current study, no correlation was encountered between TIM-3 expression and ALT except for negative correlation with CD56/ TIM-3 +ve% in decompensated group (r= –0.505, p=0.046). Also, no correlation was encountered between TIM-3 expression on all lymphocyte subsets and serum albumin or bilirubin. No correlation was found between TIM-3 expression and INR except for good positive correlation with CD56 TIM-3+ve% chronic HCV group which was good in the compensated (r=0.560, p=0.005) and strong in the decompensated (r=0.74, p=.0.001) group. This is contrary to Wu et al., **[18]** findings who studied TIM-3 in regulating the antiviral CD8+ T-cell response in Chronic Hepatitis B (CHB) patients. TIM-3 expression on peripheral virus-specific CD8+ T cells from 20 CHB patients and 20 healthy controls was determined by flow cytometry. They reported that TIM-3 expression may also indicate the severity of liver injury because its expression was markedly and positively correlated with ALT, AST, INR and TB. TIM-3 expression correlated with ALT levels (a surrogate marker for hepatic injury) suggesting that TIM-3 expression is more likely a feedback response to prevent over-activation of the host immune system than a viral strategy to counteract immune attack. This hypothesis was also supported by their previous study which showed that TIM-3 expression levels returned to normal after attenuation of liver inflammation **[19]**. In a study done by Rong et al., **[20]** TIM-3 3+CD14+ cells and TIM-3 3+CD3+CD16/CD56+ cells were analyzed by flow cytometry. Results showed that expression of TIM-3 was significantly increased on both the monocytes and NKT-like cells in CHB patients than in controls (p=0.002 and p<0.001, respectively). TIM-3 levels on monocytes and NKT-like cells were further upregulated in patients with acute liver failure. TIM-3 expression on both monocytes and NKT-like cells was positively correlated with level of ALT (r=0.59, p<0.001, and r=0.60, p<0.001). Mason et al., **[21]** analysed TIM-3 expression on NKs in chronic HCV-infected subjects, demonstrating not only elevated expression of TIM-3 on these cells but also a positive correlation between TIM-3 and NK activity; this suggests that TIM-3 on NKs is associated with activation of this innate lymphocyte population that is polarized towards cytotoxicity in chronic HCV. These findings reveal roles for TIM-3 in the regulation of NKs that might represent targets for treatment of chronic viral infections.

Vitamin D is metabolized by the liver and converted to 1,25-dihydroxyvitamin D3, which is the active form of the vitamin. Individuals with chronic liver disease may have poor con-

version from vitamin D3 or any of its other biologically active metabolites **[22]**.

In the present work, vitamin D was significantly lower in decompensated group ($p<0.001$). Severe liver disease may increase the risk of vitamin D deficiency and/or there might be a relationship between vitamin D deficiency and fibrosis. DeLuca **[23]** and Arteh et al., **[24]** also showed that low levels of 25 (OH) D are associated with fibrosis and suggested that low 25 (OH) D levels may predict hepatic decompensation and mortality in patients with chronic liver failure.

In the current study, there was no correlation between vitamin D and TIM-3 percentage expression in compensated and decompensated HCV patients. In all chronic HCV patients (n=70), negative correlation was encountered between vitamin D and CD4 TIM3 +ve% (r= –0.168, p=0.013) and strong negative correlation between vitamin D and CD14 TIM3 +ve% (r= –0.518 and p=0.000). In this work, low vitamin D level in HCV cases was found to be a significant risk factor for chronic HCV. Vitamin D has been shown to be a key regulatory element of the immune system, and its serum concentrations correlate with the severity of liver damage and the development of liver fibrosis/ cirrhosis. Furthermore, supplementation with vitamin D could be beneficial in increasing the response rate to Peg-INF-α based therapy in CHC patients **[25]**.

During chronic HCV infection, TIM-3 expression on CD4+ and CD8+ T cells is elevated, and these TIM-3+ T cells exhibit a CD 127 low CD 57 high and CD45RACC R high phenotypes, indicating the impaired function of these effector cells. Accordingly, blockade of TIM-3 expression enhances cell proliferation and promotes cytokine production **[1]**.

In conclusion, our study showed that accumulation of TIM-3+ T cells is associated with development of persistent HCV. TIM-3 expression may also indicate the severity of liver injury because its expression has been found to be higher in decompensated than compensated HCV patients. Adequate vitamin D level is a protective factor for development of CHC. The correlation between TIM-3 expression and vitamin D concentration will be critical for exploiting the therapeutic potential of TIM-3 and vitamin D for the treatment and follow-up of the disease.

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