CD 160 molecules and IL28 B genotypes in diabetic and non diabetic chronic hepatitis C patients

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Abstract Background and Aim: Despite the close relationship between hepatitis C viral infection (HCV) and diabetes mellitus type 2 (DM2), the underlying mechanism that links diabetes and HCV infection remains conjectural. During chronic hepatitis C disease, key inhibitory molecules including CD160 are up-regulated with different kinetics on CD8 cytotoxic T lymphocytes. There are two different opinions regarding the function of CD160 molecules, the first opinion suggested that the expression of CD160 on CD8 cytotoxic T cell contribute to the exhaustion and failure of immune responses, whereas the other opinion defined CD160 molecule as modulators and regulators of immune responses. Thus, we aimed to assess the pattern of expression and the role of CD160 molecules in the development and controlling of HCV associated diabetes. Also, a little of known about the genetic control of CD160 signaling pathway between T lymphocytes and antigen presenting cells. Recently, IL-28B gene polymorphisms were identified as a genetic predictor for progression of HCV and the effectiveness of antiviral therapy. IL28B polymorphisms include C/C, C/T and T/T genotypes, C/C genotype is considered as a good predictor of disease progression and therapy responses while T alleles groups were associated with bad prognosis and less response to therapy. We aimed to clarify the link between a triad, CD160 molecules, T lymphocytes immune responses and IL28B genotypes during HCV associated diabetes. **Patients & Methods:** This cross sectional study was conducted at Al-Zahraa University Hospital from June 2011 to January 2012. An informed consent was obtained from patients. The study included fourteen diabetic chronic hepatitis C (HCV) patients, fourteen non-diabetic HCV patients (without decompensated liver or hepatocellular carcinoma) and twelve healthy control subjects. The selection of HCV patients depends upon seropositive anti-HCV confirmed by positive HCV-RNA-PCR. Also, the exclusion of patients with decompensated liver disease or hepatocellular carcinoma was achieved via questionnaire, clinical examination, pelviabdominal ultrasonography and routine laboratory investigations. The diabetic HCV patients were identified in the presence of documented hypoglycemic medications or fasting blood sugar greater than 140 mg/dL on two occasions. Using flow cytometry, the patients and control groups were investigated for the expression of CD160 molecules on CD8 T lymphocytes. Using real time PCR, the diabetic and non-diabetic HCV patients were evaluated for the frequency of IL-28B genotypes. In addition, the diabetic HCV patients were estimated for glycosylated hemoglobin (Hb A1C) using high performance liquid chromatography (HPLC). **Results*:*** The diabetic and non-diabetic HCV patients showed statistically significant increase in CD160% as matched to control group. Importantly, theCD8 cytotoxic T cells of diabetic HCV group displayed higher expression of CD160% as compared to non-diabetic group. Also, we observed significant negative correlation between the frequency of CD160 molecules and the mean value of HbA1C in diabetic HCV group. As regard IL-28B polymorphisms, our result showed higher frequency of C/C genotype in diabetic HCV patients, whereas non-diabetic HCV group showed higher frequency of C/T genotype. Interestingly, our data showed an association of favorable genotype C/C with higher frequency of CD160 molecules as matched to both T alleles genotypes groups. Conclusion: we concluded that CD 160 molecules up regulation on cytotoxic CD8 T cells occur as a consequence of exaggerated immune responses and vigorous secretion of proinflammatory cytokines. Also, we clarified that the over expression of CD160 in tandem with IL28B C/C genotype is considered as a good predictor of disease progression and antiviral treatment response. In addition, we suggested the involvement of IL28 B gene in controlling T cell immune responses including CD160 signaling via HLA class-1 gene. Importantly, we confirmed the modulatory and regulatory role of CD160 molecules in controlling of HCV associated diabetes.

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1. Introduction

Hepatitis C viral infection (HCV) is common problem worldwide, affecting millions of people across all populations ***(Elawady et al., 2012)***. The highest HCV prevalence in the world occurs in Egypt at an estimated 12% among the general populations ***(Elhawary et al., 2011)***. About 30% of HCV patients are able to eliminate the virus spontaneously while the other 70% of patients develop chronic infection ***(Bengsch et al., 2007)***. Chronic HCV is specifically

and frequently associated with diabetes mellitus type 2 regardless of liver cirrhosis, it is not well known whether HCV infection leads to diabetes or vice versa ***(Antonelli et al., 2005)***. Although the initial associations between diabetes and HCV was seen in advanced liver disease, more recent reports have described an increased frequency of diabetes mellitus type 2 during inflammatory phase of HCV before development of cirrhosis ***(Elhawary et al., 2011)***.

Type 2 diabetes is a multi-system disorder, the pathophysiology of HCV associated diabetes include defect in insulin secretion, increased hepatic tumor necrosis factor alpha, excessive hepatic glucose production and insulin resistance . All of which in addition to predisposing factors such as obesity; aging and family history contribute to the development of diabetes during the course of chronic HCV ***(Costantini et al., 2012)***.

Recently, the immunogenetic pathogenesis of HCV associated diabetes have been studied, the immunological mechanisms proposed for the development of diabetes mellitus during chronic HCV infection are generally based on cytokines / CD8 T lymphocytes dependant pathways ***(Bengsch et al., 2010)***.

HCV infection is widely known to cause generalized immune activation and subsequent massive release of pro-inflammatory cytokines ultimately leading to HCV progression and developing of extra-hepatic complications such as type 2 diabetes mellitus ***(Donelly, 2012)***. Several studies have suggested over the years that the accumulation of inhibitory signaling proteins including CD160 on the surface of CD8 T lymphocytes is linked to regulation of cytokines dependant mechanisms and modulation of immune responses ***(Rio et al., 2010)***. CD160, glycophosphatidyl- inositol anchored protein was identified as modulator molecule, the interaction between CD160 molecules and their natural legends restored virus specific T cell proliferation and cytokines production ***(Baitsch et al., 2012)***. The anti-inflammatory, anti-angiogenic and regulatory role of CD160 molecules have been suggested during the course of chronic hepatitis C disease, but the mechanism that determine the role of these molecules in the pathogenesis of HCV associated diabetes have been difficult to decipher ***(Raziorrough et al., 2011 , Ciuffreda and Kim., 2012)***.

It is not clear whether the CD160 molecules act as motivation factor for the development of diabetes or it occur as consequence of inflammatory phase of chronic hepatitis C and developed DM. Also, little information is currently available about the involvement of genetic factors in controlling CD160 inhibitory signaling pathway ***(Peretz et al., 2012)***. Recently, genome wide study defined single nucleotide encode type III interferon-λ3 as IL28B gene , it has been established the potential predictive role of IL28B gene polymorphisms for the progression of HCV and antiviral therapy response .The IL28B polymorphisms include C/C ,C/T and T/T genotypes ***(Friedrich et al., 2012)***. It is not well understood to what degree and by what mechanism IL28B genotypes contributes to CD160 signaling pathway as a part of natural homeostatic immune response.

Therefore, in our study, we aimed to determine the expression pattern and the function of CD160 molecules during the course of HCV associated type 2 diabetes mellitus. Also, we tried to clarify the link between CD160 molecules and IL28B polymorphism as regard progression of the disease and the effectiveness of treatment.

2. Patients & Methods

Our study comprised fourteen diabetic HCV patients, fourteen non-diabetic HCV patients with age ranged from 36 to 57 years and twelve healthy control adult subjects. Hepatitis C viral Infection patients were diagnosed in the presence of seropositive HCV antibodies followed by HCV-RNA-PCR for confirmation of diagnosis.

All patients were subjected to the following:

A. Questionnaire (to screen the history of disease, complains, history of hematemsis or melana and medications history.
B. General clinical examination for jaundice, splenomegaly or ascites.
C. Abdominal ultrasonography.
D. Routine laboratory investigations which include fasting blood sugar, liver function, kidney functions, α feto protein, complete blood picture and prothrombine time.

The patients with decompensated liver disease or hepatocellular carcinoma or renal failure were excluded from our study.

According to the questionnaire, clinical examination and laboratory investigations, the presumptive diagnosis of decompensated liver was achieved in the presence of one of the following , history of hematemsis and or melena , ascites, reversed albumin/globulin ratio , hematological evidence of hypersplenism (pancytopenia or thrombocytopenia) or coagulopathy in the form of low prothrombine concentration (Lai *et al.*, 2011).

The diagnosis of hepatocellular carcinoma was achieved by markedly increased α feto-protein, computed tomography (CT) and magnetic resonance imaging (MRI).

The HCV patients were assigned a diagnosis of diabetes if there was documented use of

hypoglycemic medications or fasting blood sugar greater than 140 mg/dL on two occasions.

The diabetic and non-diabetic HCV patients as well as control group were evaluated for the expression of CD160 molecules on CD8 T lymphocyte using flow cytometry.

The patients groups (diabetic and non-diabetic HCV groups) were assessed for the frequency of IL-28B genotypes using real time PCR technique. In addition, the diabetic HCV patients were estimated for Hb A1C.

Sampling: Venous blood sample were taken in three EDTA vaccutainers. One vaccutainer for immunophenotyping , the second for IL28B genotyping by real time PCR and the third tube for HbA1C.

Immunophenotyping:

We used one test tube for each sample. Each tube contain 20 microliteres of conjugated phycoerythrin (PE) labeled CD160 monoclonal antibodies , 100 microliteres of the test samples was add to each tube, vortex the tubes gently.

The tubes were incubated 20 minutes at room temperature in the dark, then add 1 mL of fix and lyse mixture, vortex the tubes immediately for one second and incubated again for 10 minutes in the dark at room temperature followed by centrifugation of tubes at low speed for 5 minutes, aspiration of supernatant and resuspension of pellet in residual fluid. 2 mL of phosphate buffer saline was add to each tube, the suspension was centrifuged at low speed. The supernatant was discarded, then the residual suspension were passed through the flow-cytometer.

For assessment of CD160 expression on CD8, CD8 lymphocytes were gated.

Detection of IL-28B polymorphism:

A. Peripheral blood on EDTA was withdrawn from chronic HCV patients with and without diabetes.
B. Genomic DNA was extracted by Roche diagnostics (high pure PCR template preparation kit).

C. Preparation of specific reagents mix:

- One reagent vial with red cap contains primers and probes to run 32 light cycler reactions.
- Add 66 μL PCR – grade water to each reagent vial; mix the solution (Vortex).

D. Preparation of control DNA:

- Add 80μL PCR grade water to each colorless cap vial.
- Mix the target DNA by pipetting the solution up and down 10 times (final concentration 10^5 target molecules in 5μL use 5 μL control DNA for 20 μL PCR reagent.

E. Preparation of light cycler reaction mix:

- The reaction mix for each sample (single reaction mix)consists of the following:

1. 9.4 μL of water PCR grade (colorless cap vial, provided with the Roch master kit).
2. 1.6 μL magnesium solution (blue cap vial).
3. 2.0 μL reagent mix (parameter specific reagent containing primers and probes).
4. 2.0 μL Roch master specific reagent (enzymes) .

To calculate the total volume of reaction mix (total volume needed for all samples and control), we multiply the volume of each content by the number of samples and control.

In a cooled reaction tube, the total volume of reaction mix was prepared by gentle mix and spin down.

. Transfer 15 μL of reaction mix for each sample to Light cycler capillary.

. Add 5 μL from the DNA of each sample or control DNA to each capillary wells (containing 15μL reaction mix), then start run.

N.B:

- Negative control (template DNA was replaced by water).
- Positive control contains provided control DNA.

F. Light cycler programming:

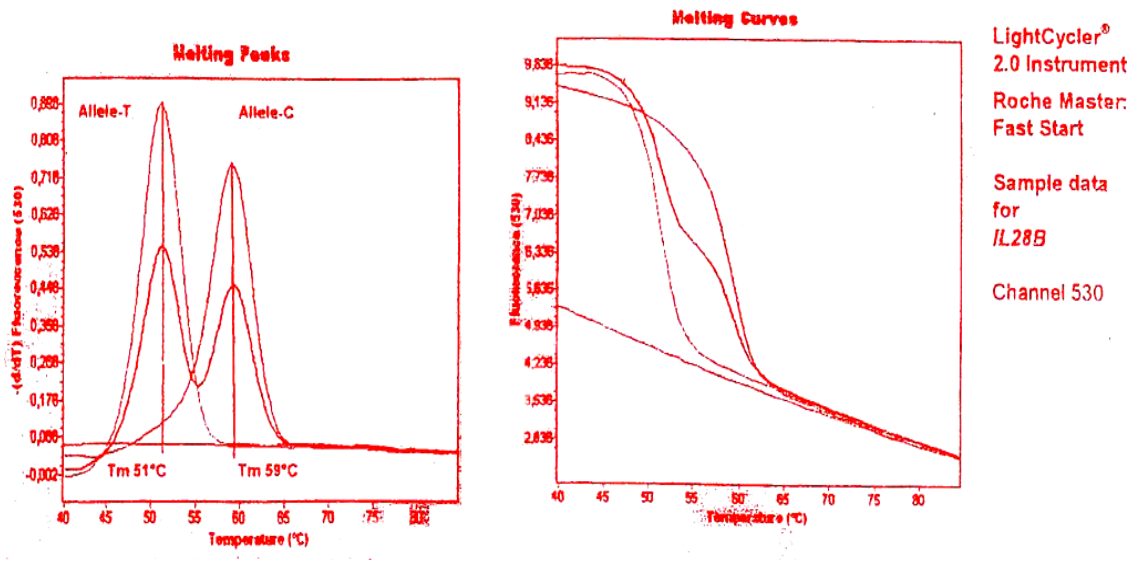
1. Denaturation: sample denaturation and enzyme activation.
2. Cycling: PCR amplification of the target DNA.
3. Melting curve analysis for identification of PCR product derived from the target DNA.
4. Cooling the instrument.

G. Data analysis:

- View IL-28B data with filter combination "Tm Calling" analysis mode. The negative control (NTC) must show no signal.

H. Inter-Pretation of Data:

- Homozygous T/T variant: Less response to therapy.
- Heterozygous C/T variant: Less response to therapy.
- Homozygous C/C variant: Higher probability of viral clearance and good response to therapy.



3. Results

Fourteen diabetic chronic HCV patients, fourteen non-diabetic HCV patients and twelve healthy control subjects were included in our study.

Table (1) shows the routine laboratory findings of diabetic and non-diabetic HCV patients: fasting blood sugar, serum bilirubine, SGPT, white blood cells and platelets counts were significantly higher in diabetic HCV group as matched with non-diabetic HCV group (*P*: <0.001 , 0.05 , <0.01 , <0.02 and <0.05, respectively).

Hemoglobin values, prothrombine concentration and albumin / globulin ratio were not statistically differing between both patients groups.

To assess the expression pattern of CD160 molecules on CD8 T lymphocytes of diabetic and non diabetic HCV patients, we compared the mean percentage of CD160 molecules between the three studied groups.

Table (2) shows the mean percentage of CD160 in both diabetic and non-diabetic HCV patients respect to controls. Our data showed statistically significant increase in CD160% of both diabetic and non-diabetic HCV patients groups as matched with control (*P*: <0.001 & <0.04, respectively).

Importantly, diabetic HCV group showed statistically significant higher CD160% as compared with non-diabetic HCV (*P*<0.05).

In addition, figures, 2a and 2 b show the frequency of CD160 molecules in non diabetic and diabetic HCV patients.

To evaluate the role of CD160 molecules in controlling the progression of diabetes during chronic hepatitis C, we correlate the mean percentage of CD160 of diabetic HCV group with the mean value of glycated hemoglobin.

Significant negative correlation between the two parameters was observed (*P* = 0.002) (Table and figure 3).

As regard IL-28B gene polymorphisms, table and figure (4) show the frequency of different IL-28B genotypes (homozygous C/C, heterozygous C/T and homozygous T/T) in both patients groups.

The diabetic HCV group showed higher frequency of C/C genotype as matched with non-diabetic HCV group (50% versus 20%, *P* <0.002).

On the other hand, the non-diabetic HCV group was associated with higher frequency of C/T genotype as compared with diabetic HCV group (60% versus 25%, *P* <0.001).

As regard T/T genotype, no significant difference between both patients groups, was observed (*P* =0.1).

In an attempt to find relationship between IL-28B gene polymorphism and CD160 molecules, we categorized the diabetic HCV patients into three groups according to their genotypes, C/C group, C/T group and T/T group.

Table and figure (5) showed that C/C genotype group was associated with higher percentage of CD160 molecules as matched with both C/T and T/T groups (*P* <0.012, *P* <0.002, respectively).

On the other hand no statistically difference between both T alleles group (C/T vs T/T) regarding the frequency of CD160 molecules (*P* = 0.5).

Table (1):Routine laboratory findings of diabetic and non-diabetic HCV cases.

	Non-diabetic HCV	Diabetic HCV	*P*-value
Fasting blood sugar (mg/dL)	97 ± 21	272 ± 81	<0.001
Serum bilirubine (mg/dL)	1.3 ± 0.6	1.8 ± 0.45	0.05
SGPT (U/l)	56 ± 14	91 ± 11	<0.01
Albumin/globulin (A/G) ratio	1.6 ± 0.54	1.75 ± 0.27	0.6
Hemoglobin (g/dL)	11 ± 3.6	10 ± 2.8	0.4
White blood cells (/mm^3)	6.9 ± 2.7	9.9 ± 4.5	<0.02
Platelet (/mm^3)	159 ± 14	190 ± 18	<0.05
Prothrombin concentration (%)	78 ± 15	80 ± 18	0.7

Table (2): Comparison between the studied groups as regard CD160%.

	Mean(CD160%)	±SD	Min.	Max.		
Control group	14.41%	±1.43	12.7%	21.6%		
Non-diabetes HCV	25.19%	±8.32	17.8%	33.8%		
Diabetic HCV	48.14%	±24.26	27.4%	70.9%		
LSD tests						
Control versus non-diabetes HCV		**Control versus diabetic HCV**		**HCV without diabetes versus HCV with diabetes**		
<0.04		<0.001		< 0.05		

Figure (2a) **Figure (2b)**

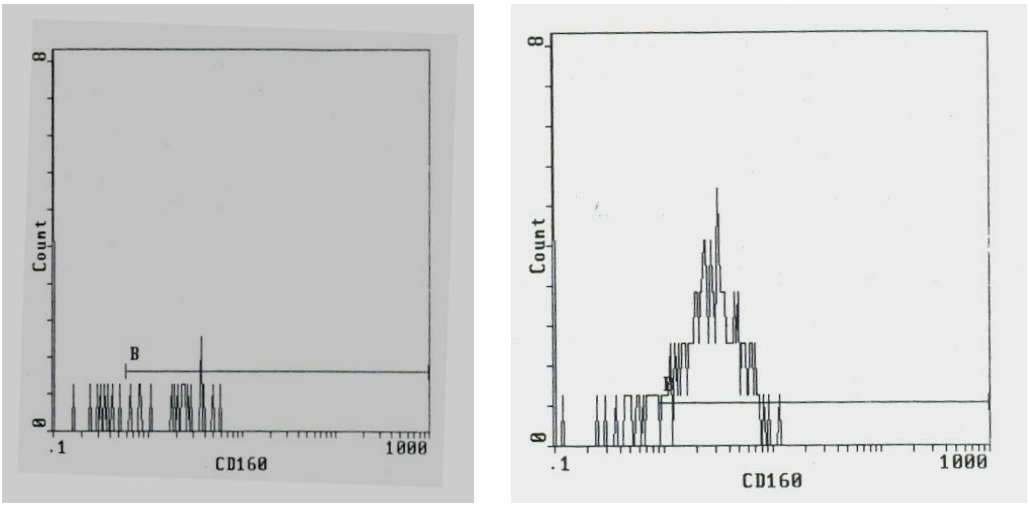


Figure (2a) : The frequency of CD 160 + cytotoxic T lymphocytes in non diabetic HCV group
Figure (2b) : The frequency of CD 160 + cytotoxic T lymphocytes in diabetic HCV group .

Table and figure (3): Correlation between CD160% and glycated hemoglobin (HbA1/C).

	CD160%	
	R	***p*-value**
HbA1/C	0.544**	0.002

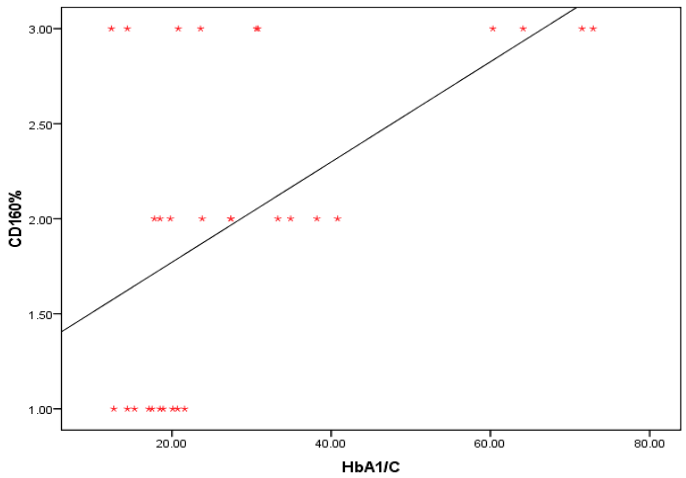


Figure (3)

Table and figure (4): Comparison between diabetic and non-diabetic HCV patients as regard IL-28B genotypes.

	Genotype C/C%	Genotype C/T%	Genotype T/T%
Diabetic HCV	50%	25%	25%
Non-diabetes HCV	20%	60%	20%
X^2		26.027	
P-value	<0.002	<0.001	0.1

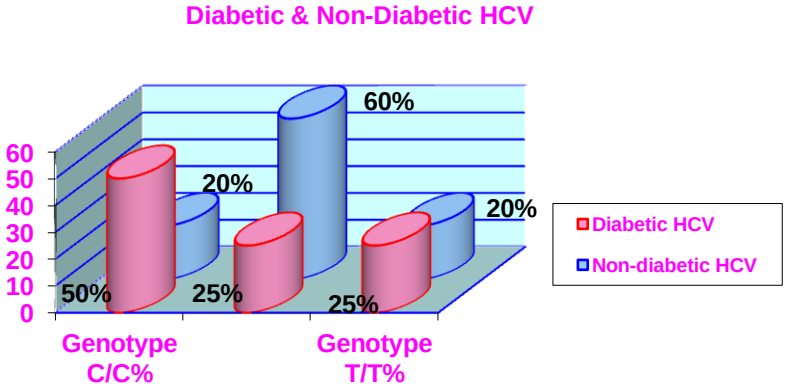


Figure (4)

Table and figure (5): Comparison between the three IL-28B genotypes as regard CD160% in HCV diabetic group.

IL28B polymorphism	CD160%		One Way ANOVA	
	Mean	±SD	F	*P*-value
C/C	56.68	±17.87	13.142	0.004
C/T	22.00	±1.98		
T/T	15.60	±2.73		
LSD tests				
C/C vs C/T	C/C vs T/T		C/T vs T/T	
P < 0.012	P < 0.002		0.553	

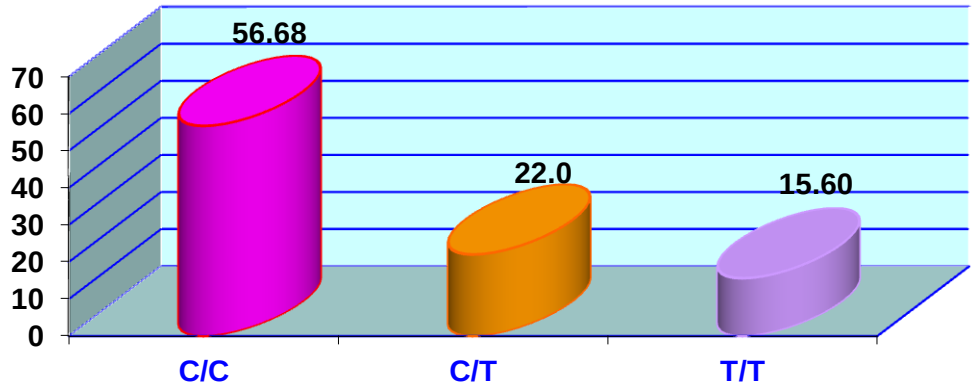


Figure (5)

4. Discussion

Since the discovery of the HCV virus as the cause of non A non B hepatitis and the finding that a minority can clear the virus while the majority cannot, there has been an intensive search for the key to successful immunity. The critical role of T lymphocytes in successful control of immune response has been observed (**Rehermann *et al.*, 2009).**

The extended up-regulation of inhibitory receptors on T lymphocytes plays many roles in the modulation of immune responses and in the prevention of tissue damage by cytotoxic T cells ***(Bengsch et al., 2010)***.

Even though the signaling cascades downstream of inhibitory molecules are likely to overlap, their function are not necessary redundant. The fact that their expression is differently regulated depending on the T cell activation status argues for distinct functions of different inhibitory receptors ***(Baitsch et al., 2012)***.

The inhibitory molecules mediate CD8 T lymphocytes hypo-responsiveness against HCV infection, CD160 and programmed cell death (PD1) have been extensively studied ***(Ciuffreda and Kim, 2012)***.

Despite of overwhelming evidence on the importance of CD160 molecules in controlling immune-response and inflammatory process during chronic hepatitis C disease, little information is available on their role in the development of extra hepatic complication such as diabetes mellitus type 2 ***(Raziorrough et al., 2011)***.

Also, the impact of these molecules on the effectiveness of antiviral therapy and their relation to IL28B as genetic predictor for progression of HCV associated diabetes and antiviral therapy response is still unclear ***(Caesano et al., 2008)***.

Our study showed higher SGPT and higher serum bilirubin in HCV diabetic cases as compared to non diabetic HCV group ,our findings were in agreement with ***Elhawary et al.*(2011**). Regarding hemogram, the diabetic HCV group showed significant increase of both white blood cells and platelets as matched with non diabetic HCV. These findings support the idea that the association of HCV and diabetes trigger exaggerated inflammatory process which subsequently result in reactive increase in white blood cells and platelets (***Lai et al* .,2011**).

In our comparative study, both diabetic and non-diabetic HCV groups displayed excessive expression of CD160 molecules on CD8 cytotoxic T lymphocytes as matched with control group.

This observation were in agreement with ***Sozzani (2012)***, who demonstrated the over expression of several inhibitory molecules including CD160 on CD8 virus specific T lymphocytes during chronic HCV viral infection.

Also, an association between HCV disease progression and increased frequency of CD160 PD1 cells has been shown by ***Bengsch et al. (2010)***.

In addition, the modulation of HCV specific immune response via expression of CD160 negative regulators on cytotoxic T lymphocytes has been observed in murine model ***(Raziorrough et al., 2011)***.

Importantly, our study showed that the frequency of CD160 cytotoxic T lymphocytes in diabetic HCV patients was higher than their frequency in non-diabetic HCV patients.

Our results may be explained by a novel triad, CD160 molecules, tumor necrosis factor-α and HCV associated diabetes. The inflammatory phase of HCV trigger vigorous secretion of tumor necrosis alpha which deregulated insulin signaling pathway causing insulin resistance and subsequently developing of type 2 diabetes mellitus (**Donelly** *et al.*, **2012).**

Tumor necrosis factor alpha secretion is regulated differentially by positive and negative signals exchange between antigen presenting cells

(APCs) and CD8 cytotoxic T lymphocytes (**Claassen** *et al.,* **2012).**

Excessive secretion of tumor necrosis alpha factor and subsequent developing diabetes mellitus type 2 trigger over expression of regulatory molecules CD160 on CD8 T lymphocytes (**Costantini** *et al.,* **2012**).

CD160 regulatory molecules initiate feed back negative regulatory signaling pathway to suppress pro-inflammatory cytokines secretion (**Sozzani** *et al.,* **2012**).

In the same context, it has been reported that simultaneous expression of inhibitory molecules including CD160 correlated with cytokines production(**Peretz** *et al.,* **2012).**

Other studies suggested an important role of CD160 in the network of co stimulatory/ inhibitory molecules that regulate CD8 T cells functions in chronic HCV patients (**Rio** *et al.,* **2010**).

In addition, ***Ciuffreda and Kim (2012)*** concluded that the expression of CD160 molecules occur as consequence of T cells activation and excessive cytokines secretion but It is not necessarily to be as signature of immune response.

Interestingly, the negative correlation between the frequency of CD160 and glycated hemoglobin (HbA1C) suggested the role of CD160 in controlling HCV associated diabetes. Recently, the potential modulatory role of CD 160 molecules have been described during the course of type 2 diabetes, CD160 regulators exhibit their effect via modulation of cytokines secretion and antiangiogenic mechanisms (***Chabot*** *et al., **2011***).

As regard IL28B polymorphisms ,it has been established that the genotypes of IL28B gene were described as C/C homozygous or heterozygous C/T or homozygous T/T. C/C genotype was associated with high probability of viral clearance whereas both T alleles genotypes ,C/T and T/T were associated with less response to antiviral therapy (***Thomas*** *et al., **2009***).

In our study , we noted that diabetic HCV group exhibit higher frequency of IL28 B genotype C/C as compared with non diabetic HCV group whereas, the frequency of genotype C/T was strikingly higher in non diabetic HCV group as matched with diabetic HCV group.

Our results were contradictory to the findings of **Friedrich** *et al.* **(2012)** who showed increased frequencies of T alleles genotypes in diabetic HCV group , while the frequency of C/C genotype was higher in non diabetic HCV patients.

The possible explanation of this contradiction is that the previous study suggested the association between diabetic HCV group and T alleles genotypes in cirrhotic HCV patients while the cirrhotic HCV patients were excluded from our study in attempt to clarify the link between IL28 B genotype polymorphisms and CD160 molecules, the diabetic HCV patients were categorized into C/C genotype group, C/T group and T/T group.

Interestingly, the C/C group was associated with higher frequency of CD160 negative regulator molecules as matched with both T alleles groups (C/T and T/T groups). It has been established that C/C genotype is considered as a good prognostic indicator for HCV disease progression and good predictor for antiviral therapy response ***(Thomas et al., 2009)***.

In the same context, it has been suggested that CD160 regulatory molecules may contribute to favorable diabetes mellitus outcomes via anti-angiogenic and anti-inflammatory effect ***(Chabot et al., 2011)***.

Also, **Nicolova** *et al.***(2009)** defined CD160+ cytotoxic T lymphocytes as functionally effector T cells characterized by gamma interferone(IFN-Y) secretion.

Moreover , the relationship between human leukocyte antigen(HLA) class 1 restricted T cells immune responses including CD160 modulatory signaling pathway and genetic predictor IL28B single nucleotide have been studied , the previous experimental studies demonstrated that the single nucleotide IL28B share with HLA class I loci in particular amino acid within binding groove of protein, suggesting that the interaction between HLA-peptide and IL28 gene is key to the mechanism underlying the impact of IL28 B polymorphism on CD160 inhibitory molecules signaling pathway ***(Timm et al., 2007; Blachburn et al., 2009 and Sozzani et al., 2012)***.

Previous studies and our findings may supported the hypothesis of involvement IL28 B gene in regulation of CD160 inhibitory signaling pathway between CD8 T lymphocyte and antigen presenting cells (APCs) during the course of chronic hepatitis C associated diabetes (***Rehermann*** .,***2009*** , ***Peretz*** *et al., **2012***).

5. Conclusion

Over all our findings highlight that the simultaneous presence of chronic hepatitis C and type 2 diabetes mellitus induce evident increase in pro-inflammatory cytokines and subsequent over expression of CD160 modulatory molecules on CD8 cytotoxic T lymphocytes ***(Rio et al., 2010 and Costantini et al., 2012)***.

Interestingly , Our findings suggested that the expression of CD160 on CD8 T lymphocytes correspond to recently activated effectors CD8 T cells which characterized by restoration of T cells function , secretion of gamma interferon and prevention of

deterious immune responses ***(Nicolova et al .,2009, Rio et al., 2010 , Ciuffreda and Kim., 2012)***.

Importantly, the association of C/C favorable genotype with over expression of CD160 molecules on CD8 T cells supported that CD160 molecules may considered as a good prognostic indicator during the course of chronic hepatitis C associated diabetes ***(Friedrich et al., 2012)***.

Also, the previous association confirmed the contribution of CD160 regulatory molecules to favorable effectiveness of antiviral therapy.

Abbreviations:

- CD = cluster differentiation
- DM = diabetes mellitus
- HCV = hepatitis C virus
- IL28B = interleukin 28 B
- PCR = polymerase chain reaction
- PD1 = programmed cell death
- TNF = tumour necrosis factor
- VS = versus

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