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Evaluation of Soluble Inducible T-cell Co-stimulator (sICOS) as a Prognostic Biomarker for Patients with Chronic Hepatitis C Virus

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Authors' contributions

This work was carried out in collaboration between all authors. Author RAED designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors HEB and MEB managed the analyses of the study. Author RAED managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Aim of the Work: This study aimed to investigate the serum level of sICOS molecule in chronic Hepatitis C Virus (HCV) patients and its association with pathological injury and HCV viral load in chronic hepatitis C patients to evaluate sICOS molecule as a novel biomarker for HCV replication, and disease progression.
Patients and Methods: The study included 40 chronic HCV-infected patients divided into two groups; group I 20 patients suffering of chronic HCV without cirrhosis, group II 20 patients with cirrhosis and group III composed of 10 healthy controls. Serum level of sICOS was analyzed by Enzyme Linked Immunosorbent Assay (ELISA). HCV viral load was estimated in all patients using real time PCR. The correlation between serum sICOS) and HCV viral load was studied.
Results: Viral load in group I ranged from 401.11 to 880.000 IU/ml with a mean value of

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521.727 ±152.225 and group II ranged from 315.49 to 1.110.000 IU/ml with a mean value of 603.298± 207.2. regard level of sICOS; it was significantly elevated in chronic HCV patients as its mean level in group I was 56.35 ± 6.75%, in group II was 69.4± 6,004% and in group III was 32±6.61% with P-value <0.001 (significant). In chronic HCV infection sICOS was positively correlated with HCV viral load.

Conclusion: sICOS serum level significantly higher in chronic HCV patients than normal healthy individuals. sICOS level in the serum was highly correlated with HCV viral load in chronic HCV-infected patients, which made sICOS a novel indicator to evaluate HCV replication and disease progression in chronic hepatitis C patients.

Keywords: sICOS; chronic HCV; HCV viral load.

1. INTRODUCTION

Hepatitis C is a liver disease caused by the hepatitis C virus. The virus can cause both acute and chronic hepatitis infection, ranging in severity from a mild illness lasting a few weeks to a serious, life long illness. 130–150 million people globally have chronic hepatitis C infection [1].

The WHO estimates that 3% of the world's population becomes infected with HCV [2].

Tight correlation between host circulating CD8+ T cell-mediated immune response and control of viral replication is classical characteristic of long-term HCV infection. CD8+T cell maturation/activation markers are expected to be associated with viral replication and disease progression in chronic HCV infection [3].

The diagnosis of HCV infection is based on the detection of anti –HCV antibody by ELISA and it is confirmed by an immunoblot assay or by the presence of HCV RNA by polymerase chain reaction (PCR) [4].

The sICOS (soluble inducible T cell co stimulator) molecule is expressed on lymphocytes, especially on T and B cells, and is an inducible inhibitory regulator of T cell activation [5]. Higher level of sICOS expression in HCV infection was documented [6].

Many co-stimulatory molecules, such as CD28, CD80, have two forms; membrane form and soluble forms. sICOS is formed from alternative mRNA splicing. Moreover, it has found that sICOS is highly associated with active lymphocytes presented in autoimmune diseases. However, positive correlation with sICOS and HCV viral load making this marker partially associated with virus replication and HCV pathogenesis, m aking sICOS an important marker in cases of vhronic HCV [7].

Previous studies have demonstrated that co-stimulators sICOS, are involved in the HCV pathogenic progress. Co-stimulator; ICOS, also assists in activating adaptive immunity, particularly by aiding antibody secretion by B cells. However, there is limited number of studies concerning ICOS in HCV patients. Ribavirin downmodulates ICOS in CD8+ T cells and assists HCV clearance, indicating that ICOS prevention is a novel choice for HCV treatment [8]. In addition, ICOS has been found to be upregulated in chronic HBV-infected cells.

Previous studies have demonstrated that there are different isoforms of sICOS from alternatively spliced messenger RNA (mRNA), and the soluble isoforms of these may regulate immune homeostasis [9]. In addition, soluble ICOS (sICOS) and have been identified to be present in numerous diseases, particularly autoimmune diseases, such as systemic lupus erythematosus (SLE) and cancer. However, the clinical significance of this marker in chronic HCV infection remains uncertain [10]. However, it will be promising to use ICOS as a marker in follow up of chronic HCV patients as it is easy to perform, more rapid , and economically could be afforded by all patients replacing polymerase chain reaction with its high cost and complex technique .

This study aimed to investigate the serum level of sICOS molecule in chronic Hepatitis C Virus (HCV) patients and its association with pathological injury and HCV viral load in chronic hepatitis C patients to evaluate sICOS molecule as a novel biomarker for HCV replication, and disease progression.

2 PATIENTS AND METHODS

This study was carried in Medical Microbiology and Immunology Department, Tanta Faculty of Medicine after approval of ethical committee in

Tanta Faculty of Medicine and a written consent from all participants on 50 subjects; 40 Cases with chronic HCV were selected from patients attending Tropical Medicine Department-Tanta University Hospital and 10 healthy individual during the period from December 2016 to May 2017.

Cases in this study were divided into three groups; Group I: Composed of 20 patients suffering from chronic HCV without cirrhosis their age ranged from 23 to 66 years, Group II: Composed of 20 patients suffering from chronic HCV with cirrhosis their age ranged from 38 to 69 years and Group III: 10 healthy individual their age ranged from 35 to 68 years as a control group.

All patients were subjected to Complete history taking, Clinical examination. Routine Laboratory investigations including Liver enzymes: AST and ALT, serum bilirubin and Hb level. Ultrasound used to evaluate HCV patients: liver cirrhosis or not.Fibroscan used to evaluate HCV patients: liver cirrhosis or not. Quantitative estimation of HCV-RNA viral load in the blood of the patients using real time-PCR, ELISA for estimation of the serum level of sICOS.

2.1 Exclusion Criteria

All cases in the study were negative for HBV and HIV and will not receive any HCV-specific antiviral therapy 6 months before the study. The healthy control individual approved to be HCV negative.

2.2 Peripheral Blood Sample

5 ml of venous blood from patients and control subjects were withdrawn with a disposable sterile plastic syringe for ELISA and Real Time PCR.

2.3 Enzyme Linked Immunosorbent Assay (ELISA)

Serum level of sICOS of all patients and the normal control group were estimated with ELISA (R&D Systems, Inc., Minneapolis, MN, USA) according to the manufacturer's instructions [11].

2.4 Quantitative Assessment of HCN RNA by Real Time PCR for HCV Patients

Quantitative detection of HCV RNA by real-time PCR was performed using the light cycler taqman master mix kit on Roche Light cycler version 2.0 (Roche Diagnostic, GmbH, Mannheim, Germany). The cycle conditions of RT-PCR include 95°C for 20 seconds, followed by a further 40 cycles at 95°C for 10 seconds, 58°C for 15 seconds and 72°C for 10 seconds. The sequence of the primer used was: forward 5'-GAAGGCAAGATGGCACTAAGCA-3'and reverse 5'-TCTCGTCTGTTGCCGGAGATAG-3' [12].

2.5 Statistics

Statistical presentation and analysis of the present study was conducted, using the mean, standard deviation and chi-square test by SPSS V.20.

3. RESULTS

This study was carried out on 50 subjects, was done over the period from December 2016 to May 2017. The study included three groups; group I included 20 subjects with chronic HCV without cirrhosis, and subjects were (12 male & 8 female, age range from 23 to 66 years and mean age of 46.15 ± 11.979), group II included 20 subjects with chronic HCV with cirrhosis, and subjects were (12 male & 8 female, age range from 38 to 69 years and mean age of 53.15 ± 8.054), and group III included 10 subject as healthy control subjects were (6 male & 4 female, age range from 35 to 68 years and mean age of 49.4 ±10.606 with P-value was 0.110, 1.000 respectively.

The results of the study showed that ALT mean level in group I was 9.92 ±1.25, in group II was 35.55± 9.98 and in group III was 10.46 ±1.09 with p-value <0.001 (significant). There was no significant difference between group I and III (Table 1).

Table 2 shows the serum level of AST as its mean level in group I was 10.42 ± .81, in group II was 35.5± 7.95 and in group III was 9.85 ±1.19 with p-value <0.001 (significant). There was no significant difference between group I and III.

Table 3 shows that there was a significant difference between group I and group II and between group II and group III as regard level of serum bilirubin as its mean level in group I was .67± .26, in group II was 6.46± 3.49 and in group III was .7 ±.24 with p-value <0.001 (significant). There was no significant difference between group I and III.

As regard the viral load among the studied group Table 4 shows that there was no significant difference between group I and group II as regard level of viral load as its mean level in group I was 521.727 ±152.225 and in group II was 603.298± 207.2 with P-value was 0.164 (insignificant).

Table 5 show the concentration of sICOS between studied groups where there was a significant difference between group I, II and group III as regard level of sICOS as its mean level in group I was 56.35 ± 6.75, in group II was 69.4± 6,004 and in group III was 32±6.61 with P-value <0.001 (significant).There was significant difference between group I&II, Group I&III and Group II&III

Table 6 shows Correlation between sICOS and other studied parameters in patients with chronic HCV: This table shows that there were a significant correlation between sICOS Level and level HCV viral load, ALT, AST, serum bilirubin.

Table 7 shows analysis of ROC curve demonstrating cutoff point of sICOS

Fig. 1 shows that there was a significant correlation between HCV viral load and sICOS.

Table 1. Level of serum ALT in the studied groups

Groups	ALT U/L						ANOVA	
	Range			Mean	±	SD	F	P-value
Group I	8.000	-	12.000	9.920	±	1.253	33.088	<0.001*
Group II	20.000	-	70.000	35.550	±	9.984		
Group III	8.500	-	12.000	10.460	±	1.093		
Tukey's Test								
I&II			**I&III**			**II&III**		
<0.001*			0.991			<0.001*		

Normal range of ALT: 6-42 mg/dl

Table 2. Level of serum AST in the studied groups

Groups	SGOT U/L						ANOVA	
	Range			Mean	±	SD	F	P-value
Group I	8.500	-	12.000	10.425	±	0.810	48.318	<0.001*
Group II	25.000	-	60.000	35.500	±	7.957		
Group III	8.000	-	12.000	9.858	±	1.190		
Tukey's test								
I&II			**I&III**			**II&III**		
<0.001*			0.985			<0.001*		

Normal range of AST: 6-42 mg/dl

Table 3. Level of serum bilirubin in the studied groups

Groups	Serum bilirubin mg/dl						ANOVA	
	Range			Mean	±	SD	F	P-value
Group I	0.240	-	1.200	0.678	±	0.266	39.806	<0.001*
Group II	2.600	-	14.500	6.468	±	3.499		
Group III	0.200	-	1.100	0.790	±	0.242		
Tukey's Test								
I&II			**I&III**			**II&III**		
0.003*			0.991			0.020*		

Normal range of bilirubin: 0.2-1.00 mg/dl

Table 4. The level of viral load in the studied groups

Groups	PCR						T-test	
	Range			Mean	±	SD	T	P-value
Group I	401.11	-	880	521.727	±	152.225	-1.419	0.164
Group II	315.49	-	1100	603.298	±	207.200		

Table 5. Concentration of sICOS among the studied studied groups

Groups	sICOS					ANOVA	
	Range			Mean	± SD	F	P-value
Group I	46.000	-	69.000	56.350	± 6.753	112.697	<0.001*
Group II	60.000	-	79.000	69.400	± 6.004		
Group III	20.000	-	40.000	32.000	± 6.616		
TUKEY'S Test							
I&II	**I&III**			**II&III**			
<0.001*	<0.001*			<0.001*			

Table 6. Correlation between and sICOS and other studied parameters in patients with chronic HCV

	sICOS	
	R	P-value
Age(Y)	0.149	0.360
PCR	0.461	0.003*
ALT	0.502	0.001*
AST	0.585	<0.001*
Serum bilirubin	0.461	0.003*

Table 7. Analysis of ROC curve showing cutoff point of sICOS

.ROC curve					
Cutoff	Sens.	Spec.	PPV	NPV	Accuracy
> 40 *	100.0	100.0	100.0	100.0	1.000

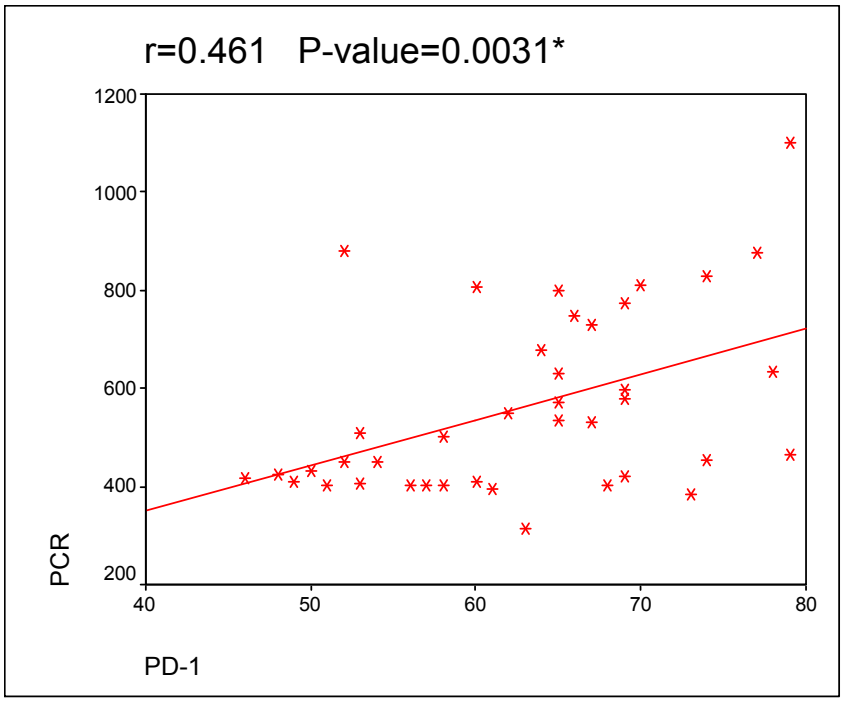

Fig. 1. Correlation between sICOS and viral load

4. DISCUSSION

Hepatitis C virus infection is considered a worldwide problem affecting more than 200 million people all over the world [11]. The WHO estimates that 3% of the world's population becomes infected with HCV [1]. Egypt has the highest HCV prevalence in the world [9]. About 12 to 15% of the total populations are infected, with HCV Genotype-4 accounting for the overwhelming majority of HCV infections [12].

This study was aimed to investigate the serum level of sICOS molecule and its association with pathological injury and HCV viral load in chronic hepatitis C patients to evaluate sICOS molecule as a novel biomarker for HCV replication, and disease progression.

HCV Viral Load is the amount of HCV viruses in a given volume of the blood (usually 1 milliliter = 1 cubic centimeter). There appears to be no significant correlation between HCV RNA levels and ALT values or histological activity in patients untreated by anti-viral therapies (Interferon). Viral load varies between infected individuals but is not a useful prognostic indicator nor does it measure the severity of virus-induced liver disease [13,14].

In this study the level of viral load in group I ranged from 401.11 to 880.000 IU/ml with a mean value of 521.727 ±152.225 and group II ranged from 315.49 to 1.110.000 IU/ml with a mean value of 603.298± 207.2 with no significant different between the two groups as regard level of viral load. So HCV viral load could not reflect the degree of liver histological damage.

In agreement with this study, Pei Liu et al. [15] found no significant connection was noted between serum HCV-RNA titer and the grades of liver necroinflammatory activity (r=0.50, P=0.667) or the stage of liver fibrosis.

In contrast, Adinolfi et al. [16] held that serum HCV-RNA titer was correlated to the severity of liver damage, which could be accelerated by high HCV load and fat degeneration.

In HCV infection, persistent infection appears to be due to weak $CD4^+$ and $CD8^+$ T-cell responses during acute infection, which fail to control viral replication [17].

Dimitra et al. [18] had found a strong $CD8^+$ T-cell response in the peripheral blood in the chronic HCV patients and also found no correlation was recorded between the numbers of CD8+lymphocytes and the degree of fibrosis.

ICOS is a costimulator that help in activating adaptive immunity, by aiding antibody secretion by B cells. The studies that investigate the serum level in chronic HCV patients is still limited. Ribavirin downmodulates ICOS in CD4+ T cells and help in HCV clearance, indicating that ICOS prevention is a novel choice for HCV treatment [19,20].

In the present study the serum level of sICOS was significantly higher in the patients with chronic HCV than healthy controls as there was significant difference between group I, II and group III as regard level of sICOS as its mean level in group I was 56.35 ± 6.75%, in group II was 69.4± 6,004% and in group III was 32±6.61% with P-value <0.001 (significant).

This came in agreement with Dongsheng et al. [21] who found that the sICOS levels were significantly higher in the HCV group compared with the normal control group. They explained that these molecules have an immunomodulatory function and Increased production of sICOS may interfere with the patient's adaptive immune function. They added that sICOS may compete and interfere with the ICOS interactions with their respective ligands, leading to immune dysregulation and a defective immune response.

As regard the correlation between sICOS and other studied parameters in patients with chronic HCV this study showed that there were a significant correlation between sICOS level and level HCV viral load, ALT, AST, serum bilirubin.

In accordance to the results of this work Dongsheng et al. [21] found that sICOS levels correlated with Total and direct bilirubin and did not correlate with ALT levels that came in reverse to this study.

This study showed that there is a significant correlation between the serum level of sICOS and HCV viral load. Dongsheng et al. [21] found this positive correlation between sICOS and level of HCV antibodies in the serum. They explained that high levels of sICOS and high levels of anti-HCV antibody are closely associated, that is explained that high level of sICOS occur as a result of shedding from the active T-cell membranes, that makes production of sICOS very important in HCV infection. The detailed

immunopathological roles of those soluble co-stimulatory molecules in HCV infection require further study.

5. CONCLUSION

sICOS serum level significantly higher in chronic HCV patients than normal healthy individuals. sICOS level in the serum was highly correlated with HCV viral load in chronic HCV-infected patients, which made sICOS a novel indicator to evaluate HCV replication and disease progression in chronic hepatitis C patients.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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