Assessed Valuate of Soluble Programmatic Cell Death Ligand 1 (PD-1) in Sera of Hepatitis C Virus in Iraqi Patients

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ABSTRACT

Objective: The present study aims at detecting the concentration of human PD-1 in samples taken from hepatitis C virus patients related to the healthy control group which studying the relation between viral load associated with (PD-1) concentration.

subject of the treatment group content from 68 samples which is selected randomly from the patients with HCV, subdivide into 32 males and 36 females aged 23-76 years with 30 healthy individuals divided 20 males and 10 females. During first April to end of June 2020.

ELISA kit from (Shanghai Yehua Biological Technology Company, China) was used to measure programmed death concentration. While Real time-PCR technique (Device Smart Cycler, USA) was used for calculating the viral load, according to Sacace Biotechnology kit. The result was a high concentration of PD-1 in patients 295.709±29.36 and 168.337 ± 80.906) compared to healthy (106.014±63.90, 110.176 ± 36.681). Respectively, and significant difference. and we found that PD1 concentration was directly proportional to viral load

Conclusion: We have found that there is an enhancement in PD-1 concentration in patients associated with healthy control groups, and We also found that PD1 concentration was directly proportional to viral load, Whenever, increased the viral load, had risen the PD 1 concentration.

Keywords: Hepatitis C Virus, HCV , PD-1, Viral Load, Chronic Hepatitis C.

INTRODUCTION

Hepatitis is organ inflammatory disease in liver, HCV infection is a worldwide health problem. According to WHO appreciation, the number of people with HCV is estimated to be approximately rate wide world is 185 million individuals, of whom 703 800 die each year (GBD, 2015; WHO, *2016*). Depending on the region and country varies Prevalence of HCV, sero prevalence for HCV is ($\leq 15\%$) in developing countries, While being less than 2% (< 2%) in developed countries. (Hajarizadeh *et al.,* 2013). In Iraq reported 1165536 thousand infection equal (3.21%) (*Gower et al.,* 2014; lavanchy, *2011;* Ghaderi-Zefrehi *et al.,* 2016).

Death Ligand 1 (PD-1), also recognized as (CD 279) is a glycoprotein, molecular weight has 55 kDa , belongs to the CD28 of trans membrane proteins (*Zhang et al*.,2004). In the T cell hybridoma subject to cell death, PD-1 or CD 279 receptor was exposed as a genetic factor up regulated , in 1992 (Ishida *et al*.,1992). The Pdcd1 gene that is positioned on second chromosome which is the encoded for the PD1 (Keir *et al*., 2008). PD-1 represent a significant co inhibitory molecule engaged in an evolution of the chronic viral infections (Xiao *et al*., 2015). The scientist Lieping Chen is the one who discovered PD-L1, known also as CD274, in 1999 (Wang *et al*., 2017). The Cd274 gene which is located on chromosome 9 is the encoded for the PD-L1 (Keir *et al*., 2008). The inhibitory effects of PD -1 on T cells are via regulating the TCR signaling out of IL-2-dependent in addition to independent mechanisms (Patsoukis *et al*., 2012). The activation of Programmed cells Death-1 supports the inhibition a proliferation (CD4+ and CD8+ T) cells, which is correlated with cell apoptosis and the suppression of IL-2 secretion. The Programmed cells Death-1 Cascade Signaling influences the T cell response at the subsequent effector phase because up-regulated expression of PD-1 was revealed after persistent antigen intraction. Furthermore, CD8+ T cells turn out to be more prone to regulation by PD-1: PD-L exposure, because they create a down level of IL-2 (Carter *et al*., 2002). During the chronic phase HCV infection, the up-regulation of PD-1 is considered one of the apparatuses responsible for T cell impairment (Golden-Mason *et al*., 2008; Rutebemberwa *et al*., 2008). Generally, T cells depend on a set of signals to develop a strong and persistent response, and the transmitted signals are:

1. Signalling by the TCR.
2. Signalling by cytokines.
3. Signalling by costimulatory molecules expressed on antigen-presenting cells and T cells. When any one or more of the above signals are lost, this cause a defect in the response of T cells to HCV infection (Mescher *et al*., 2006). The persistence of viral infection and T-cell exhaustion in chronic HCV is related to the expansion of up-regulation of PD-1. Block the PD-1

pathways by modifying immune checkpoint molecules to restore virus-specific T cell responses, is an interesting possible strategy (Hyosun *et al*., 2017). Several studies have manifested that PD-1 pathways during acute infections play a crucial function in regulating anti-microbial responses (*Brown et al*., 2010). And PD-1: PD-L1 interactions possibly working to protect the CNS from immunopathology at rabies virus infection through suppressing CD8 T cell responses (Lafon *et al*., 2008).

MATERIALS AND METHOD

the study group consisted of sixty eight samples estimated DP1 levels of HCV patients to be distributed into 32 in male group and 36 female group aged 23 - 76 years that's randomly selected, with 20 healthy individuals with 12 males and 8 females aged 19 - 62 years. And for the period start in April to end in July 2020. Blood Specimen was collected from each patient and control. PD-1 ELISA kit obtained by (Shanghai Yehua Biological Technology Company, China) was used to measure programmed death concentration. While Real time-PCR technique (Device Smart Cycler, USA) was used for calculating the viral load, according to Sacace Biotechnology kit (Issa *et al*., 2012).

RESULTS

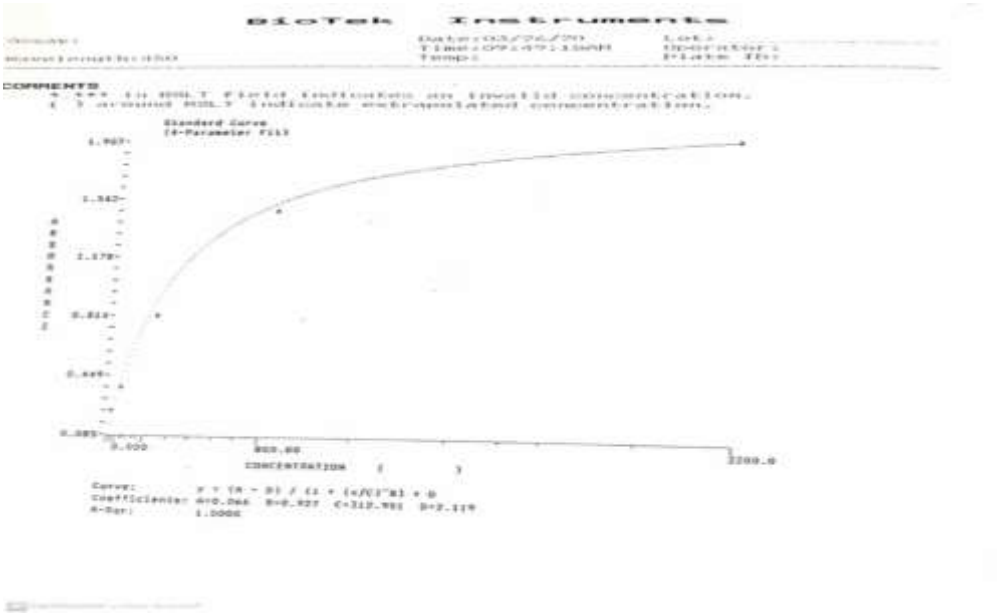


Figure 1: Curve of Concentration of PD-1

The figure 1 shown the layout of standard samples in ELISA assay through the the determine PD1 levels as first step in this test.

Anti-HCV positive samples showed a significantly higher concentration of PD1 (mean= 83.175±33.03 mg/dl) in comparison with the normal average calculated for the kit, 138 mg/dl. t-test value = 12.85, significance (2-tailed) =.000, 95% according to summarized outcome in table 2. The results in table 1. Demonstrated that it's the mean of PD1 concentrations of anti-HCV female samples (234.79±116.091) found to be no significant difference between them but clearly low than that of anti-HCV male samples (239.93±81.4796 mg/dl). F value = .068, no significance (ANOVA) = .794, mean difference is 658.613.

PD1 concentration in HCV patients found to be significantly higher than control group by sig. P ≤.000), F (408.28) and the mean± SD (295.709±29.36, 106.014±63.90). This results shown in table 2 in the sex group than in the female group (healthy category) in males. In female patients, sex group (treatment) no found to be any significant values than the male group also the age group (41-50 years). Small differences between other age groups found to be non-significant. Results are shown in figures, 2, 3, 4, 5.

Table 1: Shown the mean and SD of PD1 concentration between the studied groups according to the gender

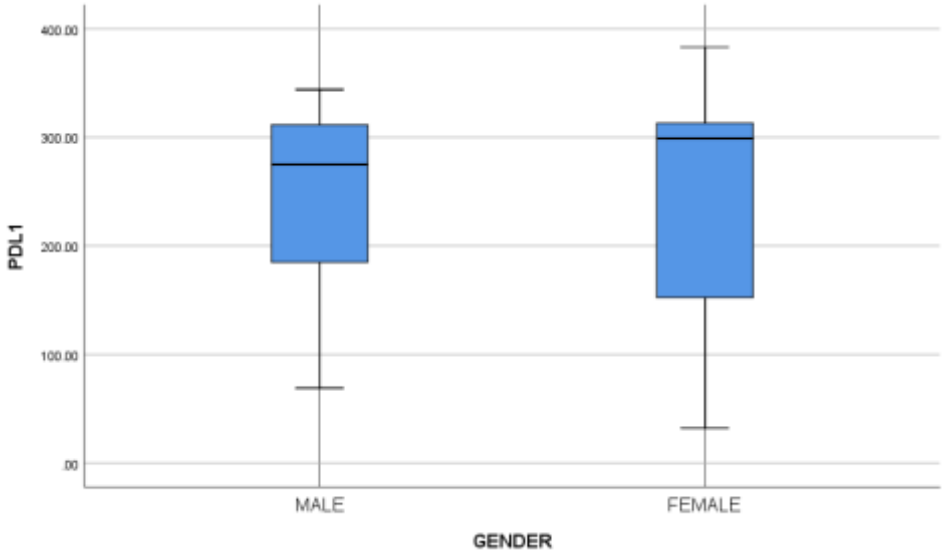
GENDER	Mean	±Std. Deviation	N	% of Total N	% of Total Sum	Minimum	Maximum
MALE	239.93	±81.4796	55	56.1%	56.7%	68.85	344.00
FEMALE	234.79	±116.091	43	43.9%	43.3%	32.00	383.00
Total	237.64	±97.664	98	100.0%	100.0%	32.00	383.00

		Sum of Sq	df	Mean Sq	F	Sig.
PDL1 * GENDER	Between Groups	658.613	1	658.613	.068	.794
	Within Groups	924538.034	96	9630.605		
	Total	925196.648	97			

Table 2: Concentration by mean of PDL1 in studied group

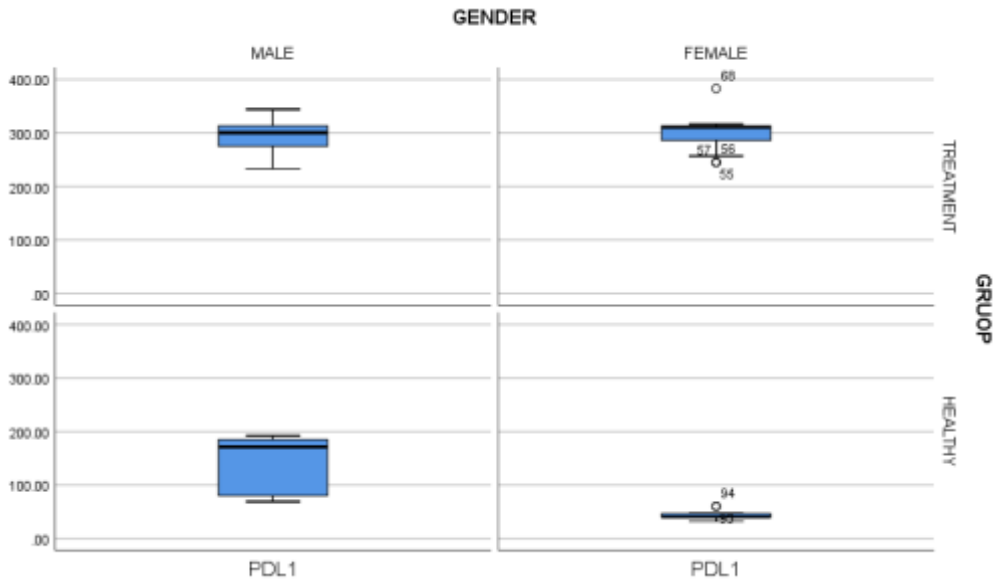
GRUOP	Mean±	Std. Deviation	N	% of Total N	% of Total Sum	Minimum	Maximum
TREATMENT	295.709	±29.36	68	69.4%	86.3%	233.00	383.00
HEALTHY	106.014	±63.90	30	30.6%	13.7%	32.00	192.00
Total	237.639	±97.67	98	100.0%	100.0%	32.00	383.00

		Sum of Sq	df	Mean Sq	F	Sig.
PDL1 * GRUOP	Between Groups	7491.745	1	749063.745	408.28	.000
	Within Groups	1761.903	96	1834.718		
	Total	9252.648	97			



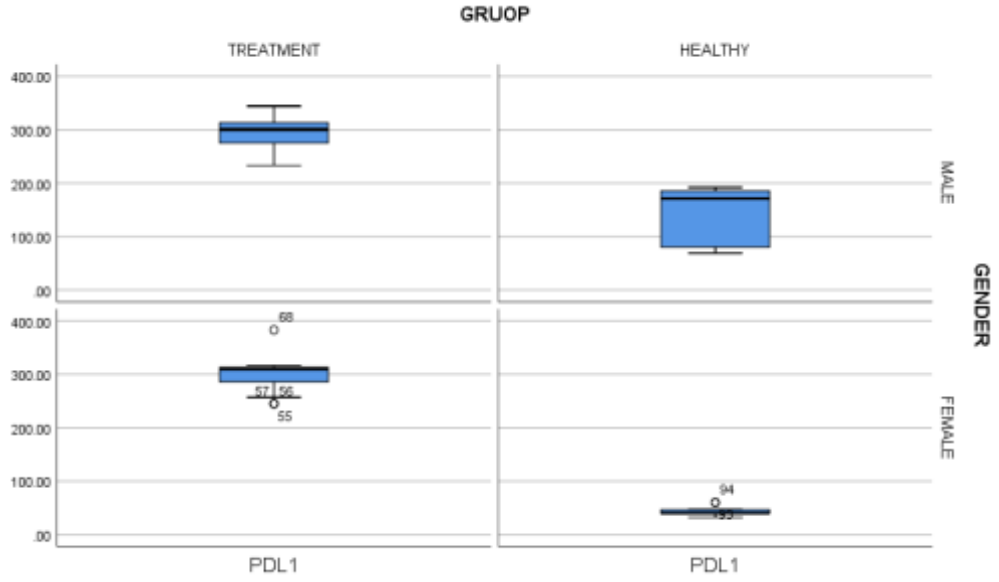
MALE	55	100.0%	FEMALE	43	100.0%

Fig 2: Average of PD1 concentrations in HCV patients and HCV male and female individuals



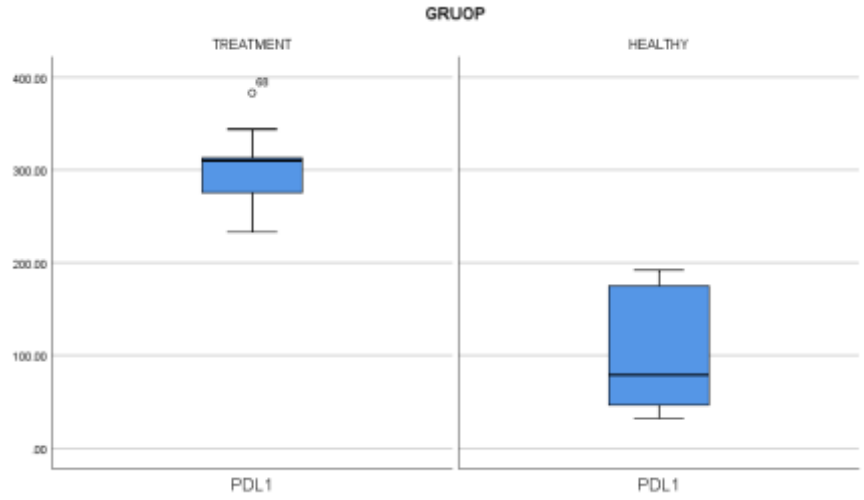
	N	Percent	N	Percent	N	Percent
PDL1	98	94.2%	6	5.8%	104	100.0%

Fig 3: Average of PD-1 concentrations in HCV patients, the control group and HCV male and female individuals



	N	Percent	N	Percent	N	Percent
PDL1	98	94.2%	6	5.8%	104	100.0%

Fig 4: Average of PD-1 concentrations in HCV patients and HCV negative individuals



	N	Percent	N	Percent	N	Percent
PDL1	98	94.2%	6	5.8%	104	100.0%

Fig 5: Average of PD-1 concentrations in HCV treatment and HCV negative individuals according to the gender

Table 3: Replicate Concentration of PD- 1 (U/ ml) between the studied groups.

groups	No.	No.%	Mean ± Std. Deviation	P-value
Patients group	68	77.27%	168.337 ± 80.906	0.00 HS
Control	20	22.63%	110.176 ± 36.681	
Total	88	100.0%	139.256 ± 58.883	

Sig. (2-tailed): (0 .000). (17.157). (df = 19-67).

DISCUSSION

The mean ± SD of serumPD1 are described in (Table 1) demonstrated a statistically significant improve in serum PD-1 ($p < 0.0001$) level hepatitis C virus patients in evaluation with the control group with percent of (43%), while the level of serum PD1 ($p < 0.01$) significantly decreased in these patients related to the control group by p value ($p < 0.001$).

Frequency of HCV infection in male sex groups showed significantly higher than that in the female group in the control category ANOVA. The small differences among other age groups for both males and females were not significant at the level of 0.05 by T-test and ANOVA test. See result in table 2.

The convergence in PD-1 levels was determined, as per the standard assembling bend. PD-1 focus demonstrated a noteworthy rise in hepatitis C infection patients serum contrasting and control group .the mean concentration of PD- 1 in hepatitis C patients was (168.337 ± 80.906), while the concentration in healthy control was (110.176 ± 36.681) as shown in the table (3).

Control of antiviral CD8+ T cell responses may be through PD1\ PD-L1 interactions (Latchman *et al*., 2004). After T cell activation, the inhibitory receptors such PD-1 can be up regulated within 24–72 hours of T cell receptors stimulation (Keir *et al., 2008*). PD-1\ PD-L1 ligation will transmit inhibitory signals to T cells, and thus cause functional exhaustion, T cell energy, and apoptosis (Wang *et al*., 2017). So, Yao *et al*., 2007 proved it.

Irrespective of the final outcome of HCV, have been reported dysfunctional in CD8 cells at the acute infection stage (Gruener *et al.,* 2001; Thimme *et al.,* 2001). In acute HCV, Exhaustion may be the mechanism immune response aimed at the impairment of T-cells reaction, which can initially go on for a number of reasons, including the fast kinetics of virus replication and subsequent prevalence of infection, in addition to T cells are exposed to elevated concentrations into antigen-exposed and continuously (Urbani *et al*., 2006).

The persistence of viral infection and T- lymphocytes exhaustion at chronic phase HCV is correlated to the development of up-regulation of PD1. Block the PD-1 pathways by modifying immune checkpoint molecules to reestablish virus-specific T cell responses, is an interesting possible strategy (Hyosun *et al*., 2017). Through chronic viral infection PD-1: PD-L1 collaborations may assist in dysfunctional of CD8+ T cells. As that Blocking this pathway perhaps furnish a means to enhance antiviral immune (Smith *et al*. 2004). In chronic infection, CD8+ T cells specific in HCV that's show a in elevate expression of PD1, but it displays absent or decrease expression of PD 1 in HCV patients with resolving the infection *(*Golden-Mason *et al.,* 2008*;* Urbani *et al.,* 2006*).*

In vitro, blocking PD-1 /PD-L1 interaction invert exhausted propagation of specified virus against T cells besides soluble cytokines production (Golden-Mason *et al*., 2007; Penna *et al*., 2007). In persistent manner to infected mice that lack CD4 T cell help, blockade of PD 1: PD-L1 pathway, restored and maintained the capability of "helpless" CD8 T cells to proliferate, release cytokines, decrease viral load and kill infected cells. Also In vivo, PD-1 \ PD-L1 blockade lead to a significant decrease in viral load (Keir *et al*., 2008). Inhibited and exhausted regulatory roles of virus-specific T cells could be partially restored, through PD-1/PD-L1 pathway blocking, and the recon quest range is in height correlating with the concentration of PD-1 in lymphocytes (Dellgren *et al., 2009;* Wei *et al., 2013*). In self-limited HCV infection, restoration of antiviral CD8 function synchronous with decrease PD-1, while continuous of CD8 dysfunction concomitant with a perseverance high expression of PD-1 by CD8 cells support a role for the PD-1/PD-L1 pathway in modulating CD8 function in conditions of sustained high concentrations of HCV antigen stimulation. This is proven via the surveillance that blockade of PD1/PDL-1 pathway promotes propagation of HCV-specific CD8 cells. And the data of this study robustly indicate that this mechanism (inhibiting the PD-1/PDL-1 pathway) can be a curative aim to reverse T lymphocytes impairment in chronic HCV (Urbani *et al*., 2006). This results consistent with our study which demonstrated that PD1 concentration was directly proportional to viral load. Also, PD-1 on virus-specific CD8 T cells may serve as a useful marker to indicate the disease severity and degree of T cell exhaustion (*D'Souza et al*., 2007). Studies of antiviral CD8 T cells have identified expression of PD-1 as a molecular signing up of exhausted T cells. And most importantly in vivo, blockade the PD-1/PD-L1 interaction with blocking antibodies versus PD-L1 or PD-1, will promote the T-cell role and decrease of viral titer level (Barber et al,. 2006). In the end, these results promote scientists' hopes that regenerating exhausted T cells by modifying the PD-1/ PD-L1 reactions may drive to effective treatment of the HCV disease.

CONCLUSION

In conclusion, this report first establish that PD-L1 was valuate in concentration of the sera of patients with viral load by COVID 19 infection, although no relationship between the PD-L1 levels and the viability of TVR- or SMV-based triple treatment therapy was well-defined. Results showed that the degree recorded of sera sPD-L1 might be associated with the movements of viral load linked with chronic hepatitis C. CHC and the production of hepatocellular carcinoma. In spite of the fact that the particular character of PD-L1 requires extra assessment, this examination suggested that PD-L1 fixation may be detailed in the resistant pathogenesis of viral load.

CONFLICT OF INTEREST: Nil

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