Zagazig University Medical Journals
www.zumj.journals.ekb.eg

Original Article

ASSESSMENT OF THE PREDICTIVE VALUE OF T-REGULATORY CELLS FOR THE RESPONSE OF CHRONIC HEPATITIS C TO SOFOSBUVIR BASED THERAPY

Monkez MoteihYousif[1], Mohammad Mohammad Sakr[1], Seham Mahrous Zaki[2], Ayman Magd-eldin Sadek[1], Osama Ibrahim Husseiny Mostafa[1]

1Internal Medicine Department, Faculty of Medicine, Zagazig University, Zagazig, Egypt
2Clinical Pathology Department, Faculty of Medicine, Zagazig University, Zagazig, Egypt

Corresponding Author:
Osama Ibrahim Husseiny Mostafa
Internal Medicine Department, Faculty of Medicine, Zagazig University, Zagazig, Egypt
usama_elhusini2011@yahoo.com

Submit Date	2019-01-25
Revise Date	2019-02-26
Accept Date	2019-02-26

ABSTRACT

Background: In Chronic Hepatitis C (CHC) disease, the role of Treg is still controversial and most studies yielded conflicting reports. Our study aimed to assess the influence of baseline T-regulatory cells on Chronic Hepatitis C (CHC) disease progression and response to treatment with direct-acting antiviral drugs (DAA).**Method:** This prospective cohort study included 120 CHC patients who are eligible to receive DAA treatment in National Committee for Combating Viral Hepatitis (NCCVH) at Al-AHRAR hospital, were subjected to routine laboratory investigation, pre and post-treatment polymerase chain reaction (PCR) analysis and flow cytometry analysis to reveal ratio percentages of T-regulatory cells subsets.

According to response to DAA into the sustained virological response (SVR) group and Non-response (NR) group included 112 and 8 patient respectively also divided into Non-Cirrhotic and Cirrhotic groups included 103 and 17 patients respectively.

Result: Comparison SVR and NR groups resulted in a significant difference between both groups with higher FOX-p3 expression among NR populations (p= 0.002). Multivariate regression analysis resulted in FOX-p3 expression as an independent factor for non-response to DAA treatment, there is a positive correlation between T-regulatory cells and the severity of liver disease. ROC curve analysis had shown a cut-off for FOX-p3 expression percentage (≥6.92) that can differentiate between SVR and NR groups.

CD25+CD4 (P =0.002) and Fox+ve Treg (P =0.018) were higher in Cirrhotic than Non Cirrhotic group. **Conclusion:** T-Regulatory cell level may predict the response to DAA treatment in CHC patients.

Keywords: Direct-acting antiviral drugs, Chronic hepatitis C, T-regulatory

INTRODUCTION

Burton et al; 2008 stated that The percentage of Tregs was higher in NR than in SVR patients before and at 24 weeks post-treatment and in early NR than in SVR patients at baseline, thereby suggesting a possible role of Tregs in virological response to combined antiviral treatment. To our knowledge, only one longitudinal study has examined the effect of combined antiviral treatment in Tregs frequency, finding no differences on the percentage of Tregs before, during or after treatment or between NR and SVR patients. (1)

Study on untreated population **Ebinuma et al; 2008** stated that another lymphocyte subpopulation studied in patients with HCV infection is the CD4+T-reg cells have an important role in either disease progression or control. The percentage of T-regs in peripheral blood in patients with chronic HCV infection is either similar or increased compared with recovered HCV patient. (2)

Akiyama et al; 2010 stated that Treg levels (CD4+CD25+Foxp3+) have already been evaluated during antiviral HCV therapy, and it has been suggested that they could predict the result of combination therapy.[3]

Given the high potency of DAAs, it is unclear whether immune response plays a significant role in viral eradication. However, in a recent study of patients treated with SOF and ribavirin, **Meissner et al; 2007** demonstrated that the resetting of intrahepatic type I interferon response was associated with SVR, suggesting a role for healthy liver in aiding HCV clearance, even in the setting of interferon-free therapy. [4]

Our study aimed to assess the influence of baseline T-regulatory cells on the response to treatment with DAA and liver disease severity.

SUBJECTS AND METHODS

This study that was achieved in Internal Medicine, laboratories of Clinical Pathology Department, Faculty of Medicine, Zagazig University and patients who are eligible to DAA treatment at (NCCVH at Al-AHRAR HOSPITAL) in the period from January 2017 to June 2018. Table 1 showed demographics for the groups studied.

Inclusion Criteria:

Patients with CHC (BY +HCV RNA by real-time PCR), Age ≥ 18 years, both sex, Naieve for treatment with DAA and Includes both Cirrhotics and Non-Cirrhotics.

Exclusion Criteria:

Treatment-experienced, Combined HCV, HIV infection, HCC or other malignancies, Pregnancy, Refusal to participate in the study, Use of immune suppressive medication.

Ethical Clearance:

Written informed consent was taken from the patients' relatives to participate in the study. Approval for performing the study was obtained from The Institutional Review Board (IRB) approval, also according to the declaration of Helsinki.

Study design: prospective cohort study

All subjects of the study were subjected to:

Full history and thorough clinical examination as well as drug prescriptions were done. General examination and local examination of different systems with gastrointestinal examination were done also.

Routine investigations were done according to the protocol of clinical pathology and laboratories of Zagazig University Hospital: Complete blood count, Liver Kidney function tests, α-fetoprotein level, PT INR and RBS.

Radiological investigation: Pelvi-abdominal ultrasound, CT if suspicious HCC.

Quantitative real-time PCR: Before the start of the treatment to detect baseline HCV RNA viral load and we followed up the patients for 12 weeks then the PCR was repeated after the end of treatment for Assessment of response to DAA treatment (SVR).

Special investigations (flow cytometry analysis)

Reveal specific markers of T-reg cells including (CD4+, CD25+, and FoxP3+) through five ml of peripheral blood (PB) were drawn from the patients. Each blood sample inserted in 2 ml EDTA tube for immunophenotyping, Flow cytometry was performed on the same day of the arrival of the specimens.

Evaluation of flow cytometry results

Primary gating of cells was performed using FSC/SSC technique to detect lymphocytes, Then CD4 + cells are identified by flow cytometer and detected as T-helper lymphocytes, CD25 cells surface marker used to identify the T-reg cells (CD4+CD25+) in the upper right quadrant (UR area).

furthermore, expression of the most specific molecule for identification of T-regulatory cells is called forkhead protein-3 called as (Fox-P3) identified by gating in the position of CD4+/CD25+ve to identify the FOXp3+ve T-regs.

Statistical Analysis:

All data were analyzed using SPSS 20.0 for Windows (SPSS Inc., Chicago, Illinois, USA.)

Continuous variables were checked for normality by using the Kolmogorov Smirnov test. One-Way ANOVA was used to compare normally distributed variables in three groups. Post-hoc Fisher's Least Significant Difference test (LSD) tests were used according to the homogeneity of variances. Percent of

categorical variables were compared using the Chi-square ($\chi 2$) test. Multivariate regression analysis was done to investigate independent predictor for both SVR and cirrhosis. Pearson product-moment correlation coefficient was used to assess the correlation between TE and study parameters if data is parametric while Spearman's rank correlation coefficient (Spearman's rho) was calculated to assess the correlations between MPV and various study parameters.

Receiver operating characteristic (ROC) curve analysis was used to identify optimal cut-off values of. < 0.05 was considered statistically significant (S), and $p \geq 0.05$ was considered none statistically significant (NS).

RESULTS

Comparing SVR and NR groups , NR group shows higher (CTP, APRI, and FIB-4) and platelets , albumin bilirubin levels ,While no differences between both groups regarding age , gender , BMI , HCV RNA viral load, ALT ,AST or ribavirin use , flow-cytometry analysis shown that FOX-p3+ve Tregs are significantly lower in the SVR group , +ve correlation between FOX-p3+ve Tregs and markers of liver disease severity scores (CTP , APRI, FIB-4) , ALT, AST, α-Fetoprotein but -ve correlation with Platelets count while no correlation with either Age, BMI, baseline HCV RNA viral load or MELD score after that multivariate regression analysis resulted had shown FOX-p3+ve Tregs as independent predictor for SVR in CHC patients with DAA therapy with cutoff value= 6.92% with sensitivity= 91% ,specifity= 98% ,+ve predictive value =98% and –ve predictive value = 36.4 % by ROC analysis (Table 2, 4, 5).

There is statistical significant difference between Non-Cirrhotic and both Cirrhotic child(A) , child (B) groups in liver functions like higher ALT, AST, α-Fetoprotein, and INR also higher (CTP , APRI and FIB-4 scores) but lower platelets count and albumin level while no significant difference regarding Age, gender, BMI, baseline HCV RNA viral load, by flow cytometry analysis we found that all Tregs subsets are significantly lower in Non-Cirrhotic compared to Cirrhotic CHC patients (Table 3).

Table 1. Demographics of the studied group.

	The studied Population (n=153)	
	No	%
Age (Years) *Median (Range)*	46±11	
Sex *Male* *Female*	 62 58	 52% 48%
Presence of cirrhosis *Non Cirrhotic* *Cirrhotic*	 103 17	 86% 14%
BMI (Kg/m^2) *Mean± SD*	29.7±6.1	
Diabetes *No* *Yes*	 102 18	 75% 15%
Hypertension *No* *Yes*	 107 13	 89.2% 10.8%
Ischemic heart No yes	 118 2	 97.5% 2.5%

Table 2. Comparison between SVR and NR population.

		SVR	NR	Test	P
Age		46.73±11.54	49±12.23	-0.535*	0.594
Sex	Male	58 (52%)	4 (50%)	0.010**	0.603
	Female	54 (48%)	4 (50%)		
BMI		29.6±6.01	30.65±6.27	-0.436*	0.664
HCV viral load (IU/ml)		363734±571889	518279±404959	-0.427*	0.670
Platelets (103/ µl)		195.52±61.95	120.38±53.14	3.34*	0.001
Child-Pugh score		5.19±0.54	5.88±0.83	-3.861*	0.024
MELD score		5.59±1.47	6.15±1.04	-1.052*	0.295
APRI score		0.67±0.64	1.32±0.71	-2.35*	0.020
FIB- 4		1.98±1.55	3.62±2.26	-2.79*	0.006
CD4+ cells		73.55±6.53	74.19±8.34	0.333*	0.740
(CD25+ / CD4+)		15.40±2.53	15.70±2.34	0.356*	0.731
(CD25+ / Foxp3+)		5.61±1.35	7.55±1.66	-3.985*	0.002

* Numerical data calculated by T.test.
** Categorical data calculated by CHI-square test.
A p value <0.05 was considered statistically significant(S).

Table 3. Comparison between Non-Cirrhotic, Cirrhotic child (A) and Cirrhotic child (B) population.

		Non-Cirrhotic	**Cirrhotic Child (A)**	**Cirrhotic Child (B)**	**Test**	**P**
Age		46.58±12.03	49.83±8.28	46±7.84	0.436*	0.647
Sex	Male	56(54.3%)	4(33.3%)	2(40%)	2.189**	0.355
	Female	47(45.7%)	8(66.7%)	3(60%)		
BMI		29.68±6.1	29.51±5.25	29.65±6.97	0.004*	0.996
HCV Viral load (IU/m)		1204927± 1301844	738401± 541195	1163821± 1048369	0.820*	0.443
ALT (U/L)		50.73+26.39	74.92+26.13	51.40+14.3	4.99*	0.008
AST (U/L)		45.65+19.54	74.82+31.97	57.40+19.16	11.43*	0.001

Table Continue					
	Non-Cirrhotic	**Cirrhotic Child (A)**	**Cirrhotic Child (B)**	**Test**	**P**
Platelets(10^3/ µl)	205.73±55.04	109.85±39.9	89.8±31.49	28.24*	0.000
α-FETOPTN	5.25±3.22	8.69±4.49	9.60±3.64	9.224*	0.001
Child-Pugh score	5.11+0.31	5.58+0.51	7.00+0.23	85.96*	0.002
MELD score	5.55+1.51	6.01+1.1	6.25+1.09	0.963*	0.385
APRI score	0.62+0.38	1.94+1.02a	1.74+0.69a	46.34*	0.001
FIB- 4	1.68+1.06	4.59+2.4a	4.50+1.94a	36.71*	0.003
CD4+ cells	74.69+3.59	75.56+4.30	75.41+2.37	0.378*	0.686
(CD25+ / CD4+)	14.90+2.13	18.98+2.21a	17.55+2.73a	24.836*	0.002
(CD25+/Foxp3+)	5.38+1.09	8.07+1.05a	7.59+1.27a	35.374*	0.018

* Numerical data calculated by T.test.
** Categorical data calculated by one way Anova .
p-value <0.05 was considered statistically significant(S).

Table 4 Correlation of (CD25+ / Foxp3+) with HCV viral load and other laboratory biochemical parameters and liver fibrosis indices (by pearson-correlation coefficient) of study population (n=120).

	Total population (n=120)		
	r	P	
Age	0.08	0.382	(NS)
BMI	0.119	0.197	(NS)
HCV viral load	-0.014	0.877	(S)
ALT	0.237	0.009	(S)
AST	0.195	0.033	(S)
α.FETOPTIEN	0.190	0.038	(S)
Platelets count	-0.421	0.003	(S)
Child-Pugh score	0.406	0.004	(S)
MELD score	0.131	0.154	(NS)

r = Spearman's rank correlation coefficient
*= Pearson correlation coefficient
A *p* value <0.05 was considered statistically significant(S).

Table 5 Binary logistic regression analysis of best predictor for sustained virological response.

Variables in the Equation							
		B	Wald	Sig.	Exp(B)	95% C.I.for EXP(B)	
						Lower	Upper
Step 1[a]	BMI	-3.374-	.000	.989	.034	.000	9.765E±206
	cirrhosis	230.462	.001	.976	1.225E±100	.000	.
	Comorbid		.000	1.000			
	HCVCount	.000	.001	.982	1.000	.997	1.003
	ALT-c	-.300-	.000	.996	.741	.000	1.496E±051
	AST-c	.535	.000	.997	1.708	.000	7.868E±106
	α.fPTN	-16.506-	.001	.981	.000	.000	.
	PLT	1.837	.001	.978	6.280	.000	2.749E±056
	FIB4	-12.801-	.000	.993	.000	.000	.
	APRI	51.941	.000	.992	36118014	.000	.
	MELD	-40.473-	.001	.977	.000	.000	.
	CD4	-.655-	.000	.999	.519	.000	.
	CD25foxp3	-42.645-	.001	.978	.000	.000	.
	Constant	496.258	.000	.996	3.327E±215		
Step 12[a]	PLT	.030	5.477	.019	1.031	1.005	1.057
	CD25/foxp3	-1.771-	7.121	.008	.170	.046	.625
	Constant	8.899	3.682	.055	7327.895		

Table 6 ROC curve analysis shows "**Cutoff -level**" at which SVR was achieved.

Cut-off values	AUC	Asymptotic Sig. B	PPV % (95% CI)	NPV % (95% CI)	Sens. % (95% CI)	Spec. % (95% CI)
Total CD4 ≥74.19	0.531	0.780	94.5	14.6	95.3%	11.8%
CD4/CD25 ≥16.71	0.781	0.591	93.5	11.8	73%	50%
FOX.p3 Tregs ≥6.92	0.946	.0001	100	36.4	91%	98%

DISCUSSION

Unitt et al; 2005 stated that impaired T-reg functions are associated with many liver disease including viral hepatitis ,autoimmune liver disorders and increased incidence of hepatocellular carcinoma. [5]

Our study proved that there was expansion of different classes of T-reg cells in peripheral blood of CHC Patients this goes with **Barjon et al; 2015** who stated that during the natural course of chronic HCV infection, increased numbers of T-reg cells have been observed both in peripheral blood and the liver. [6]

In this study revealed a SVR rate about (93.6%), the previous is similar to what reported by **Webster DP et al; 2015** who informed that treatment with direct-acting antiviral (DAA) drugs, either alone or in combination with IFN-α, have achieved sustained virological response (SVR) in over 90% of treated patients. [7]

Comparison both SVR and NR groups had revealed that NR groups were higher in child-pough , APRI , FIB-4 scores ,While no differences between both groups regarding age , gender , BMI , HCV RNA viral load, ALT ,AST or ribavirin addition and that concordant to study by **Christoph Werner et al;2016** who considered sex, age, baseline viral load, and early virological kinetics were found not to be significant as predictors of SVR12 , thus costly "in-between" measurements of HCV viral load possibly are expendable. [8]

Although there was an increased value of ALT & AST in NR compared to SVR group but this increase wasn't statistically significant, in converse to **Billerbeck et al; 2007** who studied INF-associated SVR, reported that there was a significant increase in the value of ALT & AST in non-responder group compared to responder one.[9]

Regarding AFP level as an important marker of hepatic inflammation, our results showed marked difference in its level between SVR and NR groups this goes with **Hayashi et al; 2000** who stated that persistence of ALT elevation is a convenient marker for identifying an increased risk for HCC. In fact, the risk of HCC was significantly higher for SVR patients whose post-interferon treatment AFP or ALT levels were high (≥ 10 ng/mL and ≥ 40 U/L, respectively). [10]

Our results had shown that treatment with (SOF, DCV ± R) for 12 week achieved SVR percentage about (93.6%) this goes **Backus et al; 2015** who revealed that Patients with SOF, DCV ± R as combination partners (86%-93%) have been reported, containing hard-to-treat patients liver cirrhosis, portal hypertension and post-liver transplantation. [11]

Our Regression analysis showed that platelets number and expression ratio of FOX-p3 is an independent factors for treatment failure others showed Data on HCV genotype, liver disease severity, and first and second line DAA regimens were prospectively collected in consecutive patients who reached the 12-week post-treatment and retreatment evaluations from January 2015 to December 2016 in 23 of **The PITER network centers** revealed that Among 3,830 patients with advanced fibrosis (F3) or cirrhosis, 139 (3.6%) failed to achieve SVR. So that study concluded that ,Genotype 3, bilirubin levels >1.5mg/dl, platelet count <120,000/mm3 and the sofosbuvir ± ribavirin regimen (SOF±RBV) were independent predictors of failure by logistic regression analysis. [12]

Our results showed that NR group shows high T-reg levels and elevated serum alanine transferase as a marker of hepatic inflammation also T-regs may protect the host from liver destruction by suppressing immunoreactive T cells but in converse to our results, **Manigold et al; 2007** stated that the frequency of n-T-reg in chronic hepatitis was not related to the grade of inflammation or the serum level of ALT. [13]

Our study which compared pretreatment level of T-reg (BT) levels in both SVR Vs NR groups ,revealed that there is an elevated level of FOX-p3+ve T-regs (7.55±1.66%) in NR compared to SVR group (5.61±1.35%) then by ROC curve estimation that 6.92% cutoff level for can differentiate SVR and NR groups with sensitivity= 91% ,specifity= 100% this goes with INF-based study by **Burton et al; 2008** who stated

that The percentage of regulatory T cells was higher in non-responder patients, An increase in the percentage of T-regs during antiviral treatment was observed in both NR and SVR patients. The comparison of NR vs SVR at BT showed that NR had a significantly higher percentage of T-regs BT (NR: 3.42 ± 1.33% vs SVR: 2.49 ± 1.1%; P = 0.025). [(1)]

Our results revealed that addition of RBV don't affect SVR out of 112 patients 32 didn't receive RBV had achieved SVR compared to 4 out of 8 patients who received RBV didn't achieve SVR (p-value=0.208) , **Kumada et al; 2014** stated combination of sofosbuvir and daclatasvir DCV has been safe and effective, both, in previously treated and untreated HCV-patients with. In previously untreated HCV-infected patients, SVR-12 of 98 % was achieved with no significant impact of the duration of the treatment (12 wk vs 24 wk) or the addition of RBV. [(14)]

Also results by **Deterding et al; 2015** who stated that patients with liver cirrhosis show much lower response rates (87% with liver cirrhosis, 97% without; P = 0.005), and especially those with advanced portal hypertension (platelets <100/nL), or high MELD score (≥10) show significantly lower SVR12 rates than patients without (P < 0.0001, uni-and multivariate analysis). [(15)]

Marked differences in the T-reg counts were noticed between Non_Cirrhotic group, Cirrhotic child (A) and child (B) also there is strong positive correlation between levels of FOX-p3+ve T-regs and ALT, AST and AFP levels so ,There is a proven correlation between the number of T-reg and occurrence of hepatic injury and cirrhosis this explained by **Hartling et al; 2012** who informed that Persistent HCV-specific cytotoxic T-cell responses in the liver have been associated with the development of hepatic inflammation which may ultimately lead to liver cirrhosis. One of the potential mechanisms that might modulate hepatitis C virus (HCV)-specific immune responses is the inhibitory role of the regulatory T cells. [(16)]

No correlation between baseline FOX-P3+ve T-regs and baseline viral load (r = -0.014, P = 0.877) this in concordance **Cabrera et al; 2004** who informed that trend towards a positive correlation between HCV-RNA level and CD81 expression levels on CD19+ B lymphocytes was observed at baseline also the percentage of T-reg level at baseline did not correlate with baseline HCV-RNA levels . [(17)]The previous in converse with **Smyth MJ et al; 2006, Burton et al; 2008** whom had shown a positive correlation was detected between CD25 +ve CD4 T cell frequency and HCV RNA titer, whereas an inverse relation was found with liver inflammatory activity. They indicated that CD25+ CD4 T cells can respond directly to HCV antigens and suppress the HCV-specific immune response in a dose-dependent cell contact manner.[(18,1)]

But according to **Xu et al; 200**6 T-reg considered to be enemy to our immunity, the proof may be the positive correlation between the HBV or HCV viral load and upregulation of CD4+CD25+ T-reg count. [(19)]

In conclusion:

T-regulatory lymphocyte plays an important role in the natural course of HCV infection, response to treatment, and sequels of the infection with a strong correlation of the high levels of T-reg with the hepatic injury, cirrhosis in addition to the treatment failure.

Limitations of the study:

The high cost of the flow cytometry kits and no available studies on the post-treatment level of T-regs.

Declaration of interest
The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.
Funding information
None declared

REFERENCES

1. **Burton JR, Klarquist J, KyungAh Im , Sue Smyk-Pearson1 , Lucy Golden-Mason , Nicole Castelblanco et al ;** Prospective analysis of effector and regulatory CD4+ T cells in chronic HCV patients undergoing combination antiviral therapy. J Hepatol 2008; 49(3): 329–338.
2. **Ebinuma H , Nobuhiro Nakamoto ,Yun Li, David A. Price, Emma Gostick, Bruce L. Levine, et al.** Identification and in vitro expansion of functional antigen-specific CD25+ FoxP3+ regulatory T cells in hepatitis C virus infection. J Virol 2008; 82(10): 5043–5053.
3. **Akiyama M, Ichikawa T, Miyaaki H, Motoyoshi Y, Takeshita S, Ozawa E, et al.** Relationship between regulatory T cells and the

combination of pegylated interferon and ribavirin for the treatment of chronic hepatitis type C. Intervirology. 2010;53(3):154-60.

4. **Meissner EG, Bon D, Prokunina-Olsson L, Wei Tang, Henry Masur, Thomas R. O'Brien, et al.** IFNL4- DeltaG genotype is associated with slower viral clearance in hepatitis C, genotype-1 patients treated with sofosbuvir and ribavirin. J Infect Dis 2014; 209: 1700–4.
5. **Unitt E , Rush brook, Marshall A, Davies S, Gibbs P, Morris LS ,et al.,** Compromised lymphocytes infiltrate hepatocellular carcinoma: the role of T-regulatory cells. Hepatology 2005, 41(4):722–30.
6. **Barjon C, Dahlqvist G, Calmus Y, Conti F et al**; Role of regulatory T-cells during hepatitis C infection: From the acute phase to post-transplantation recurrence. Dig Liver Dis 2015; 47:913–917.
7. **Webster DP, Klenerman P, Dusheiko GM.** Hepatitis C. Lancet (London, England). 2015; 385 (9973):1124+35.
8. **Christoph R Werner, Julia M Schwarz, Daniel P Egetemeyr ,Robert Beck, Ulrich M Lauer ,** Christoph P Berg **et al;** Second-generation direct-acting-antiviral hepatitis C virus treatment: Efficacy, safety, and predictors of SVR12 World J Gastroenterol 2016 September 21; 22(35): 8050-8059 ISSN 1007-9327 (print) ISSN 2219-2840
9. **Billerbeck E1, Bottler T , Thimme R et al;** Regulatory T cells in viral hepatitis. World J Gastroenterol. 2007 Sep 28; 13 (36):4858-64.
10. **Hayashi J, Furusyo N, Ariyama I, Sawayama Y, Etoh Y, Kashiwagi S.** A relationship between the evolution of hepatitis C virus variants, liver damage, and hepatocellular carcinoma in patients with hepatitis C viremia. J Infect Dis 2000; 181: 1523-1527 [PMID: 10823749 DOI: 10.1086/315431]
11. **Backus LI, Belperio PS, Shahoumian TA, Loomis TP, Mole LA.** Real-world effectiveness of ledipasvir/sofosbuvir in 4,365 treatment naive, genotype 1 hepatitis C-infected patients. Hepatology. 2016 Aug;64(2):405-14.
12. **PITER network**, Incidence of DAA failure and the clinical impact of retreatment in real-life patients treated in the advanced stage of liver disease, Interim evaluations from the PITER network, PLOS One. 2017 Oct 4; 12(10):e 0185728.
13. **Manigold T, Eui-Cheol Shin, Eishiro Mizukoshi, Kathleen Mihalik, Krishna K. Murthy, Charles M et al;** Foxp3+CD4+CD25+ T cells control virus-specific memory T cells in chimpanzees that recovered from hepatitis C. Blood, 2006, 107(11):4424–32.
14. **Kumada H, Suzuki Y, Ikeda K, Chayama K, Karino Y, Toyota J et al;** Daclatasvir plus asunaprevir for chronic HCV genotype 1b infection. Hepatology 2014; 59: 2083-2091 [PMID: 24604476 DOI: 10.1002/hep.27113].
15. **Deterding K, Höner Zu Siederdissen C, Port K, Solbach P, Sollik L, Kirschner J, et al**; Improvement of liver function parameters in advanced HCV-associated liver cirrhosis by IFN-free antiviral therapies. Aliment Pharmacol 2015; 42: 889-901 [PMID: 26250762 DOI: 10.1111/apt.1334].
16. **Hartling HJ, J. C. Gaardbo, A. Ronit, L. S. Knudsen, H. Ullum, B. Vainer . et al**; CD4 (±) and CD8 (±) regulatory T cells (Tregs) are elevated and display an active phenotype in patients with chronic HCV mono-infection and HIV/HCV co-infection. Scand J Immunol. 2012; 76:294–305. [PubMed: 22671952].
17. **Cabrera R, Zhengkun Tu, Yiling Xu,Roberto J. Firpi, Hugo R. Rosen,Chen Liu, et al**; An immunomodulatory role for CD4 + CD25+ regulatory T lymphocytes in hepatitis C virus infection. Hepatology 2004;40:1062–1071
18. **Smyth MJ, Teng MW, Swann J, Kyparissoudis K, Dale I. Godfrey, Yoshihiro Hayakawa et al;** CD4+CD25+ T regulatory cells suppress NK cell-mediated immunotherapy of cancer. J Immunol 2006, 176: 1582-1587.
19. **Xu D , Junliang Fu, Lei Jin, Hui Zhang, Jing-Min Zhao, Bin Zhang et al.,** Circulating and liver residentCD4+CD25+regulatory T cells actively influence the antiviral immune response and disease progression in patients with hepatitis B. J Immunol, 2006,177(1):739–47.

To cite this article: Yousif MM, Sakr MM, Zaki SM, Sadek AM, Husseiny OI. Assessment of the Predictive Value of T-Regulatory Cells For The Response of Chronic Hepatitis C To Sofosbuvir Based Therapy.,Egypt.ZUMJ 2019;25(3);350-358,DOI: 10.21608/zumj.2019.7623.1035