Original Research Article

Sofosbuvir and Daclatasvir improve Platelet Factor 4 levels in Chronic Hepatitis C Patients

ABSTRACT

Background: Background: Egypt has the highest hepatitis C virus (HCV) prevalence worldwide, with an estimated overall prevalence of 21.9% among adults with predominance of genotype 4, so great efforts were directed to study its consequences and different lines of treatment of chronic HCV. One of its common sequences is decreased platelet count and impaired platelet function. DAAs are a new therapeutic line for chronic HCV infection proved its efficacy and safety. Sofosbuvir-daclatasvir are used in treatment of genotype 4 which is common in Egypt and reported some benefits to the patients after completing treatment such as improvement of platelet count and liver functions.

Objective: In our study we aimed to evaluate the effect of HCV on platelet function and to determine whether it is affected after treatment with sofosbuvir-daclatasvir. So we estimated PF4 which is a platelet derived molecule involved in inflammatory and immune processes and can be used as an indicator of platelet function in cases of chronic HCV patients before and after treatment and compare it with healthy controls.

Methods: This study was carried on chronic HCV patients who had been receiving DAAs (sofosbuvir-daclatasvir) at Internal Medicine Department at Tanta University Hospital over a period of 6 months (between February 2020 to August 2020). The study was performed on 25 patients with chronic HCV infection and 25 normal volunteers as a control group. All patients were subjected to history taking, examination, investigations (CBC, liver functions, renal functions, pelvi abdominal ultrasound, TSH, AFP, PCR for HCV and PF4 by ELISA).

Results: A significant decrease in PF4 was observed in chronic HCV patients which increased after treatment with sofosbuvir and daclatasvir.

Conclusion: Chronic HCV infection is associated with decreased serum level of platelet factor 4 (which is an indicator of platelet function). After treatment with DAAs (sofosbuvir-daclatasvir) serum level of platelet factor 4 increases. This means that chronic HCV infection is associated with impaired platelet function which improved after treatment with DAAs (sofosbuvir-daclatasvir)

Keywords: hepatitis C virus, antithrombin

INTRODUCTION

Egypt has the highest hepatitis C virus (HCV) prevalence worldwide, with an estimated overall prevalence of 21.9% among adults.[1]

HCV-induced inflammatory and immunological phenomena in the liver tissues are assumed to be directly responsible for in-vivo platelet activation.[2]

Accordingly, activated platelets are considered to play a significant role in the pathogenesis of liver damage by stimulating fibrogenesis and mitogenesis of the liver cells.[3]

Platelet factor 4 (PF4), also known as chemokine CXCL4, is released from the alpha-granules of activated platelets as a complex with a chondroitin sulfate proteoglyan carrier.[4]

It disappears rapidly from plasma as it transfers to higher affinity heparin sulfate on endothelial cells, inhibiting local antithrombin (AT) activity and thus promoting coagulation.[5]

Generally, the role of platelets in the pathophysiology of viral diseases, such as inflammation, vasculogenesis, and tissue repair, might lead to a relatively better understanding of platelet function disorders in viral liver cirrhosis.[6]

Several oral DAA combination regimens for the treatment of genotype 4 HCV were evaluated in many studies, which reported high SVR rates with few side effects.[7, 8]

Based on a multi-center prospective study, combined sofosbuvir plus daclatasvir with or without ribavirin for12–24 weeks appears to have favorable outcomes with high rates of sustained virological response and safety profile in the treatment of Egyptian patients with genotype 4 HCV infection.[9]

AIM OF THE WORK

The aim of this work was to assess the effect of sofosbuvir plus daclatasvir with or without ribavirin on PF4 as a marker of platelet activation.

PATIENTS AND METHODS

The study was carried out on 25 patients who were positive for HCV by PCR. The patients were recruited from the outpatient clinic and wards of Internal Medicine Department, Tanta University Hospital between the periods of February 2020 to August 2020. Also 25 healthy volunteers were enrolled as a control group. An informed written consent was obtained from all participants in this research. The study was approved by the ethical committee of Tanta University, Faculty of Medicine under number (31995/12/17).

Inclusion criteria: Patients aged ≥ 18 years who were positive for HCV by polymerase chain reaction (PCR).

Exclusion criteria: Patients with any of the following were excluded from the study: other types of hepatitis (e.g HBV or autoimmune hepatitis), platelet count < 100×10^3 cell/mm^3, Child **-** Pugh score B and C, renal impairment (serum creatinine >2.5 mg/dL or estimated glomerular filtration rate <30 mL/min/1.73 m^2), hepatocellular carcinoma, thyroid disease, elevated serum level of alpha-feto protein, or patients receiving anti-platelet drugs e.g. aspirin, clopidogril. Pregnant females were also excluded.

Methods:

All the participants have been subjected to: Full history taking, thorough clinical examination, laboratory tests e.g: complete blood count (CBC). PCR for HCV before treatment and the end of treatment, aspartate aminotransferase (AST), alanine aminotransferase (ALT), serum albumin, serum bilirubin, International normalized ratio (INR), Alpha-feto protein, blood urea, serum creatinine, TSH and PF4 by ELISA (we used a double sandwich ELISA to assay the level of PF4 in samples. By adding PF4 to monoclonal antibody enzyme which is pre-coated with PF4 monoclonal antibody, incubation, then adding PF4 antibodies labeled with biotin, and combined with streptavidin-HRP to form immune complex, then carrying out incubation and washing to remove the uncombined enzyme. Then adding chromogen solution, A and B, the color of the liquid changes into blue and at the effect of acid, the color finally becomes yellow. The Chroma of color and the concentration of PF4 of sample were positively correlated). Pelvi-abdominal ultrasound was performed to determine the state of the liver**)**

Treatment regimens

All the recruited patients were non-cirrhotic and treatment naive patients, they were treated with Sofosbuvir 60 mg /d orally plus Daclatasvir 400 mg /d orally for 12 weeks. Ribavirin was not added to the treatment protocol as none of the patients were cirrhotic nor treatment experienced. Treatment duration was extended for 3 months after which PCR for HCV was repeated to evaluate response.

Follow up of the patients

Follow-up HCV RNA by PCR was done at week 4 and at 3 months (the end of therapy), while PF4 was assessed before the beginning of treatment and another reading at the end of treatment.

Statistical analysis of the data

Data were fed to the computer and analyzed using IBM SPSS software package version 22 (Armonk, NY: IBM Corp) Qualitative data were described using number and percent. The Kolmogorov-Smirnov test was used to verify the normality of distribution. Quantitative data were described using range (minimum and maximum), mean and standard deviation. Significance of the obtained results was judged at the 5% level. The following tests were performed: ANOVA test was used for comparison among more than two means in quantitative data, Chi-square was used to compare qualitative parameters. The correlation between PF4 and some prognostic markers was performed using the Pearson correlation test. Receiver Operating Characteristic (ROC) curve was used for assessment of sensitivity, specificity and best cutoff value for PF4. Univariate and multivariate analysis were estimated using Cox proportional hazard regression analysis.

RESULTS

The demographic data and presence of chronic diseases are reported in **Table (1).**

When we studied the laboratory parameters, there was a significant decrease of Hb level in patients after receiving treatment compared to control group and patients before receiving treatment (P2=0.001, P3=0.001 respectively), while there was no significant difference in the following parameters: platelet count, TLC, ALT, AST, Bilirubin, albumin and INR in the control group when compared to patients before and after receiving treatment. As for PF4, it was significantly lower in HCV patients before receiving treatment when compared to control group (P1 value = 0.001). After treatment, PF4 increased significantly when compared to pre-treatment levels (P3 value = 0.001). But there was no significant difference in PF4 in HCV patients after receiving treatment when compared to control group (P2 value=0.627) **Table (2).**

Negative significant correlation was observed between PF4 before treatment with age, ALT and PCR for HCV (P = 0.048, 0.045, 0.001 respectively). On the other hand, there was no significant correlation between PF4 before treatment and remaining studied laboratory parameters including Hb level, platelet count, TLC, AST, serum bilirubin, serum albumin and INR. After treatment, no significant correlation was observed between serum PF4 after treatment and any of the studied parameters **Table (3).**

Univariate analysis revealed that age, ALT and PCR for HCV were the most important effectors on PF4 (P values= 0.037, 0.025, 0.001, respectively) while in multivariate analysis only PCR was the independent factor affecting PF4 (P value= 0.015) **Table (4).**

On performing ROC curve analysis, the best cutoff value of PF4 was 120 (IU/nm^3), with 84%sensitivity and 60%specificity **Table (5), Figure (1).**

Table (1): Demographic data of participants and presence of chronic diseases.

Parameter		Cases	Control	Test	P value
Sex	Male (%)	11 (44%)	15 (60%)	X^2: 1.279	0.258
	Female (%)	14 (56%)	10 (40%)		
Age (years)	Mean± SD	49.36 ± 12.2	45.7 ± 10.2	T: 1.147	0.256
DM	Yes/ No	0/25	0/25	–	–
HTN	Yes /No	2/23	0/25	2.079	0.149
Cardiac diseases	Yes /No	0/25	0/25	–	–
Thyroid diseases	Yes/ No	0/25	0/25	–	–
Cirrhosis	Yes /No	0/25	0/25	–	–

DM: diabetes mellitus. HTN hypertension.

Table (2): Comparison between some laboratory parameters in controls and patients (before and after receiving treatment)

Parameter	Control	HCV+ve before TTT	HCV+ve after TTT	F. test	p. value	
Hb (gm/dl)	13.06 ± 1.29	13.16 ± 1.20	11.65 ± 1.12	7.254	0.001*	P1= 0.778 P2= 0.001* P3= 0.001*
PLT ($\times 10^3/mm^3$)	251.63± 74.35	265.48 ± 80.39	277.96 ± 85.96	0.961	0.402	P1= 0.530 P2= 0.310 P3= 0.598
WBCs ($\times 10^3/mm^3$)	6.94 ± 1.99	6.98 ± 2.02	7.30 ± 2.09	0.526	0.684	P1= 0.937 P2= 0.526 P3= 0.528
ALT (unit /l)	24.53± 6.39	22.48 ± 6.90	22.56 ± 7.73	0.638	0.497	P1=0.281 P2= 0.332 P3= 0.969
AST (unit /l)	28.61 ± 7.93	27.12 ± 8.04	29.28 ± 9.08	0.597	0.524	P1= 0.513 P2= 0.782 P3=0.378
Bilirubin (gm/dl)	0.86 ± 0.16	0.90 ± 0.19	0.94 ± 0.18	0.743	0.297	P1= 0.425 P2= 0.114 P3= 0.414
Albumin (mg/dl)	4.13 ± 0.41	4.09 ± 0.45	4.06 ± 0.38	0.532	0.625	P1=0.744 P2= 0.534 P3= 0.786
INR	1.07 ± 0.07	1.04 ± 0.08	1.02 ± 0.07	1.012	0.236	P1= 0.165 P2= 0.162 P3=0.970
Urea	25.13 ± 6.29	26.52 ± 7.96	24.96 ± 7.08	0.532	0.618	P1=0.49 P2=0.92 P3=0.46
Creatinine	0.96 ± 0.20	1.02±0.21	1.3 ± 0.20	1.117	0.298	P1=0.30 P2=0.16 P3=0.78
PF4 (IU/nm^3)	195.8 ± 66.79	111± 54.2	186.75± 64.03	10.33	0.001*	P1=0.001* P2= 0.627 P3= 0.001*

P1: P value between control group and positive HCV cases before receiving treatment.
P2: P value between control group and positive HCV cases after receiving treatment.

P3: P value between Positive HCV cases before and after receiving treatment.
Hb: Heamoglobin, Plt. Platelets, WBCs: white blood cells.
*: statistically significant.

Table (3): Correlation between PF4 in HCV patients before and after receiving treatment with age and other laboratory data.

PLT factor 4	Before treatment		After treatment	
	R	P	R	P
Age	-0.396	0.048*	0.353	0.083
Hb	-0.309	0.133	-0.292	0.157
Plt	0.103	0.625	-0.172	0.411
WBCs	-0.244	0.239	0.035	0.869
ALT	-0.397	0.045*	0.205	0.326
AST	0.218	0.295	0.229	0.272
Bilirubin	-0.016	0.939	-0.244	0.240
Albumin	-0.067	0.750	-0.016	0.941
I.N.R	0.191	0.360	0.018	0.931
Urea	0.062	0.768	0.053	0.802
Creatinine	0.184	0.378	0.087	0.680
PCR	-0.627	0.001*		

r: Spearman coefficient

*: Statistically significant at $p \leq 0.05$

Hb: Hemoglobin, Plt: Platelets, WBCs: white blood cells, ALT: alanine aminotransferase. AST: aspartate aminotransferase. INR: international normalized ratio, PCR: Polymerase chain reaction.

Table (4): Univariate and multivariate analysis of Age and other laboratory parameters.

	Univariate		Multivariate	
	OR (95% CI)	**P value**	**OR (95% CI)**	**P value**
Age	**1.954 (1.201 – 6.352)**	**0.037***	**2.951 (0.589 – 8.561)**	**0.129**
Hb	**0.632 (0.185 – 4.326)**	**0.109**		
Plt	**0.715 (0.352 – 3.265)**	**0.328**		
WBCs	**0.857 (0.268 – 8.521)**	**0.218**		
ALT	**2.634 (1.854 – 9.852)**	**0.025***	**1.529 (0.267 – 4.526)**	**0.095**
AST	**1.302 (0.824 – 4.329)**	**0.367**		
Bilirubin	**0.324 (0.109 – 2.638)**	**0.542**		
Albumin	**1.965 (0.589 – 3.634)**	**0.297**		
I.N.R	**2.521 (0.749 – 9.327)**	**0.197**		
Urea	**0.697 (0.198 –8.521)**	**0.362**		
Creatinine	**0.559 (0.052–12.305)**	**0.281**		
PCR	**3.652 (2.318 – 8.564)**	**0.001***	**1.643 (1.109 – 5.631)**	**0.015***

Hb: Hemoglobin, Plt: Platelets, WBCs: white blood cells, ALT: alanine aminotransferase. AST: aspartate aminotransferase. INR: international normalized ratio, PCR: Polymerase chain reaction.

*: Statistically significant at $p \leq 0.05$

Table (5): Receiver Operating characteristics curve (ROC curve) for PF4 as an indicator of platelet function in HCV patients.

	Cutoff	AUC	Sensitivity	Specificity	PPV	NPV	Accuracy	P value
PLT factor 4	120	0.818	84	60	68	79	72	0.001*

AUC: area under the curve. NPV: negative predictive value. PPV: positive predictive value.

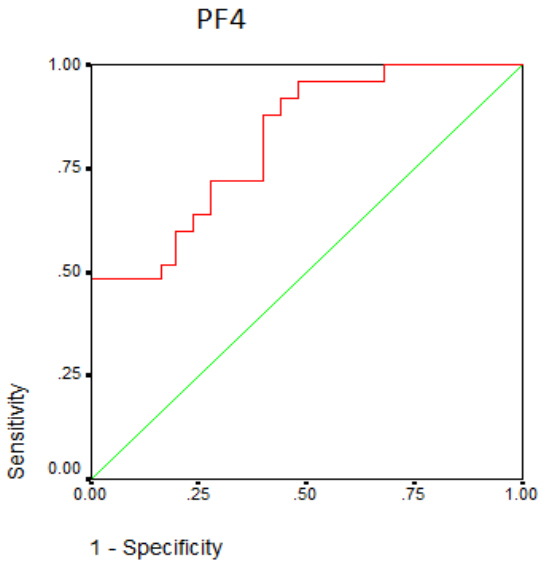

Figure (1): Receiver Operating characteristics curve (ROC curve) for PF4 as an indicator of platelet function in HCV patients.

DISCUSSION

Globally, Hepatitis C Virus (HCV) is a leading cause of liver cirrhosis and hepatocellular carcinoma. It affects 180 million people worldwide; about 3% of the world population. Egypt has the highest prevalence of HCV. The number of annual new infection is about 150 000. Genotype 4 is the predominant genotype in Egypt; as it represents 90% of infected Egyptians.[2, 3]

In 2011, the emergence of Directly Acting Antivirals (DAAs), represented a major advance in treatment of HCV.[4] These drugs targeted the viral proteins implicated in viral replication. Among DAA drugs, sofosbuvir and daclatasvir showed promising efficacy and may even bring some unexpected benefits to the patients such as improvement of liver functions, platelet count, child pugh score in some cases.[5]

Sofosbuvir is a nucleotide analogue which is HCV NS5B polymerase inhibitor and daclatasvir is HCV NS5A replication complex inhibitor. Both drugs achieved high efficacy and safety in genotype 1, 2, 3 and 4 when administered orally once daily in a dose of 60 mg and 400 mg, respectively for 12 or 24 weeks[5, 6, 7, 8, 9, 10]

Patients with chronic HCV infection are known to have acquired platelet dysfunction, which is proposed to be implicated in the development of variceal bleeding which is a major cause of morbidity and mortality in chronic HCV patients [4]. Several steps of platelet activation were shown to be impaired, attributed to both intrinsic and extrinsic defects resulting in platelet hypofunction; however, the underlying mechanisms remain speculative [11].

Recent analysis of the secreted platelet proteom, upon platelet activation, has detected numerous chemokines including ligand 4 (CXCL4) known as Platelet factor 4 which was used as an indicator of platelet function in previous studies.

The main objective of our study was to assess the effect of direct acting antiviral treatment (sofosbuvir-daclatasvir) on PF4 as an indicator of platelet function.

Our study was conducted on 25 chronic HCV patients receiving direct acting antiviral drugs (sofosbuvir-daclatasvir) for 12 weeks at Tanta University Hospital and 25 normal control subjects.

For demographic data our study population included 25 chronic HCV cases, 14 females and 11 males. Age of patients was in the range of 29 to 82 years and 25 normal control subjects, 15 males and 10 females. Age of the controls was in the range of 20 – 72 years. In our study HCV infection affects different age groups and this was in agreement with (El-Ghitany at al, 2019) [12] who reported that HCV infection affects different age groups but its prevalence increases dramatically with age. In our study there was female predominance with a percentage of 56% in contrast to (El-Ghitany et al, 2019) [12] that reported male predominance with a percentage of 54.5% from total number of 1625 HCV antibody +ve patients.

In our study there were no diabetic, cardiac, thyroid nor cirrhotic patients. There were 2 hypertensive patients in +ve HCV cases. In normal control subjects they were free from any chronic disease.

There was insignificant difference between our normal control subjects and +ve HCV cases for laboratory data while other studies reported increased levels of AST, ALT, bilirubin, INR and decreased albumin level in +ve HCV cirrhotic patients when compared to normal control subjects such as (Elsharkawy et al. 2017)[13] & (Mohamed et al. 2021)[14] and this may be due to involvement of larger number of positive HCV cases in these studies and involvement of many cirrhotic patients (Child B,C) among these cases, while in our study the included cases were all non-cirrhotic.

When we assessed the effect of sofosbuvir and daclatasvir on different laboratory data we found a significant decrease of Hb level after treatment with sofosbuvir and daclatasvir and when compared to normal control subjects. This was in agreement with (Abdel-Aziz et al. 2021) [15], (Emilio et al, 2018) [16], (Haiyan et al, 2020) [17] and (Elsharkawy et al. 2017) [13], Meanwhile, there was insignificant difference of platelet count after receiving treatment in contrast to (Abdel-Aziz et al. 2021) [15], (Soliman et al, 2021) [18] & (Elsharkawy et al. 2017) [13] who documented an increased platelet count after receiving treatment. In our study there was no significant change of other studied laboratory data i.e. TLC, ALT, AST, bilirubin, albumin, and INR after receiving treatment. Meanwhile, other studies as (Abdel-Aziz et al. 2021) [15] reported increased levels of serum bilirubin after treatment with sofosbuvir and daclatasvir. Also (Elsharkawy et al. 2017) [13] documented that there was a rise in bilirubin and INR after treatment. In contrast, (Deterding et al. 2021) [19] documented that there was a significant improvement of liver functions i.e AST, ALT, bilirubin, albumin and INR after treatment with sofosbuvir and daclatasvir. (Mohamed et al. 2021)[14] as well, reported improved liver functions after sofosbuvir-daclatasvir treatment. This may be due to involvement of larger number of cases in this study and presence of cirrhotic patients among these cases.

Several studies reported the efficacy of DAAs with sustained virological response in the majority of cases. (Ali et al. 2021)[20] reported that more than 96.3 % of cases achieved SVR 12 weeks after treatment with sofosbuvir and daclatasvir. (Ahmed et al. 2021)[21] reported that 96% have achieved SVR 12 weeks after treatment with sofosbuvir and daclatasvir. Also (Elsharkawy et al. 2017)[13] reported 93.4% had achieved SVR after treatment. This was in agreement with our study which denoted that 100% of cases had achieved SVR 12 weeks after receiving treatment.

In our study we used PF4 as an indicator of platelet function and we observed that there was significant decrease in serum levels of PF4 in cases of chronic HCV infection before receiving treatment when compared to normal control subjects (P value = 0.001). There is no any previous study to assess effect of chronic HCV infection on PF4, but (Drescher et al. 2021)[22] reported that PF4 significantly decreased in patients and mice with acute liver injury. Other studies such as (Sirvastava et al, 2008)[23] documented increased levels of PF4 in other infections as an inflammatory mediator e.g. Experimental cerebral malaria. Also, (Ojha et al, 2019)[24] reported increased level of PF4 in dengue and Japanese encephalitis virus infection in which PF4 promotes rapid replication and propagation of the virus. (Nielsen et al. 2021)[25] reported platelet dysfunction with HCV infection using a multiple Platelet function Analyzer, assessing aggregation after stimulation with adenosine diphosphate test (ADP test), arachidonic acid (ASPI test), ristocetin in high concentration (RISTO test), and thrombin receptor agonist peptide (TRAP test). Also,

(Ghozlan et al. 2014)[11] reported platelet hypofunction (using platelet function analyzer-100) in chronic HCV cases compared to normal control subjects.

Also we noticed a significant increase in PF4 after receiving treatment with sofosbuvir and daclatasvir (P value 0.001). There are no previous studies to assess the effect of sofosbuvir and daclatasvir on PF4. But, (Nielsen et al, 2021)[25] reported improvement of platelet functions after receiving treatment with DAAs including sososbuvir and daclatasvir regimen.

We assessed the effect of several factors on PF4 in +ve HCV cases and we found that there were some factors (Age, ALT and PCR for HCV) having a negative significant relationship with the value of PF4 in cases of HCV before receiving treatment (P values = 0.048, 0.045, 0.001 respectively). Univariate analysis of the studied parameters revealed that the same 3 factors (age, ALT and PCR for HCV were the most important effectors on platelet factor 4. On the other hand, multivariate analysis of these factors revealed that only PCR for HCV was the independent factor affecting platelet factor 4, while there was insignificant relation between these factors and the change of PF4 after receiving treatment.

Our study revealed that at cutoff value of 120 (IU/nm^3) the sensitivity of PF4 was 84%, specificity was 60%, Positive predictive value was 68, negative predictive value was 79, Accuracy of the test was 72%. (P value= 0.001).

CONCLUSION

PF4 can be used as an indicator of platelet function in chronic HCV infection as its level was lower than normal in positive HCV cases and improved with DAAs with an 84% sensitivity and 60%specificity at a cutoff value of 120 (IU/nm^3).

COMPETING INTERESTS DISCLAIMER:

Authors have declared that no competing interests exist. The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

REFERENCES

1. **Guerra J, Garenne M, Mohamed MK, et al.** HCV burden of infection in Egypt: results from a nationwide survey. J Viral Hepat. 2012; 19:560–567.
2. **Witters P, Freson K, Verslype C, et al.** Blood platelet number and function in chronic liver disease and cirrhosis. Aliment PharmacolTher 2008; 27:1017–1029.
3. **Panasiuk A, Zak J, Kasprzycka E, et al.** Blood platelet and monocyte activations and relation to stages of liver cirrhosis. World J Gastroenterol 2005; 11:2754–2758.
4. **Stringer SE, Galagher JT.** Specific binding of the chemokine platelet factor 4 to heparin sulfate. J BiolChem 1997; 272:20508–20514
5. **Sachais BS, Higazi AA, Cines DB, et al.** Interactions of platelet factor 4 with the vessel wall. SeminThrombHemost. 2015; 30:351–358.
6. **Witters P, Freson K, Verslype C, et al.** Blood platelet number and function in chronic liver disease and cirrhosis. Aliment PharmacolTher 2008; 27:1017–1029.

7. **El Raziky M, Gamil M, Hammad R, et al.** Treatment of hepatitis C genotype 4 patients with simeprevir and sofosbuvir: preliminary results from a phase IIa, partially randomised, open-label trial conducted in Egypt (OSIRIS). Hepatology. 2015; 62:145.
8. **Asselah T, Hassanein TI, Qaqish RB, et al.** Efficacy and safety of ombitasvir/ paritaprevir/ ritonavir co-administered with ribavirin in adults with genotype 4 chronic hepatitis C infection and cirrhosis (Agate-I). Hepatology. 2015; 62:119.
9. **Ahmed O, Elsebaey M, Fouad M, et al.** Outcomes and predictors of treatment response with sofosbuvir plus daclatasvir with or without ribavirin in Egyptian patients with genotype 4 hepatitis C virus infection. Infection and Drug Resistance. 2018; 11: 441–445.
10. **Asmaa, Naglaa Allam, Aisha Elsharkawy, et al.** Hepatitis C Infection in Egypt: Prevalence, Impact and Management Strategies. Hepat Med. 2017; 9: 17–25
11. **Ghozlan M, Saad A, Eissa D, et al.** Evaluation of platelet dysfunction in viral liver cirrhosis (relationship to diseaser severity). Ehj.net. 2013; 2: 63-65
12. **El-Ghitany E, Farghaly A.** Geospatial epidemiology of hepatitis C infection in Egypt 2017 by governorate. Heliyon. 2018; 5:260-263
13. **Elsharkawy A, Eletreby R, Fouad R, et al.** Impact of different sofosbuvir based treatment regimens on the biochemical profile of chronic hepatitis C genotype 4 patients. Expert Review of Gastroenterology & Hepatology. 2017; 11: 773-778
14. **Mohamed S, Amr SH, Mohamed M, et al.** Sofosbuvir and Daclatasvir Plus Ribavirin Treatment Improve Liver Function Parameters and Clinical Outcomes in Egyptian Chronic Hepatitis C Patients. National library of medicine. 2021; 29: 1368-1372.
15. **Abdel-Aziz, Asmaa M, Mohamed A, et al.** Effect of Sofosbuvir plus Daclatasvir in Hepatitis C Virus Genotype-4 Patients: Promising Effect on Liver Fibrosis. Journal of clinical and experimental hepatology. 2021; 8: 15-22.
16. **Emilio M, Héctor M, Antonio C, et al.** Anaemia predictors in patients with chronic hepatitis C treated with ribavirin and direct-acting antiviral agents. European Journal of Hospital Pharmacy. 2018; 25:132-137
17. **Haiyan Z, Lei L, Zhouhua H, et al**. Direct-acting Antiviral in the Treatment of Chronic Hepatitis C: Bonuses and Challenges. Int J Med Sci. 2020; 17:892-902
18. **Soliman Z, El Kassas M, Elsharkawy.** Improvement of platelet in thrombocytopenic HCV patients after treatment with direct-acting antiviral agents and its relation to outcome. Platelets. 2021; 32:383-390.
19. **Deterding KC.** Höner zu Siederdissen, K. Port, et al. Improvement Of Liver Function Parameters In Advanced HCV-Associated Liver Cirrhosis By IFN-Free Antiviral Therapies. National library of medicine. 2021; 42:889-901.
20. **Alaa Aboelela Hussein, Emad Farah, Mohamed Kholef, et al.** Effect Of Sofosbuvir Plus Daclatasvir On Virological Response And Liver Function Tests As A Line Of Treatment For HCV Related Cirrhosis (A Prospective Cohort Study). Egyptian liver journal. 2020; 27: 33-36.
21. **Ahmed, Ossama Ashraf, Eslam Safwat, et al**. Sofosbuvir Plus Daclatasvir in Treatment of Chronic Hepatitis C Genotype 4 Infection in A Cohort Of Egyptian Patients: An Experiment The Size Of Egyptian Village. International journal of hepatology. 2018; 19:125-128.
22. **Drescher C, Hannah K, Elisa FB, et al.** Platelet Factor 4 Attenuates Experimental Acute Liver Injury In Mice. Front. Physiol. 2019; 12: 345-350.
23. **Kalyan Srivastava, Ian A. Cockburn, AnneMarie Swaim, et al.** Platelet Factor 4 Mediates Inflammation in Experimental Cerebral Malaria. Cell Host & Microbe. 2008; 4: 179-187
24. **Ojha A, Bhasym A, Mukherjee S, et al.** Platelet factor 4 promotes rapid replication and propagation of Dengue and Japanese encephalitis virus. EBioMedicine. 2019; 3: 332-347.

25. **Nielsen T, Nick K, Sofie J, et al.** Impaired Platelet Aggregation and Rebalanced Hemostasis in Patients with Chronic Hepatitis C Virus Infection. International journal of molecular sciences. 2017; 18: 153.