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MOLECULAR AND BIOCHEMICAL STUDIES ON HEPATITIS C PATIENTS RELAPSED AFTER SOFOSBUVIR TREATMENT

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ABSTRACT

Hepatitis C viral infection is one of the most common diseases in Egypt in the 20th century that leaded to liver diseases such as liver cirrhosis or hepatocellular carcinoma (HCC). sofosbuvir is one of the most successful direct-acting antiviral (DAA) that play an important role in treatment of HCV. Fifty thousand patients with HCV were admitted in Mabaret El-Asafra hospital, Alexandria, Egypt, during a duration period extended from Oct 2015 to Oct 2017. All patients treated with sofosbuvir and ribavirin for 6 months as first line therapy according Egyptian health authorities. At end treatment period with first line of therapy during the follow-up checking detected HCV by quantitative PCR in seventy patients. Liver enzymes for seventy patients were estimated after relapse. Seventy patients were treated with combined therapy sofosbuvir and simeprevir as second line therapy. Liver enzymes and PCR were estimated after treated with second line therapy. All our cohort treatment experienced was between 1st and 2nd therapy including monitoring for their liver functions after 1st treatment, during relapse period and after receiving the 2nd therapy. Patients that were treated with combination of sofosbuvir and ribavirin at the first therapy achieved SVR after 24 weeks, a decrease in liver functions (ALT & AST) 94% achieving normal values, while 6% had mid elevation in ALT; 84% had normal values of AST and 16% had abnormal results with a negative PCR results. After following up (4 w to 48w) 36 % had normal ALT, 64% had abnormal results; 11.2 % had normal AST, 89% were abnormal. Patients were relapsed in 48weeks, and their viral load ranged from 7.0 X 10^1 to 9.90 X 10^6IU/m. After receiving the 2nd therapy, patients achieved SVR in a period ranged from 4 weeks to 12 weeks with no apparent changes in ALT, AST values, while the viral load was negative in all patients. Combination therapy (sofosbuvir and simeprevir) for 12 weeks in patients with genotype 4 infection is an effective regimen with an overall SVR rate of 100%.

Keyword: Hepatitis c Virus; sofosbuvir; simeprevir; ribavirin and HCV relapsed patients.

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INTRODUCTION

Hepatitis C virus (HCV) infection is one the most causes of chronic liver disease worldwide[1]. The long period of HCV infection is leaded to extensive liver fibrosis and liver cirrhosis with or without hepatocellular carcinoma (HCC). The number of chronically infected persons worldwide is estimated to be about 180 million , but most are not known of their infection[2]. Hepatitis C viral infection is endemic in Egypt with the highest distribution rate in the world. It is known that the treatment of antischistosomal to mass population with tartar emetic injections (from 1950s to 1980s) led to widespread infection by reusing the same medical equipment's. In the Nile Delta the infection rates were around 26% while in the Upper Egypt the infection rates were around 28% relatively [3]. HCV genotype 4 strains were around 20% of all cases of chronic HCV infection worldwide. There are around 15% of population in Egypt, where estimated may have chronic hepatitis C, over 90% of the infections have been reported to be HCV genotype [4-7]. The HCV was transmitted by three main methods, as well as other blood borne HCV [8]. First HCV infection transfers through blood contact from infected person to normal person or by using the same tools of the infected person such as shaved tools, tattoo tools or transporting of blood. Second, the poor sterilization techniques which led to high frequency of HCV and other blood borne diseases that transmitted to people that work in these places. Third is reuse of the same medical equipment between

people such as injection syringes, which was one of the major causes of many diseases' transmission such as HCV from 1950s to 1980 and epidemic HIV in the in the early- to mid-1980s [9].

Complications of HCV

Hepatitis C viral infection is one of the most causes of liver disease. It is major significant "precursor" for fibrosis, cirrhosis, and ultimately, hepatocellular carcinoma, but it is important to understand this is only in long-term, chronic cases[8 and10]. 85% of Egyptian patients whose infected with HCV still life, leading to chronic hepatitis [8 and11]. Hepatocellular carcinoma, Liver cirrhosis, liver failure, hematemesis from esophageal varies and hepatic encephalopathy are the major causes of death [8 and 12]. HCV may complicate the course of sadomasochistic and vice versa with a perhaps synergistic effect. A long-term study showed that complications occurred at a much faster rate in those with co infection with around 48% having cirrhosis, compared with 15% in those who had HCV alone and 0% in the group with schistosomiasis alone [8]. The aim of the present study was to evaluation the first line therapy (sofosbuvir and ribavirin) used for treatment HCV patients according Egyptian health authorities and estimation used combination between (ofosbuvir and simeprevir) for treatment relapsing cases.

MATERIALS AND METHODS

Description of Cases study

Fifty thousand patients with HCV were admitted in Mabaret El-Asafra hospital, Alexandria, Egypt, during a duration period extended from Oct 2015 to Oct 201. All patients were treated with sofosbuvir and ribavirin for 24 weeks as first line therapy according Egyptian health authorities. After completing 24 weeks from treatment with first line of therapy during the follow-up checking detected HCV by quantitative PCR in seventy patients. We study these cases with its history and the drug that they took it in the first line therapy and treated with second line therapy combination from (sofosbuvir and simeprevir) for 12 weeks. Liver enzymes (ALT and AST) data obtained from their previous results after first line therapy of treatment and recovery and it was in normal range.

Specimen: Human serum or plasma collected in the anticoagulant EDTA or serum Gel tube. Our samples were taken on serum Gel tubes to avoid any contamination. For virus detected 650µl used to PCR for each sample per test and 300µl for liver enzymes per test in every stage.

Estimation of liver enzymes

Liver enzymes were estimation during relapse period and after treatment with second line for 12 weeks. Samples were collected for each patient and measured their liver enzymes in the same day to keep stability of the enzymes. Biochemical tests measured using the full automated system of Roche/Hetachi Cobas c 311, Cobas c 501/502 analyzers [13].

Detection of HCV

HCV RNA was measured with Polymerase Chain Reaction Technique **(PCR)** using the full automated system of Roche Cobas AmpliPrep and COBAS TaqMan 48 Analyzer assay V.2.0 (lower limit of detection 15 IU/ml) [14 and 15]. The COBAS AmpliPrep/COBAS TaqMan HCV Test is an automated nucleic acid amplification test for the quantitation of HCV RNA in human serum or plasma, using the COBAS AmpliPrep Instrument for nucleic acid extraction and reagent preparation, and the COBAS TaqMan 48 Analyzer for amplification and detection. HCV-RNA levels were quantified with a lower limit of detection of 15 IU/ml at all sites. All patients' results after 1st and 2nd line of treatment was target not detected. During relapse all the levels were quantified with International unit per ml (IU/mL) [16].

RESULTS

Distribution of the studied patients according to the time to relapse

Result described the time to relapse as being the time between achieving complete response following the first line of therapy and the detection of the virus by quantitative PCR during the follow-up checking visits. The time to relapse of our 70 patients ranged from 4.0 to 48.0 weeks after completion of the 24 weeks therapy (sofosbuvir and ribavirin), with a mean

value of 16.97 ± 7.81weeks. The patients turned negative to HCV RNA after receiving the second line of therapy (sofosbuvir +simeprevir) at different time points that ranged from 4.0 to 12.0 weeks with a mean value of 11.26 ± 1.84weeks. Table (1)

Table (1): Distribution of the studied cases according to time of relapse after achieving complete response.

	Min. – Max.	Mean ± SD.
Time to relapse (week)	4.0 – 48.0	16.97 ± 7.81
Time to CR after the 2nd therapy(week)	4.0 – 12.0	11.26 ± 1.84

Relation between time to relapse and gender

Recorded data revealed that, the time to relapse ranged from 8.0 – 32.0weeks with the mean value of 16.36 ± 6.21weeks in males, whereas the time to relapse ranged from 4.0 – 48.0weeks with a mean value of 18.0 ± 10.01weeks in females. Statistical analysis showed no statistically significant difference in the time to relapse between males and females HCV patients. (p = 0.627). (Table 2)

Table (2): Relation between the gender and the time to relapse

Time to relapse (weeks)	Gender		U	*p*
	Male (n= 44)	Female (n= 26)		
Min. – Max.	8.0 – 32.0	4.0 – 48.0	533.0	0.627
Mean ± SD.	16.36 ± 6.21	18.0 ± 10.01		

U, *p*: U and *p* values for **Mann Whitney test** for comparing between the two groups

Distribution of the studied patients according to complete virological response (CR)

At end of 2ndtherapy.

by the complete virological response: a negative HCV viral load. Results showed that the virological response at 4th week (0 – 4) at end of 2nd therapy was 2.9%. Between the fourth and eighth week, (>4 – 8 weeks) CR was 12.9%. Between the eighth and the twelfth week, (>8 – 12 weeks) CR was 84.3%. By the twelfth week (>12 weeks) CR was achieved in all the patients, Figure (2).

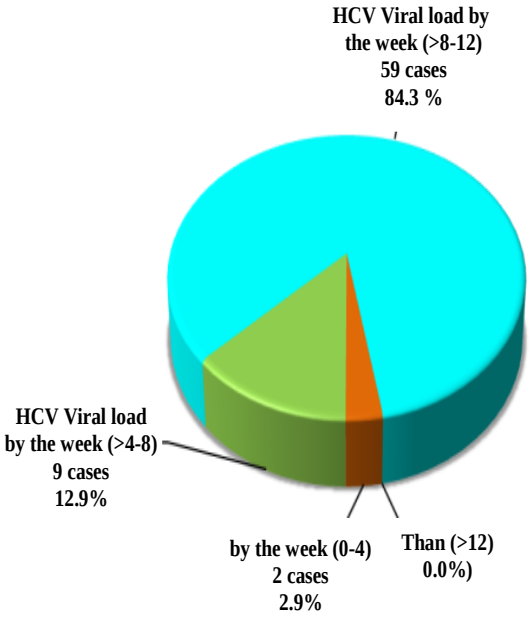

Figure (2): Distribution of the studied cases according to virological response (VR) at end of 2ndtherapy (n= 70)

Distribution of the studied cases according to the liver enzymes ALT and AST (U/L)

Integrity of the hepatic cells was assessed by assessing the liver enzymes serum AST and ALT. The normal range of the liver enzymes was:

ALT (Male: up to 41 and Female: up to 32), AST (Male: up to 40 and Female: up to 33).

A. At the end of 1st therapy (Sovaldi (Sofosbuvir) Plus Ribavirin)

Recorded data showed that the serum ALT level at the end of 1st therapy was within the normal range in 94.3% of the total patients with a mean of 17.55 ± 7.58 U/L, and a range of 7.0 to 41.0U/L, and in 44 males (62.85%) it ranged from 7 to 41 U/L with a mean of 18.32 ± 7.78 U/L, while in 22 females (31.42%) it ranged from 7.0 to 31 with a mean of 16.0 ± 7.08 U/L. In four (4) female patients representing 5.7% of the total patients, ALT level was elevated and a range of 33-40.0 U/L with a mean of

36.25 ± 2.87 U/L .AST level was within the normal range in 84.3% of the total patients with a mean of 20.56 ± 7.13U/L, and a range of 9.0 to 38.0U/L, in males with a mean of 22.27 ± 7.54 U/L, while in females it ranged from 11.0 to 27.0 U/L with a mean of 16.67 ± 4.07 U/L . In 15.7% of the patients, AST level was elevated with a mean of48.0 ± 7.16 U/L. In three (3) males patients representing 4.28%of the total patients, AST level was elevated and a range of 43-67 U/L with the mean of 52.33±12.86. In 8 female patients representing 11.4%, of the patients, AST was elevated and the ranged of 41 to 52 U/L with the mean of 46.38 ± 3.85 U/L Table (3), Table (4) & Table (5).

B. AST and ALT levels during relapse

It was found that the serum ALT level during relapse period within the normal range in25 patients in both males and females 35.7% with a mean of 35.52±5.29, and ranged from 32 to 41 in 20 males (28.5%) with a mean of 37.70 ± 2.77 U/L, while in 5 females (7.1%) it ranged from 22-30 U/L with a mean of 26.80 ± 3.56 U/L. In 45 of the patients (64.3%) were abnormal with a mean of 45.89±7.28 U/L of total patients, and in 24 males (34.3%) it ranged from 42 to 67 U/L with mean of 48.46 ± 6.87 U/L, while in 21 females (30%) it ranged from 33 to 56 U/L with the mean of 42.95 ± 6.73 U/L. AST level was within the normal range in 8 patient's (11.42%) both males and females ranged from 26 to 40 U/L with a mean of 34.25 ± 4.89U/L, and it ranged in 5 males (7.14%) from35 to 40 U/L with a mean of 37.40 ± 2.30, while it ranged in 3 females (4.28%) from 26 to 31 U/L with a mean of 29.0 ± 2.65. AST level was with abnormal range during relapse period in 62 of patients in both males and females (88.50%) with a mean of 50.27 ± 8.60 U/L ranged from 38 to 81 U/L of total patients. And it ranged in 39 males (55.71%) from 41 to 81 with a mean of 50.95 ± 8.76U/L, while it ranged in 23 females (32.85%) from 38 to 65 U/L with a mean of 49.13 ± 8.41 U/L . Table (3), Table(4)& Table (5).

C. AST and ALT at the end of 2nd therapy (Sovaldi(Sofosbuvir) Plus Sempirvir)

It was clear that the serum ALT level at the end of 2nd therapy was within the normal range in (94.3%) of the total patients with a mean of 19.95 ± 6.59 UL, and a range of 9.0 to 41.0U/L, and in 43 males (61.42%) it ranged from 9 to 41 U/L with a mean of 20.21 ± 7.20 U/L, while in 23 females (32.85%) it ranged from 12 to 29 with a mean of 19.48 ± 5.38 U/L. In three (3) female patients representing (4.28%) of the patients, ALT level was elevated and a range of 33-41.0 U/L with a mean of 37.67 ± 4.16 U/L .AST level was within the normal range in 84.3% of the total patients with a mean of 24.80 ± 7.10U/L, and a range of 12.0 to 40.0U/L in 41 males (58.57%) with a mean of 26.20 ± 7.81 U/L, while in 18 females (25.71%) it ranged from 15.0 to 29.0 U/L with a mean of 21.61 ± 3.63 U/L . In 15.7% of the patients, AST level was elevated with a mean of 48.09 ± 9.57 U/L. In three (3) male's patients representing 4.28%of the total patients, AST level was elevated and a range of 43 to 74 U/L with the mean of 54.67±16.86. In 8 female patients representing 11.4%, of the patients, AST was elevated and the ranged of 38 to 51 U/L with the mean of 45.63 ± 4.90 U/L figure (3) , Table (3), Table(4)& Table(5).

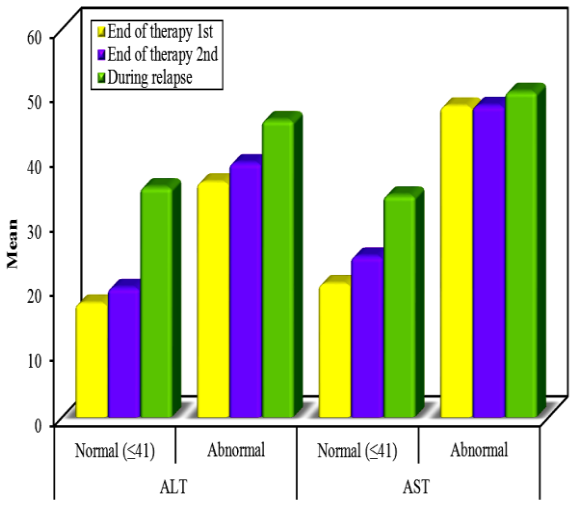

Figure (3): ALT and AST serum levels during therapy and relapse for total patients.

Table (3): ALT and AST serum levels during therapy and relapse

	End of therapy		During relapse (n = 70)
	1st (n = 70)	2nd (n = 70)	
ALT			
Normal (≤41)	**(n = 66)**	**(n = 66)**	**(n = 25)**
Min. – Max.	7.0 – 41.0	9.0 – 41.0	22.0 – 41.0
Mean ± SD.	17.55±7.58	19.95±6.59	35.52±5.29
Median	16.5	19.0	37.0
Abnormal	**(n = 4)**	**(n = 4)**	**(n = 45)**
Min. – Max.	33.0 - 40.0	33.0– 44.0	33.0 – 67.0
Mean ± SD.	36.25±2.87	39.25±4.65	45.89±7.28
Median	36.0	40.0	44.0
AST			
Normal (≤40)	**(n =59)**	**(n =59)**	**(n =8)**
Min. – Max.	9.0 – 38.0	12.0 – 40.0	26.0 – 40.0
Mean ± SD.	20.56±7.13	24.80±7.10	34.25±4.89
Median	19.0	24.0	35.0
Abnormal	**(n =11)**	**(n =11)**	**(n =62)**
Min. – Max.	41.0 –67.0	38.0 – 74.0	38.0– 81.0
Mean ± SD.	48.0 ±7.16	48.09±9.57	50.27±8.60
Median	47.0	47.0	48.5

Table (4): ALT and AST serum levels during therapy and relapse for males

Males	End of therapy		During relapse (n = 44)
	1st (n = 44)	2nd (n = 44)	
ALT			
Normal (≤41)	**(n = 44)**	**(n = 43)**	**(n = 20)**
Min. – Max.	7.0 – 41.0	9.0 – 41.0	32.0 – 41.0
Mean ± SD.	18.32 ± 7.78	20.21 ± 7.20	37.70 ± 2.77
Median	18.0	20.0	38.0
Abnormal	**(n = 0)**	**(n = 1)**	**(n = 24)**
Min. – Max.			42.0 – 67.0
Mean ± SD.	-	44.0	48.46 ± 6.87
Median			46.50
AST			
Normal (≤40)	**(n = 41)**	**(n = 41)**	**(n = 5)**
Min. – Max.	9.0 – 38.0	12.0 – 40.0	35.0 – 40.0
Mean ± SD.	22.27 ± 7.54	26.20 ± 7.81	37.40 ± 2.30
Median	23.0	25.0	38.0
Abnormal	**(n = 3)**	**(n = 3)**	**(n = 39)**
Min. – Max.	43.0 – 67.0	43.0 – 74.0	41.0 – 81.0
Mean ± SD.	52.33 ± 12.86	54.67 ± 16.86	50.95 ± 8.76
Median	47.0	47.0	49.0

Table (5): ALT and AST serum levels during therapy and relapse for females

Females	End of therapy		During relapse (n = 26)
	1st (n = 26)	2nd (n = 26)	
ALT			
Normal (≤32)	**(n =22)**	**(n =23)**	**(n =5)**
Min. – Max.	7.0 – 31.0	12.0 – 29.0	22.0 – 30.0
Mean ± SD.	16.0 ± 7.08	19.48 ± 5.38	26.80 ± 3.56
Median	14.0	19.0	29.0
Abnormal	**(n =4)**	**(n =3)**	**(n =21)**
Min. – Max.	33.0 – 40.0	33.0 – 41.0	33.0 – 56.0
Mean ± SD.	36.25 ± 2.87	37.67 ± 4.16	42.95 ± 6.73
Median	36.0	39.0	42.0
AST			
Normal (≤33)	**(n = 18)**	**(n = 18)**	**(n = 3)**
Min. – Max.	11.0 – 27.0	15.0 – 29.0	26.0 – 31.0
Mean ± SD.	16.67 ± 4.07	21.61 ± 3.63	29.0 ± 2.65
Median	15.50	21.50	30.0
Abnormal	**(n = 8)**	**(n = 8)**	**(n = 23)**
Min. – Max.	41.0 – 52.0	38.0 – 51.0	38.0 – 65.0
Mean ± SD.	46.38 ± 3.85	45.63 ± 4.90	49.13 ± 8.41
Median	46.0	46.50	46.0

D. Correlations between the liver enzymes AST and ALT

The results of the present study revealed a significant direct correlation between the serum ALT & AST levels (r = 0.918, p<0.001). Table (6) & Fig. (4). There was no a significant correlation between the serum ALT and AST levels and time to relapse. Similarly, there was no significant correlation between ALT, AST levels, neither with HCV levels nor age of the patients. (Table 7)

Table (6): Correlation between ALT and AST (n= 70).

	ALT	
	r_s	P
AST	0.918*	<0.001*

rs: Spearman coefficient

*: Statistically significant at p ≤ 0.05

Table (7): Correlation between age and ALT, AST and viral load (n= 70)

	Age	
	r_s	P
ALT	-0.031	0.798
AST	-0.181	0.331
Viral load	-0.096	0.430

r_s: Spearman coefficient

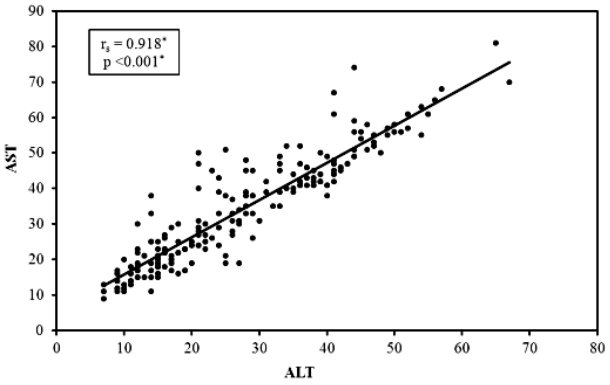

Figure (4): Correlation between ALT and AST (n= 70)

VI - Distribution of the studied cases according to the HCV viral load

According to gender at the end of treatment of 1st therapy (Sovaldi (sofosbuvir) plus ribavirin) **:**

All patients were treated, and the PCR results were Target Not Detected.

According to gender during relapse:

It was found that the Serum HCV levels during relapse ranged from7.0X 10^1 to 9.90 X 10^6IU/ml with a mean value of 786.0 ± 2009.22IU/ml in females. Meanwhile, its level ranged from8.40X $10^2$to 4.70X 10^5 with a mean value of 692.0 ± 1013.29IU/ml in males. The statistical analysis revealed that, there was no statistically significant between the mean HCV levels between males and females of HCV patients. (p=0.535). (Table 7).

According to gender at the end of treatment of 2nd therapy (sovaldi (sofosbuvir) plus sempirvir**):**

All patients with different ages, genders and viral load values were treated and the PCR results was **Target Not Detected.**

DISCUSSION

Egypt has the highest prevalence of hepatitis C virus HCV in the world with Genotype 4 a being the most predominant subtype [4 and 17]. HCV is a single strand RNA that encodes a large polyprotein. It is the main major of liver cirrhosis and hepatocellular carcinoma in Egypt, which lead to significant morbidity and mortality [18 and 19]. Pegylated interferon (PEG-INF) combined with Ribavirin (RBV) was the standard treatment for chronic hepatitis C infection [20 and 21]. This treatment associated with serious side effects, high medical cost, particularly when used for a long time to achieve SVR. Sofosbuvir (Sovaldi) is a new drug for chronic HCV genotypes 1, 2, 3 and 4 infection treatment that is approved by the food and drug administration (FDA). (Sofosbuvir is an antiviral drug which prevents the replication of HCV RNA by inhibiting the RNA enzyme. The treatment regimens may include Sofosbuvir plus ribavirin with or without PEG-IFN [22]. Previously HCV genotype 4 was treated by ribavirin plus PEG-IFN for 48 weeks accompanied by severe side effects [23].

In 2014, a new era of oral antiviral therapy against HCV started. Sofosbuvir plus Simperevir was approved by European Association for the Study of the Liver (EASL) guidelines as one of treatment indication for HCV infection [24].

In the present study 70 Patients chronically infected with HCV genotype 4 have received combined treatment of (Sofosbuvir plus ribavirin) for 24 weeks, the virological response to the combination therapy was evaluated in 70 patients by measuring the viral load by quantitative PCR, the serum hepatic enzymes ; ALT, AST levels. This combination therapy reduces the time to achieve SVR as compared to old standard therapy (PEG-INF) plus Ribavirin [13 and 25].

The mean age of males and females and range were 46.26 years, (62.9%) were men, and (37.1%) were females, and the viral load in all patients was negative. At the end of 1st treatment , Our data showed that the serum ALT level was within the normal range in 94.3% of the total patients with a mean of 17.55 ± 7.58 U/L, and a range of 7.0 to 41.0U/L, and in 44 males (62.85%) it ranged from 7 to 41 U/L with a mean of 18.32 ± 7.78 U/L, while in 22 females

Table (8): Relation between genders with viral load and age

	Gender		Test of sig.	P
	Male (n= 44)	Female (n= 26)		
PCR IU/ml				
Min. – Max.	8.40X10^2 – 4.70 X10^6	7.0 X 10^1– 9.90 X 10^6	U= 521.0	0.535
Mean ± SD.	692.0 ± 1013.29	786.0 ± 2009.22		
Median	200.0	185.0		

U, p: U and p values for **Mann Whitney test** for comparing between the two groups

(31.42%) it ranged from 7.0 to 31 with a mean of 16.0 ± 7.08 U/L. In four (4) female patients representing 5.7% of the total patients, ALT level was elevated and a range of 33-40.0 U/L with a mean of 36.25 ± 2.87 U/L .AST level was within the normal range in 84.3% of the total patients with a mean of 20.56 ± 7.13U/L, and a range of 9.0 to 38.0U/L, in males with a mean of 22.27 ± 7.54 U/L, while in females it ranged from 11.0 to 27.0 U/L with a mean of 16.67 ± 4.07 U/L . In 15.7% of the patients, AST level was elevated with a mean of 48.0 ± 7.16 U/L. In three (3) males patients representing 4.28%of the total patients, AST level was elevated and a range of 43-67 U/L with the mean of 52.33±12.86. In 8 female patients representing 11.4%, of the patients, AST was elevated and the ranged of 41 to 52 U/L with the mean of 46.38 ± 3.85 U/L.

Recorded data showed that the SVR rate was 100% which is comparable with other real-life Egyptian studies as SVR was 92 % [26], and in another study SVR rate was nearly 95% in patients without cirrhosis [26 and 27].

During follow up; some patients were relapsed and became infected with HCV. The viral load in all patients was detected and all patients had positive results. HCV RNA in males ranged from 8.40 x 10^2 IU/ml to 4.70 x 10^6 IU/ml whereas in females ranged from 7.0 x 10^1 IU/ml to 9.9x 10^6 IU/m in a period ranged from 4 weeks to 48 weeks; during this period of relapse ALT & AST enzymes were measured. Rapid relapse was detected whereas some patients relapsed after the end of treatment with 4 weeks. This result is in agreement with a previous study while 84% of patients relapsed by week 4 post treatment and 9% relapsed between week 4 and week 12 post treatment [12].

During relapse, our data showed that the serum ALT level within the normal range in25 patients in both males and females 35.7% with a mean of 35.52±5.29, and ranged from 32 to 41 in 20 males (28.5%) with a mean of 37.70 ± 2.77 U/L, while in 5 females (7.1%) it ranged from 22-30 U/L with a mean of 26.80 ± 3.56 U/L. In 45 of the patients (64.3%) were abnormal with a mean of 45.89±7.28 U/L of total patients, and in 24 males (34.3%) it ranged from 42 to 67 U/L with mean of 48.46 ± 6.87 U/L, while in 21 females (30%) it ranged from 33 to 56 U/L with the mean of 42.95 ± 6.73 U/L . AST level was within the normal range in 8 patient's (11.42%) both males and females ranged from 26 to 40 U/L with a mean of 34.25 ± 4.89U/L, and it ranged in 5 males (7.14%) from35 to 40 U/L with a mean of 37.40 ± 2.30, while it ranged in 3 females(4.28%) from 26 to 31 U/L with a mean of 29.0 ± 2.65. AST levels was with abnormal range during relapse period in 62 of patients in both males and females (88.50%) with a mean of 50.27 ± 8.60 U/L ranged from 38 to 81 U/L of total patients. And it ranged in 39 males (55.71%) from 41 to 81 with a mean of 50.95 ± 8.76U/L, while it ranged in 23 females (32.85%) from 38 to 65 U/L with a mean of 49.13 ± 8.41 U/L.

ALT has been widely reported as present in the liver as a major source. Elevation of serum ALT is found in hepatitis, Cirrhosis. Obstructive jaundice, Alcohol abuse and hepatocellular carcinoma (HCC). AST is widely distributed in many tissues, liver, kidney, cardiac and muscles. Elevation serum level of this enzyme is found in disease involving these tissues. Hepatobiliary diseases, such as cirrhosis, metastatic carcinoma and viral hepatitis also increase serum AST level. In Myocardial infarction and kidney diseases, patients have elevated level of serum AST. AST is higher than ALT as AST enzyme is detected in the cytoplasm and mitochondria of the hepatic cell while ALT was detected in the cytoplasm only; ALT is more liver specific enzyme than AST. Increasing of both liver enzymes as a result of reactivation of HCV RNA and this result were comparable with another study [28].

The 70 patients received a Combination of Sofosbuvir (400 mg orally once per day) plus simeprevir (400 mg orally once per day) as a 2nd therapy for treatment of HCV during relapse in a period that ranged from 4 weeks to 12 weeks. These combinations were recommended by EASL Guidelines against HCV genotype 4 [29].

Furthermore, it was reported that this treatment regimen has been successful for a 12-week. Using the combination therapy of SOF and SMV, in our study a rapid virological response was detected after week 4 in two patients, and an 8 weeks treatment regimen was

achieved in 9 patients and these results were similar to previous reported studies [30-32].

All 70 patients were treated with the 2nd therapy, the viral load (HCV RNA) results were measured by PCR and their results became negative. At the end of 2nd therapy data of biochemical tests showed that the serum ALT level was within the normal range in (94.3%) of the total patients with a mean of 19.95 ± 6.59 UL, and a range of 9.0 to 41.0U/L, and in 43 males (61.42%) it ranged from 9 to 41 U/L with a mean of 20.21 ± 7.20 U/L, while in 23 females (32.85%) it ranged from 12 to 29 with a mean of 19.48 ± 5.38 U/L. In three (3) female patients representing (4.28%) of the patients, ALT level was elevated and a range of 33-41.0 U/L with a mean of 37.67 ± 4.16 U/L .AST level was within the normal range in 84.3% of the total patients with a mean of 24.80 ± 7.10U/L, and a range of 12.0 to 40.0U/L in 41 males (58.57%) with a mean of 26.20 ± 7.81 U/L, while in 18 females (25.71%) it ranged from 15.0 to 29.0 U/L with a mean of 21.61 ± 3.63 U/L . In 15.7% of the patients, AST level was elevated with a mean of 48.09 ± 9.57 U/L. In three (3) male's patients representing 4.28%of the total patients, AST level was elevated and a range of 43 to 74 U/L with the mean of 54.67±16.86. In 8 female patients representing 11.4%, of the patients, AST was elevated and the ranged of 38 to 51U/L with the mean of 45.63 ± 4.90 U/L.

ALT was abnormal in 64.3 % of patients with a mean of 45.89±7.28 U/L and after starting treatment with 2nd therapy for 12 weeks ALT was normalized with a mean of 19.95±6.59 U/L. These results are agreemented with perumail *et al*[33].

Combination therapy for 12 weeks with sofosbuvir & simperevir in patients with HCV genotype 4 infection was an effective regimen with an overall SVR rate of 100% compared to another study of SVR rate 92 % [30]. AST level in serum was elevated in 11patients after receiving the 2nd therapy. These 11 patients were the same 11 patients that were abnormal after 1st treatment and this elevation may be due to other diseases such as kidney, heart and muscles. Our study revealed that sofosbuvir reduces the viral load and HCV replication through its inhibition of HCV NS5B polymerase function. but its direct effect on the HCV that make inflammation or damage to hepatic cells and cause releasing of these enzymes and elevation in serum level. Sofosbuvir is a pyrimidine nucleotide analogue that has a direct effect on HCV which inhibits that's required for viral replication and reduce the viral load gradually during the treatment period till reach the complete recovery and become negative. Then the specific liver enzymes decreased and return to the normal levels in serum which linked with liver organ [34].

CONCLUSION:

Results of this study revealed that treatment period with sofosbuvir plus simperevir reduced the viral load period of HCV rather than the treatment period of sofosbuvir plus ribavirin. Sofosbuvir hasn't direct effect on liver enzyme (ALT & AST), but it affect on viral by inhibit its replication and then liver enzymes return to normal level.

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الملخص العربي

تعد العدوى الفيروسية لالتهاب الكبد الوبائي من أكثر الأمراض شيوعًا في مصر في القرن العشرين والتي تؤدي إلى أمراض الكبد و مرض تليف الكبد على سبيل المثال سرطان الكبد . السوفوسبوفير (السوفالدي) هو واحد من أنجح العلاجات لمضادات الفيروسات المباشرة التي تلعب دورًا مهمًا في علاج فيروس الالتهاب الكبدي (سي) .وتهدف دراستنا إلى إظهار التأثير البيولوجي الجزيئي والكيميائي الحيوي لـ سوفوسبوفير (سوفالدي) على مرضى التهاب الكبد الوبائي سي الذين انتكسوا بعد العلاج .وشملت عينة الدراسه 70 مريضًا (يمثلون 0.14 ٪ من إجمالي الحالات) المصابين بعدوى التهاب الكبد الفيروسي المزمن ، تتراوح أعمارهم بين 21 إلى 70 عامًا ، وتم تجميع العينات في عامين من أكتوبر 2015 إلى أكتوبر 2017 ممن يتم استقبالهم فى مختبر مبرة العصافره حيث انه استقبل حوالي 50.000 مريض في هذه الفتره . اهتمت الدراسة الحالية بدراسة الحالات مع التاريخ المرضى والدواء المتناول في المرحلة الأولى من العلاج وهو جرعات من الريبافيرين و سوفوسبوفير .تم الحصول على بيانات إنزيمات الكبد **ناقل امين الالنين وناقل امين الاسبارتات** من تقاريرهم السابقة بعد المرحلة الأولى من العلاج والتعافي وكانت ضمن المعدل الطبيعي. تم جمع عينات المرضي أثناء الانتكاس وتم قياس مستوي **ناقل امين الالنين وناقل امين الاسبارتات** في نفس اليوم للحفاظ على استقرار الإنزيمات ، باستخدام اجهزة ال Roche / Hetachi Cobas c 311 ، Cobas c 501/502 . تم قياس حمض النووي الريبي لفيرس (سي) باستخدام تقنية تفاعل البلمرة المتسلسل باستخدام اجهزة Roche Cobas AmpliPrep و COBAS TaqMan 48 V.2.0 حيث الحد الأدنى للقياس 15 وحدة دولية / مل .بعد المرحلة الثانية من العلاج ، قمنا بجمع العينات من المرضي وقياس إنزيمات الكبد وتفاعل البلمرة المتسلسل بنفس الاجهزة السابقه .كانت جميع المعلومات الخاصه بالتاريخ المرضي بين العلاج الأول والثاني بما في ذلك مراقبة وظائف الكبد بعد العلاج الأول ، وخلال فترة الانتكاس وبعد تلقي العلاج الثانيز المرضى الذين تم علاجهم بمزيج من السوفوسبوفير و الريبافيرين في العلاج الأول حققوا شفاء بعد 24 أسبوعًا من استقبال العلاج ، انخفاض إنزيمات الكبد **ناقل امين الالنين وناقل امين الاسبارتات** و94 ٪م من المرضي يحققون قيمًا طبيعية ، في حين أن 6٪ لديهم ارتفاع معتدل في **ناقل امين الالنين** ؛ 84٪ لها قيم طبيعية **وناقل امين الاسبارتات** و 16٪ لها نتائج غير طبيعية مع نتائج السلبيه للتفاعل البلمره المتسلسل. بعد المتابعة (4 - 48 أسبوعًا) 36٪ لديهم **ناقل امين الالنين** عادي ، 64٪ كان لديهم نتائج غير طبيعية ؛ 11.2٪ كان لديهم**ناقل امين الاسبارتات** عادي ، 89٪ كانوا غير طبيعيين. المرضى في 48 أسبوعًا تراوحت تركيزاتهم الفيروسية من 7.0* 10^1 الي 9.90* 10^6 وحدة دوليه / مل .بعد تلقي العلاج الثاني ، حقق المرضى نتيجه سلبيه في فترة تراوحت من 4 أسابيع إلى 12 أسبوعًا دون أي تغييرات واضحة في قيم **ناقل امين الالنين وناقل امين الاسبارتات بالمقارنه في الفتره التي كانت بعد تلقي العلاج الاول** . كشفت دراستنا أن السوسبوفير يقلل من التركيز الفيروسي وتكاثر لفيرس ألتهاب الكبد الوبائي (سي) .سوفوسبوفير هو نظير نيوكليوتيد البيريميدين له تأثير مباشر على التهاب الكبد الفيروسي الذي يمنع التكاثر الفيروسي ويقلل التركيز الفيروسي تدريجيًا خلال فترة العلاج حتى يصل إلى الشفاء التام ويصبح سلبيًا. مع انخفاض إنزيمات الكبد المحددة وتعود إلى المستويات الطبيعية .كما كشفت نتائج هذه الدراسة أن فترة العلاج بمزيج من السوسبوفير (السوفالدي) والسمبريفير (اوليسو) قللت فترة التركيز الفيروسي لألتهاب الكبدي الوبائي (سي) الي (12 اسبوع) بدلاً من فترة علاج بمزيج من السوفوسبوفير(السوفالدي) والريبافيرين اتي كانت تستغرق 24 اسبوع. لم يؤثر السوفوسبوفير(السوفالدي) بشكل مباشر على إنزيم الكبد ناقل امين الالنين وناقل امين الاسبارتات ، ولكنه يؤثر على الفيروس عن طريق ايقاغ تكاثره ومن ثم تعود إنزيمات الكبد إلى المستوى الطبيعي.