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STUDY THE EFFECT OF HEPATIC TRIPLE THERAPY COMBINATION (INTERFERON, SOVALDI & RIBAVIRIN) ON GENOTYPE 4 HEPATITIS C VIRAL STRAIN THAT DIFFUSE IN EGYPT

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1. Abstract

This study aimed to evaluate the effect of hepatic therapy combination (interferon, sovaldi & ribavirin) on type 4 hepatitis C viral strain by comparing the results of liver function tests before and after treatment. The levels of serum Interleukin 10 (IL.10) which is an important cytokine with an anti-immune, anti-inflammatory and anti-fibrotic function (Grove *et al*., 2000) also was measured for all subjects to give an indication of liver inflammation. The statistical analyses of liver functions were varied among the tested cases. The investigated cases included 40 patient and 10 healthy individuals as control group. The individuals of this study were classified into 3main groups, **Control group**: 10 individuals looks like healthy, free from diseases and not taken medications with negative PCR results, **Group No. 1**:40 hepatic patients (chronically infected) with positive HCV-PCR results and **Group No. 2**: 40 chronic hepatic patients treated by hepatic therapy (interferon, sovaldi & ribavirin).The results of PCR analysis of group No.2 (after the end of treatment course) became negative in all members where all members of group No.1 were positive PCR. The statistical analysis of liver functions showed that there were no significant differences between group No.1 and group No.2 in the results of S.G.P.T, S.G.O.T, Albumin and alkaline Phosphatase, low significant difference between results of group No.1 and group No.2 in the analysis of total and direct bilirubin and moderate significant difference in the results of gamma glutamyl Transaminase where the results of analysis of group No.2 were relatively better than the results of group No.1. The previous results indicating that the therapy combination (interferon, sovaldi & ribavirin) has a remarkable effect on HCV genotype 4 that spread in Egypt.
Keywords: *S.G.P.T, S.G.O.T, Bilirubin, Gamma Glutamyle Transaminase, PCR.*

2. Introduction

Liver is a wedge-shaped reddish brown organ present in vertebrates and some other animals. In human, liver is located in the upper right quadrant of the abdominal cavity to the right of stomach, overlying gallbladder and directly resting below the diaphragm (Cotran et al., 2005). At birth the liver comprises roughly 4% of body weight and is at average 120g. Over the course of development it will increase to 1.4–1.6 kg, but will only take up 2.5–3.5% of body weight (Clemente et al 2011).The various functions of the liver are carried out by the liver cells or hepatocytes. Role of liver in human body can be classified mainly into **five main functions**:**1-Metabolic function,** as carbohydrates metabolism, where the liver synthesizes and stores approximately 100gm of glycogen via glycogenesis, (the formation of glycogen from glucose). When needed, the liver releases glucose into the blood by performing glycogenolysis, (the breakdown of glycogen into glucose (Marieb et al., 2012). In protein metabolism, liver is responsible for the mainstay of protein metabolism and plays a key role in amino acids production (W. Jelkmann 2011). **2- Synthetic functions,** liver is the factory of protein production in the body and albumin considered as the main liver protein. **3-Detoxification functions,** as the liver breaks down ammonia into urea as part of the urea cycle, and the urea is excreted in the urine (Marieb et al., 2012). **4-Storage functions,** as the liver stores a multitude of substances, including glucose (in the form of glycogen),Vitamin A(1 – 2 years' supply), vitamin D(1 – 4 months' supply),vitamin B12(1–3 years' supply), vitamin K, iron, and copper.**5-Immunological functions,** liver is responsible for immunological effects, reticuloendothelial system of liver contains an immunologically active cells, acting as 'sieve' for antigens carried to it via portal system. To evaluate the efficiency of liver and insure that the liver is optimally does its functions, liver function tests (LFT) must be conducted. Liver function tests are helpful screening tools which are an effective modality to detect hepatic dysfunction. Since the liver performs a variety of functions, so no single test is sufficient to provide complete estimate of function of liver. Liver function tests are classified into three main groups:**1-Tests of the liver's capacity to transport organic anions and to metabolize drugs** (eg: Serum bilirubin, urine bilirubin & urobilinogen).Bilirubin is an endogenous anion derived from hemoglobin degradation from the RBC. When the liver function tests are abnormal and the serum bilirubin levels more than17µmol/L, that suggest underlying liver disease (Friedman et al., 2003). **2-Tests**

that detect injury to hepatocytes (serum enzyme tests, eg: Aminotransferases (AlT & AST) also called (S.G.P.T & S.G.O.T), alkaline phosphatase, gamma glutamyl transpeptidase, 5 nucleotidase, leucine aminopeptidase).The aminotransferases (formerly transaminases) are the most frequently utilized and specific indicators of hepatocellular necrosis. ALT is primarily localized to the liver but the AST is present in other wide variety of tissues like kidney, heart, brain & skeletal muscles. **3-Tests of the Liver's biosynthetic capacity** (eg: Serum proteins, albumin, prealbumin, serum ceruloplasmin, procollagen III peptide, a 1 antitrypsin, alpha feto protein, prothrombin time). The liver is the major source of most the serum proteins. The parenchymal cells are responsible for synthesis of albumin, fibrinogen and other coagulation factors and most of a and b globulins. All of these parameters are helpful to evaluate the efficiency of liver in performing its biological functions. In addition, the liver resembles a central organ of cytokines activity due to the fact that it hosts hepatocytes, which are highly susceptible to the activity of cytokines in a variety of physiological and pathophysiological processes. Moreover, the non-parenchyma cells of the liver, in particular Kupffer cells (KCs), the resident tissue macrophages of the liver, are able to synthesize a variety of cytokines that may act systemically on any other organ of the body, or in a paracrine manner on hepatocytes and other non-parenchymal liver cells (Eur J 2001).Cytokines are a broad and loose category of proteins (5-20 kDa) that are important in cell signaling. Cytokines include chemokines, lymphokines, and tumour necrosis factors (TNF), interferons and interleukins (Williams *et al.*, 2006).Interleukins (IL) are a group of cytokines that were first seen to be expressed by white blood cells (leukocytes) (Brocker *et al*., 2010). IL-10 is a recently described natural endogenous immunosuppressive cytokine that has been identified in human and other organisms (Gesser *et al*., 1997).IL-10 is an immunoregulatory cytokine that plays an important role in the development of the infectious disease (Opdale *et al*., 2003)

Aim of study: This study was carried out to evaluate the effect of hepatic triple therapy combination (interferon, sovaldi & ribavirin) on genotype 4 hepatitis C viral strain by comparing the results of liver function analysis before and after treatment. (As all of researches and studies of this treatment were conducted on viral strain found in the United States (genotype 1), not on the viral strain that diffuse in Egypt (genotype 4) (Murphy DG, *et al*., 2007).

3. Material and Method

3.1 Collection and preparation of blood samples:

Blood samples were collected from patients of El-Mahalla liver hospital in sterilized conditions after obtaining an informed consent. Samples were centrifugated at 2000 rpm to obtain blood serum then all stored at -20 ^{o}to avoid freeze-thaw cycles (Thavasu *et al.*, 1992)

3.2Determination of routine liver functions:

The routinely liver functions analysis which conducted on all hepatic patients at El-Mahalla liver hospital include (S.G.P.T &S.G.O.T), Albumin, (Total Bilirubin &Direct Bilirubin),were carried out according to Friedman *et al*., (2001),Young (2001),Tietz (1995),respectively.

3.3 Determination of Alkaline Phosphatase (ALP):

Add 500 µl R_1 to 500 µl R_2, mix and incubate for 30 seconds at 37^{o} c. adjust the instrument to zero with distilled water. Add 10 µl sample. Read sample increase and extinction at time after 60/120/180 seconds. Calculate (ΔE/min) at wave length 405 nm by using optical path 1 cm light path (Henry *et al*., 1964).

3.4 Determination of Gamma Glutamyle Transaminase (γ G.T):

Add 20 µl sample to 220 µl R_1, mix and wait for 5 minutes. Add 55 µl R_2, mix and incubate for 50 seconds. Measure the change of absorbance per minute (ΔE/min) during 159 seconds at wave length 405 nm and temperature 37^{o} c using optical path 1 cm light path (Sewell *et al*., 2005).

3.5 Determination of IL-10:

Aliquot 0.1 ml per well of the 500 pg/ml, 250 pg/ml, 125 pg/ml, 62.5 pg/ml, 31.3 pg/ml, 15.6 pg/ml, 7.8 pg/ml human IL.10 standard solutions into the precoated 96 plate. Add 0.1 ml of the sample diluent buffer into the control well (zero well). Add 0.1 ml of each properly diluted sample of human cell culture supernates serum or plasma (heparin, EDTA, citrates) to each empty well. Seal the plate with the cover and incubate at 37^{o} C for 90 min. Remove the cover, discard plate content and plot the plate into paper towels and don't let the wells completely dry at any time. Add 0.1 ml of biotinylated anti-human IL.10 antibody working solution into each well and incubate the plate at 37 ^{o} C for 60 min. Wash plate 3 times with 0.01 ml TBS or 0.01 ml PBS and each time let washing buffer stay in wells for 1 min. Discard the washing buffer and plot the plate onto paper towel. Discard the solution in the plate without touching the side wells and plot the plate onto paper towel. Soak each well with at least 0.3 ml PBS or TBS for 1-2 min and repeat washing for three times. Add 0.1 ml of prepared ABC working solution into each well and incubate the plate at 37 ^{o} C for 30 min. Wash plate five times with 0.01 ml TBS or PBS and each time let washing buffer stay at in the wells for 1-2 min. Discard the washing buffer and plot the plate onto paper towel. Add 90 µl of prepared TMB color developing agent into each well and incubate the plate at 37 ^{o} C in dark for 20-25 min.

Add 0.1 ml of prepared TMB stop solution into each well, the color changes into yellow immediately. Read the OD absorbance at 450 nm in microplate reader within 30 min after adding the stop solution (Alamartine *et al*., 2003)

3.6 Statistical analysis:

All statistics were performed and plotted using GraphPad Prism® (Prism 6 for windows) v6.01, 2012 (© 1992-2012 Graph Pad Software Inc., California, USA).

4. Results

4.1 Table1: Results of males in Control group:

As the subjects of control group appeared healthy, free from diseases and not taken medications, the results of all conducted analysis were within normal range and the minimum and maximum results of the conducted analysis were as the following: S.G.P.T ranged from 17 to 27 (g/dl), S.G.O.T ranged from 11 to 26 (g/dl), Albumin (Alb.) from 3.8 to 4.2 (g/dl), Total bilirubin (T. Bili.) from 0.3 to 0.6 (mg/dl), Direct bilirubin (D. Bili.)from 0.1 to 0.2 (mg/dl), Alkaline phosphatase (ALP.) from 107 to 269 **(U/L)**, Gama Glutamyle Transaminase (γ GT) from 21 to 41 **(U/L)**,Inter leukin 10 (IL.10) from 19 to 24(PG/ml)and PCR results were negative in all members.

Case No.	Age (year)	S.G.P.T (g/dl)	S.G.O.T (g/dl)	Alb. (g/dl)	T. Bili. (mg/dl)	D. Bili. (mg/dl)	ALP. (U/L)	γ G.T (U/L)	IL.10 (Pg/ml)
1	37	25	19	3.8	0.6	0.1	189	21	24
2	28	19	14	4.1	0.4	0.2	107	32	19
3	28	27	26	4	0.5	0.1	269	27	19
4	31	17	11	4.2	0.3	0.1	152	41	23

4.2 Table 2: Results of females in Control group:

Concerning normal females, S.G.P.T ranged from 21 to 33 (g/dl), S.G.O.T ranged from 16 to 29 (g/dl), Albumin (Alb.) from 3.9 to 4.8 (g/dl), Total bilirubin from (T. Bili.) 0.4 to 0.8 (mg/dl), Direct bilirubin (D. Bili.) from 0.1 to 0.3 (mg/dl), Alkaline phosphatase (ALP.) from 124 to 231 **(U/L)**, Gama Glutamyle Transaminase (γ GT) from12 to 25**(U/L)**,Inter leukin 10 (IL.10) from 18 to 32 (PG/ml)and PCR results were negative in all members.

Case No.	Age (year)	S.G.P.T (g/dl)	S.G.O.T (g/dl)	Alb. (g/dl)	T. Bili. (mg/dl)	D. Bili. (mg/dl)	ALP. (U/L)	γ G.T (U/L)	IL.10 (Pg/ml)
5	19	22	27	4	0.6	0.2	124	12	17
6	25	29	29	4.2	0.4	0.1	168	17	18
7	20	33	23	3.9	0.8	0.1	204	24	26
8	30	21	16	4.8	0.7	0.1	231	25	16
9	29	31	28	4.3	0.6	0.2	209	19	16
10	17	28	28	4.6	0.8	0.3	179	21	22

4.3 Table 3: Results of males in group No. 1:

Members of group No. 1 were infected by Hepatitis C Virus as the results of PCR showed, that lead to abnormal results of some liver functions which appear during the checked out of minimum and maximum results as the following :S.G.P.T ranged from 13 to 51 (g/dl), S.G.O.T ranged from 14 to 78 (g/dl), Albumin (Alb.) from 2.90 to 4.12 (g/dl), Total bilirubin (T. Bili.) from 0.73 to 2.57 (mg/dl), Direct bilirubin (D. Bili.) from 0.20 to 1.28 (mg/dl), Alkaline phosphatase (ALP.) from 112 to 601**(U/L)**, Gama Glutamyle Transaminase (γ GT) from21 to 133**(U/L)**,Inter leukin 10 (IL.10) from61 to 129 (PG/ml) and PCR results were positive in all members.

Case No.	Age (year)	S.G.P.T (g/dl)	S.G.O.T (g/dl)	Alb. (g/dl)	T. Bili. (mg/dl)	D. Bili. (mg/dl)	ALP. (U/L)	γ G.T (U/L)	IL.10 (Pg/ml)
1	43	35	52*	3.21*	2.32*	1.28*	422*	21	87
2	20	21	20	3.61	0.86	0.34	294	71*	93
3	21	44*	37	3.44*	1.22*	0.21	128	65*	119
4	59	19	37	3.52	0.73	0.20	172	61*	79
5	61	20	18	3.50	0.86	0.34	306	133*	115
6	56	33	49*	3.43*	1.31*	0.25	422*	21	91
7	50	24	35	3.21*	1.20*	0.20	320*	93*	77
8	43	33	21	3.52	1.08	0.20	229	61*	129
9	39	21	49*	4.12	0.82	0.33	112	115*	114
10	59	19	57*	3.76	1.77*	0.78*	601*	114*	69
11	55	15	16	3.33*	1.1*	0.31	330*	38	99
12	60	34	57*	2.90*	1.33*	0.90*	501*	112*	67
13	44	31	44*	3.41*	1.42*	0.24	504*	89*	124
14	48	13	14	3.36*	1.22*	0.34	504*	71*	82
15	27	17	39*	3.43*	2.57*	0.66*	219	107*	61
16	65	48*	78*	3.12*	1.54*	0.59*	539*	127*	114
17	34	51*	31	2.96*	1.43*	0.62*	333*	28	86
18	64	24	43*	3.33*	1.73*	0.84*	407*	112*	85

4.4 Table 4: Results of females in group No. 1:

Concerning infected females in group No.1,S.G.P.T ranged from 21 to 58 (g/dl), S.G.O.T ranged from 17 to 55(g/dl), Albumin (Alb.) from 2.65 to 4.47 (g/dl), Total bilirubin (T. Bili.) from 0.62 to 2.55 (mg/dl), Direct bilirubin (D. Bili.) from 0.25 to 1.10 (mg/dl), Alkaline phosphatase (ALP.) from 147 to 494**(U/L)**, Gama Glutamyle Transaminase (γ GT) from20 to 135**(U/L)**,Inter leukin 10 (IL.10) from 51 to 113 (PG/ml) and PCR results were positive in all members.

Case No.	Age (year)	S.G.P.T (g/dL)	S.G.O.T (g/dl)	Alb. (g/dl)	T. Bili. (mg/dl)	D. Bili. (mg/dl)	ALP. (U/L)	γ G.T (U/L)	IL.10 (Pg/ml)
19	62	27	44*	3.39*	1.21*	0.39*	198	72*	51
20	57	19	37*	3.7	1.05	0.25	494*	99*	90
21	45	24	32	3.52	0.62	0.31	147	82*	88
22	50	22	41*	2.65*	2.55*	0.99*	266	69*	64
23	62	24	22	3.33*	1.27*	0.35*	226	83*	86
24	54	29	43*	2.97*	1.40*	0.51*	567*	118*	86
25	38	26	30	3.44*	1.19*	0.36*	212	109*	109
26	40	30	17	3.36*	1.22*	0.43*	420*	46*	74
27	43	27	54*	3.50	1.01	0.31	464*	45*	91
28	50	22	28	3.41*	1.30*	0.45*	468*	20	59
29	53	23	34*	3.65	0.85	0.29	227	66*	51
30	22	17	21	3.23*	1.31*	0.42*	317*	52*	76
31	47	24	33*	3.24*	1.06*	0.38*	320*	109*	92
32	30	41*	22	3.44*	1.21*	0.50*	349*	69*	58
33	39	10	22	3.38*	1.66*	0.42*	306	119*	71
34	63	38*	55*	3.29*	2.11*	1.10*	317*	63*	78
35	37	17	39*	3.36*	1.85*	0.96*	389*	71*	51
36	43	40*	41*	3.17*	1.90*	0.56*	275	125*	66
37	36	23	41*	3.25*	1.63*	0.83*	183	108*	97
38	55	58*	40*	4.47	0.86	0.25	252	109*	71
39	39	21	10	3.33*	1.63*	0.55*	367*	135*	113
40	34	21	25	3.22*	1.44*	0.44*	363*	116*	61

4.5 Table 5: Results of males in group No. 2:

Concerning males in group No. 2,S.G.P.T ranged from 21 to 49 (g/dl), S.G.O.T ranged from 12 to 59 (g/dl), Albumin (Alb.) from 2.59 to 3.98 (g/dl), Total bilirubin (T. Bili.) from 0.56 to 1.87 (mg/dl), Direct bilirubin (D. Bili.) from 0.0 to 0.76 (mg/dl), Alkaline phosphatase (ALP.) from 115 to 461 **(U/L)**, Gama Glutamyle Transaminase (γ GT) from19 to 97**(U/L)**,Inter leukin 10 (IL.10) from 43 to 65 (PG/ml) and all PCR results became negative.

Case No.	Age (year)	S.G.P.T (g/dl)	S.G.O.T (g/dl)	Alb. (g/dl)	T. Bili. (mg/dl)	D. Bili. (mg/dl)	ALP. (U/L)	γ G.T (U/L)	IL.10 (Pg/ml)
1	43	33	38	3.46*	1.72*	0.76*	395*	26	49
2	20	25	19	3.84	0.92	0.33	416*	85*	60
3	21	39	29	3.51	1.04	0.25	117	49	43
4	59	21	31	3.71	0.91	0.15	131	49	50
5	61	27	26	3.66	0.86	0.22	286	97*	55
6	56	31	37	3.33*	1.12*	0.17	176	80*	55
7	50	28	31	3.29*	0.96	0.31	304	44	63
8	43	29	12	3.61	0.77	0.26	336*	57*	53
9	39	29	31	3.98	0.56	0.0	115	69*	53
10	59	26	59*	3.83	1.42*	0.51*	397*	79*	49
11	55	24	18	3.50	0.82	0.09	316*	19	49
12	60	31	38	2.59*	1.09	0.51*	441*	76*	50
13	44	29	26	3.59	0.99	0.23	461*	62*	55
14	48	26	32	3.22*	0.85	0.29	298	24	59
15	27	49*	38	3.52	1.87*	0.49*	126	66*	55
16	65	36	51*	2.89*	1.26*	0.34	392*	62*	49
17	34	42*	32	3.13*	1.33*	0.30	314*	29	65
18	64	27	29	3.56	1.59*	0.61*	352*	54*	62

4.6 Table 6: Results of females in group No. 2:

Concerning females in group No. 2,S.G.P.T ranged from 16 to 51 (g/dl), S.G.O.T ranged from 17 to 69 (g/dl), Albumin (Alb.) from 2.34 to 4.41 (g/dl), Total bilirubin (T. Bili.) from 0.51 to 2.11 (mg/dl), Direct bilirubin (D. Bili.) from 0.20 to 0.89 (mg/dl), Alkaline phosphatase (ALP.) from 129 to 723(IU/ml), Gama Glutamyle Transaminase (γ GT) from28 to 114 (IU/ml),Inter leukin 10 (IL.10) from 42 to 65 (PG/ml) &All PCR results became negative.

Case No.	Age (year)	S.G.P.T (g/dl)	S.G.O.T (g/dl)	Alb. (g/dl)	T. Bili. (mg/dl)	D. Bili. (mg/dl)	ALP. (U/L)	γ G.T (U/L)	IL.10 (Pg/ml)
19	62	24	31	3.50	0.96	0.29	214	61*	50
20	57	19	32*	3.49*	1.31*	0.41*	401*	114*	50
21	45	17	30	4.31	0.51	0.27	129	61*	50
22	50	26	45*	2.90*	2.11*	0.77*	219	51*	43
23	62	18	29	3.42*	1.03	0.44*	219	61*	42
24	54	29	36*	2.34*	1.00*	0.30	482*	88*	53
25	38	21	31	3.52	0.84	0.20	234	100*	57
26	40	24	29	3.49*	0.87	0.24	166	29	50
27	43	16	33*	3.66	0.99	0.31*	409*	34*	45
28	50	24	29	3.50	1.05*	0.39*	371*	28	45
29	53	51*	37*	3.52	0.91	0.32*	212	39*	49
30	22	19	27	3.41*	1.09*	0.37*	298	39*	45
31	47	20	29	3.29*	0.92	0.34*	316*	62*	53
32	30	37*	19	3.51	1.14*	0.39*	212	52*	45
33	39	19	28	3.19*	1.34*	0.37*	723*	104*	65
34	63	44*	69*	3.46*	1.81*	0.89*	319*	59*	55
35	37	22	31	3.54	1.66*	0.56*	333*	59*	42
36	43	29	27	3.21*	1.45*	0.50*	261	84*	45
37	36	22	41*	3.46*	1.18*	0.44*	317*	88*	49
38	55	23	18	4.41	0.79	0.21	216	74*	62
39	39	19	17	3.61	1.37*	0.42*	317*	84*	59
40	34	24	31	3.49*	1.13*	0.32*	302	61*	62

4.8 Table 7: ANOVA test between groups:

The following table showed that, the statistical results (mean & St. deviation) of liver functions tests in group No.2 (after treatment) were relatively better than the results of group No.1 (before treatment), also the results of Tukey's Test showed that no or low significant difference between the group No.1 and group No.2 in the results of liver function tests as the following:

Item	Control group (Mean ± St. Dev.)	Group No. 1 (Mean ± St. Dev.)	Group No. 2 (Mean ± St. Dev.)	ANOVA test	
				F	P-Value
S.G.P.T	25.20 ±5.30	27.13 ±10.63	27.48 ± 8.43	0.243	0.7843(ns)
S.G.O.T	22.10± 6.60	35.70± 14.39	31.90± 10.67	5.074	0.0082(**)
Alb.	4.19 ± 0.31	3.38± 0.30	3.46± 0.37	23.38	< 0.0001(***)
T. Bili.	0.57 ± 0.17	1.37 ± 0.46	1.13± 0.35	16.93	< 0.0001 (***)
D. Bili.	0.15 ± 0.07	0.49± 0.27	0.36± 0.17	10.52	< 0.0001 (***)
ALP.	183.2 ± 48.6	336.8± 126.3	301.1± 120.9	6.779	0.0018(**)
γ G.T	23.9 ± 8.15	83.1± 33.29	61.4 ± 23.6	19.91	< 0.0001 (***)
IL. 10	20.0± 3.52	84.3± 21.0	52.25± 6.55	94.40	< 0.0001 (***)

Tukey's Test

Item	Control Group & Group No. 1	Control Group & Group No. 2	Group No. 1 & Group No. 2
S.G.P.T	(ns)	(ns)	(ns)
S.G.O.T	(**)	(ns)	(ns)
Albumin	(***)	(***)	(ns)
T. Bilirubin	(***)	(***)	(*)
D. Bilirubin	(***)	(*)	(*)
ALP.	(**)	(*)	(ns)
γ G.T	(***)	(***)	(**)
IL. 10	(***)	(***)	(***)

(ns)Nonsignificant difference, (*) Low significant difference, (**) Moderate & (***) High significant difference

5. Discussion

This study was carried out to evaluate the effect of hepatic triple therapy combination (interferon, sovaldi & ribavirin) on type 4 hepatitis C viral strain by comparing the results of liver function analysis before and after treatment. (As all of researches and studies of this treatment were carried out on the viral strain found in the United States (genotype 1) and not on the viral strain that diffuse in Egypt (genotype 4)(Murphy DG, *et al.*, 2007). The individuals of this study were divided into three main groups; **Control group**: 10 individuals looks like healthy, free from diseases and not taken any medications with negative PCR results, **Group No. 1**:40 hepatic patients (chronically infected) with positive HCV-PCR results and **Group No. 2**: 40 chronic hepatic patients treated by hepatic therapy combination (interferon, sovaldi & ribavirin), complete dose for three months. After the end of treatment course (three months), all individuals of group No.2 became negative PCR results where the subjects of group No.1 (before treatment) were positive PCR that indicating initially effect of therapy on genotype 4 viral strain. The levels of serum Interleukin 10 (IL.10) also measured for all individuals of the study to monitor the level of liver inflammation. The statistical analysis (mean and standard deviation) of IL-10 in control group were (20.0± 3.52), this result considered as low levels of IL-10 when compared with the same results of subjects in group No.1 whom developed chronic hepatitis C infection (84.3± 21.0). The variation between the two results showed that the level of IL-10 influenced significantly by chronic hepatic infection of patients in group No.1.The statistical analysis (mean and standard deviation) of IL-10 in group No.2 were (52.25± 6.55) indicating that after treatment course, the levels of liver inflammation became lower that lead to decreased in levels of IL-10. Subjects of group No. 1 developed chronic hepatic infection as the detectable viral replication present in results of PCR analysis which characterize chronic stage of infection where minimal or no symptoms during the initial few decades (Nelson *et al.*, 2011). Also chronic stage of hepatitis C can be associated with fatigue (Ray *et al.*, 2009), mild cognitive problems (Forton *et al.*, 2005) and after several years the chronic infection may cause cirrhosis or liver cancer (Rosen 2011). During chronic stage, the liver enzymes are normal in 7-53% (Nicot 2004). That agreed with the results of this study as the results of S.G.P.T, S.G.O.T were within normal ranges or mild increase in some cases as showed in table of group No.2.The statistical analysis of liver function results showed that the mean & standard

deviation of S.G.P.T results of group No.1 (27.13 ± 10.63) and after treatment course the mean & standard deviation of S.G.P.T results of group No.2 became (27.48 ± 8.43), the comparison between the two results in Tukey's Test showed no significant difference between the two results. The statistical results (mean & standard deviation) of S.G.O.T, Albumin & Alkaline phosphatase also showed no significant difference between group No.1 & group No.2, indicating no elevation of the levels of mentioned biomarkers after treatment course. The statistical analysis (mean & standard deviation) of Total Bilirubin in group No. 1 (1.37 ± 0.46) and in group No.2 (1.13 ± 0.35) and the Tukey's Test showed low significant difference (*) between the two result. The statistical analysis of Direct Bilirubin also showed low significant difference (*) between results in group No.1 & group No.2 and the results of total Bilirubin and direct Bilirubin after treatment were better than before treatment as showed on the table 9 of ANOVA Test. Concerning Gamma Glutamyle Transaminase (γ G.T), the statistical analysis (mean & st. deviation) of (γ G.T) in group No.1 (83.1± 33.29) and became better after therapy (61.4 ± 23.6) in group No.2.

6. Conclusion

The results of PCR analysis in group No.2 (after treatment) became negative in all individuals where all individuals in group No.1 (before treatment) were chronically hepatitis C infected. Also liver function tests of group No.2 (after treatment) were better than the results of group No.1 (chronically infected patients before treatment) which indicating that the treatment combination (sovaldi, interferon & ribavirin) has a remarkable effect on genotype 4 viral strain that common in Egypt.

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