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Impact of gene polymorphism on Egyptian HCV patients under direct antiviral Drugs

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ABSTRACT

Hepatitis C viral infection is with the highest prevalence rate in Egypt. Direct Acting Antivirals (DAAs) is now the standard of care for HCV infection treatment. Here we assess the predictive value of single-nucleotide polymorphisms (SNP) rs2596542 C/T in chromosome 6 located in MHC class I on the response to DAAs in chronic HCV infected Egyptian patients. This study was performed on 70 Egyptian patients positive for HCV; classified into two groups. Group I: (33 patients) were received combination therapy Sofosbuvir (Sovaldi) 400 mg/day plus Declatasvir 60 mg once daily, Group II: (37 patients) were received Ombitasvir 25 mg, Paritaprevir 150 mg and Ritonavir 100 mg/day plus Ribavirin 15 mg/kg/day for 12 weeks. HCV level by (RT-PCR), MICA single nucleotide polymorphism of rs2596542, ALT, AST, total bilirubin, serum albumin, fasting blood sugar, HbA1C %, CBC, serum creatinine, AFP were performed in all volunteer patients. Results showed that group II responded with 100% in CT- SNP genotype. However both CC and TT- SNP genotypes responded with 83.33% and 91.67% respectively. There was no observed significant association between SNP rs2596542 C/T and all clinical parameters except AFP that give positive significant correlation in the CC genotype.

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1. INTRODUCTION

Hepatitis C is the disease of liver caused by hepatitis C virus (HCV), it can result in chronic liver disease, hepatic fibrosis, cirrhosis, hepatocellular carcinoma (HCC), and liver-related mortality worldwide [1]. HCV is a RNA virus known to infect humans and chimpanzees, causing similar disease in these 2 species [2]. It can be classified into 7 genotypes and 67 subtypes [3]. In Egypt, hepatitis C is acknowledged as the most endemic viral infection with the highest prevalence rate in the world embracing a major health problem in the country [4, 5], HCV genotype-4 (GT-4) represents the predominant genotype in Egypt [6].

The discovery of different classes of direct acting antiviral (DAAs) represented a revolution in the management of chronic hepatitis C virus infection [7]. It has recently been reported that a single nucleotide polymorphism (SNP) rs2596542 located in the major histocompatibility complex class I chain-related gene A (MICA) promoter region was significantly associated with the risk for hepatitis C virus induced hepatocellular carcinoma [8]. MICA is a membrane protein that acts as a ligand for NKG2D to initiate anti-tumor effects through NK and CD8+ T cells [9]. MICA is released into the serum via cleavage at the trans-membrane domain by matrix metalloproteinases. [10, 11] The human MICA gene is located on the short (p) arm of chromosome 6 (6p21.33) and belongs to one of the members of the MIC family [12]. It is ~15.5 kb in size, ~46.4 kb centromeric to HLA-B gene [13]. The objective of the present study is to assess the predictive value of Single-nucleotide polymorphisms (SNP) rs2596542 C/T on chromosome 6 located in the major histocompatibility complex (MHC) class I chain-related gene A (MICA) on the response to direct acting antivirals (DAAs) in chronic HCV infected Egyptian patients.

2. Patients and methods:

The present study included 70 subjects of Egyptian patients positive for HCV RNA in serum (by RT-q PCR assay). The HCV patients' volunteers were divided into two groups: Group I: (33) patients were received combination therapy Sofosbuvir (Sovaldi) 400 mg/day plus delectasvir 60 mg once daily for a course of 12 successive weeks. Group II: 37 patients were received ombitasvir 25 mg, paritaprevir 150 mg and ritonavir 100 mg/day plus Ribavirin 15 mg/kg/day for 12 weeks. All samples were recruited from the HCV research and treatment unit in Faculty of Medicine, Ain Shams research institute (MASRI), Cairo-Egypt in period between March 2017 and October 2018.

Biochemical analysis

Quantitative measurement of HCV RNA

The polymerase chain reaction (PCR) assays were performed in 96-well fast plates in a total reaction volume of 10 μ L / well using 20 ng of genomic DNA. The PCR reaction was carried out using a TaqMan genotyping master mix (Applied Biosystems: Foster City, CA, USA).

Genotyping of MICA single nucleotide polymorphism of rs2596542

DNA samples from HCV patient were genotyped for SNP rs2596542 C/T using 7500 HT Fast Real Time PCR System (Applied Biosystems, Foster City, CA, USA). The SNP rs2596542 of the MICA gene was determined by using TaqMan® Predesigned SNP Genotyping Assays.

Liver function biomarkers assays

Assessment of ALT and AST enzymes were applied using Human Gesellschaft für Biochemica und Diagnostica mbH (Germany) laboratories diagnostic kits, according to the method of **Schumann & Klauke**. [14] Evaluation of total bilirubin and albumin were applied using Human Gesellschaft für Biochemica und Diagnostica mbH (Germany) laboratories diagnostic kits, according to **Van den Bergh**

& Muller, [15] and Dumas *et al.*, [16] respectively.

Blood glucose & HbA1c levels

Serum glucose level was determined enzymatically using GOD-POD kits, according to Young, [17]. Quantitative determination of glycosylated hemoglobin (HbA1c) in blood was carried out by ion exchange resin method using a kit provided by NS Biotec.

CBC Analysis: Complete blood count was carried out by the sysmex® Automated Hematology Analyzer KX-21N. Creatinine and AFP levels Serum Creatinine using Creatinine Assay Kit (Abcam, Cambridge, UK), and alpha fetoprotein (AFP) on subjects' sera using a Beckman CX4 chemistry analyzer (NY; USA).

Statistical analysis

The analysis was done using the statistical package for the social science (SPSS software version 12) on a personal computer. The following analyses were performed according to the technique described by Daniel, and Bailey, [18, 19].

3. Results

Results of MICA SNP rs 2596542C/T genotyping

Real time PCR system displays clusters for the three possible genotypes (Allele1 homozygous (CC) (red), allele 2 homozygous (TT) (blue), and allele 1/2 heterozygous (CT) (green) and a cluster for the negative controls (black). The frequencies of SNP rs2596542 C/T were TT (33.8%), CT (43.1%), and CC (23.1%) (figure 1). Age and gender distribution of the studied populations are presented in table (1). MICA SNP rs 2596542C/T genotypes with HCV viral load before and after treatment in the two groups were presented in table (2) Table (3) showed SNP genotypes association with viral response in HCV patients treated with sofosbuvir (Sovaldi) 400 mg/day plus declatasvir (drug1), also patients treated

with ombitasvir 25 mg, paritaprevir 150 mg and ritonavir100 mg/day plus ribavirin 15 mg/kg/day (drug2).

The results of biochemical parameters:

The results showed that there was a significant decrease in serum ALT and AST after 12 weeks of treatment within the CT-SNP genotype in group (I) as compared to before treatment levels, also there was a significant decrease in serum ALT within the TT and CT- SNP genotypes in group (II). A significant reduction was observed in serum AST within the CT- SNP genotype in group (II) (Figure 2). There was a significant decrease in serum total bilirubin after treatment compared to before treatment levels within the TT and CT- SNP genotypes in group (I) while a significant increase in serum albumin level was detected after treatment within the TT- SNP genotype in group (I) (Figure 3). There was a highly significant reduction in hemoglobin (Hb) level was detected in all C/T SNP genotypes in both group (I) and group (II), except TT- SNP genotype in which there was a highly significant increase in Hb level (Figure 4). Also there was no significant change in WBCs count, Platelets count, fasting blood sugar and Hb A1C % concentrations in the studied groups (table 4). There was a significant reduction in serum creatinine concentration after treatment when compared to before treatment within the CT- SNP genotype in group (I) and significant decrease in serum alpha fetoprotein (AFP) level after treatment as compared to the level before treatment within the TT- SNP genotype in group (II) (Figure 5).

Correlation between C/T genotypes with biochemical parameters

By examining the influence of the SNP rs2596542 on the disease outcomes by correlating the three SNP C/T genotypes with biochemical parameters, it was found that there was no observed significant association between this SNP and some biochemical parameters such as total

bilirubin, serum albumin, serum creatinine, HB, HbA1c %. However, there was a statistical positive significant correlation between HCV level in the CC –genotype and AFP ($p < 0.05$) (**Figure 6**)

4. Discussion

The treatment of HCV GT-4 is affected by many factors that must be precisely evaluated and optimized before treatment initiation [20]. The viral genotype is one of the most important factors affecting the duration and outcome of antiviral treatment [21]. A recent study [8] reported that a single nucleotide polymorphism (SNP) rs2596542 located in the major histocompatibility complex class I chain-related gene A (MICA) promoter region to be significantly associated with HCV induced HCC. Also, The GWAS found that a previously unidentified locus in the 50 flanking region of MICA, which is found 4.7 kb upstream of the MICA gene on chromosome 6p21 (rs2596542) to be strongly associated with HCV [22]. MICA is a natural human ligand for NKG2D [9], to activate antitumor effects of natural killer (NK) cells and CD8⁺ T cells [23]. The single nucleotide polymorphism SNP at restriction site (rs2596542C/T) has an absolute linkage within the MICA promoter region and may alter the binding of stress inducible transcription factors [22]. Tong *et al* hypothesized that the SNP rs2596542 could affect the expression of MICA or initiate pathways related with tumor development [24]. In this study, the frequencies of rs2596542 TT, CT, and CC were (33.8%), (43.1%) and (23.1%) respectively, the heterogeneous CT genotype at SNP rs2596542 more frequent than the CC genotype, this result agree with the result obtained by Mohamed *et al*, [22] who reported that Egyptians had the heterogeneous CT genotype at SNP rs2596542 more frequent than the protective CC genotype. In the present study we found that prevalence of chronic HCV infection is higher in males than females in the studied groups about (60%) for males. This

observation was in agreement with that reported by Niu *et al*, [25] who found that the prevalence of HCV infection in males significantly higher than that of females. The investigation of the associations between SNP (rs2596542C/T) with the response to DAAs in chronic HCV infected Egyptian patients was examined. It is noticed that the majority of patients gave negative PCR results at the end of treatment this means high SVR was achieved after completing the administration of DAAs. The study showed that, The HCV patients treated with Sofosbuvir (Sovaldi) 400 mg/day plus dechlorasvir 60 mg once daily for a course of 12 successive weeks (Group I), responded with 100% in both CC and TT- SNP genotypes, However CT – genotype responded with 91.67%. This goes in agreement with Ahmed *et al*, [26] who conclude that Sofosbuvir and Daclatasvir are effective in treatment of chronic HCV genotype 4 infections with minimal adverse events. The HCV patients treated with Ombitasvir 25 mg, paritaprevir 150 mg and ritonavir 100 mg/day plus Ribavirin 15 mg/kg/day for 12 weeks (Group II), responded with 100% in CT- SNP genotype, However Both CC and TT- SNP genotypes responded with 83.33% and 91.67% respectively. These results agree with those in the AGATE-II trial, Egyptian patients with genotype 4 HCV with and without cirrhosis who received Ombitasvir / paritaprevir / ritonavir plus RBV for 12 weeks had an SVR12 rate of 95%, and those with cirrhosis who received 24 weeks of treatment had an SVR12 rate of 97% [27]. Regarding the comparison between the pre-treatment and post treatment biochemical parameters among the three SNP C/T genotypes in the two studied groups; it was found that a significant improvement occurred at the levels of serum alanine transaminase (ALT) and aspartate transaminase (AST) activities.

5. Conclusions

The combination treatment of Sofosbuvir (Sovaldi) 400 mg/day plus dechlorasvir 60 mg

once daily for a course of 12 successive weeks has high response (100%) in both CC and TT- SNP genotypes. Using of a combination of Ombitasvir plus Paritaprevir plus Ritonavir with ribavirin was highly effective in CT- SNP genotype (100% SVR). However; both CC and TT- SNP genotypes responded with 83.33% and 85.71% respectively. It was concluded that there was no observed significant association between this SNP and some biochemical parameters such as total bilirubin, serum Albumin, serum creatinine, HB, HbA1c% However, there was a positive significant correlation between HCV level in the CC – genotype and the platelets count and AFP.

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Table (1):- Age & Gender distribution of the studied populations.

Parameter \ Group		Total (n=65)	TT (n=22)	CT (n=28)	CC (n=15)
Age (years)	Mean ± S.D	45.02 ± 13.16	46.8 ± 14.17	42.89 ± 11.76	43.53 ± 13.84
Gender	Male	39 (60%)	13	18	8
	Female	26 (40%)	9	10	7

Table (2):- HCV viral load before and after treatment

viral load & genotype	Before treatment		After treatment	
	Group I	Group II	Group I	Group II
High viral load > 2×10 ⁶ (IU/ml)				
TT	5	4	-	-
CT	7	3	-	-
CC	5	4	-	1
Moderate viral load < 2×10 ⁶ (IU/ml)				
TT	5	8	-	1
CT	5	13	1	-
CC	4	2	-	-

Table (3):- SNP genotypes association with viral response in treated HCV patients

Parameter \ Group		Drug1 TT (n=10)	Drug1 CT (n=12)	Drug1 CC (n=9)
SVR after 12 Weeks of treatment. n (%)	Drug 1 Responder	10 (100%)	11 (91.67%)	9 (100%)
	Drug1 Non-responder	0 (0%)	1 (8.33%)	0 (0%)
		Drug2 TT (n=12)	Drug2 CT (n=16)	Drug2 CC (n=6)
	Drug 2 Responder	11 (91.67%)	16 (100%)	5 (83.33%)
	Drug2 Non-responder	1 (8.33%)	0 (0%)	1 (16.67%)

Table (4):- WBCs and Platelets count in the studied groups.

Groups			Group I			Group II		
			TT (n= 10)	CT (n= 12)	CC (n= 9)	TT (n= 12)	CT (n= 16)	CC (n= 6)
WBCs (10 ³ /μL)	Mean ± S.D	Before treatment	7 ± 4.2	7.6 ± 2.5	4.7 ± 1.3	6.8 ± 1.2	6.9 ± 2	5.9 ± 1.6
		After treatment	7.2 ± 4.2	7.7 ± 2.4	4.4 ± 0.9	7 ± 1.3	7 ± 2	5.3 ± 0.9
	p-value	0.187	0.167	0.169	0.252	0.164	0.363	
Platelets (10 ³ /μL)	Mean ± S.D	Before treatment	207 ± 53.2	300.1 ± 87.9	199.6 ± 28.6	237.1 ± 51.3	220.3 ± 34.9	274.2 ± 76.5
		After treatment	215.9 ± 60.7	295 ± 96.1	170.7 ± 24.4	241.7 ± 54.5	218.4 ± 41.9	288.5 ± 73.4
	p-value	0.343	0.339	0.103	0.339	0.680	0.363	

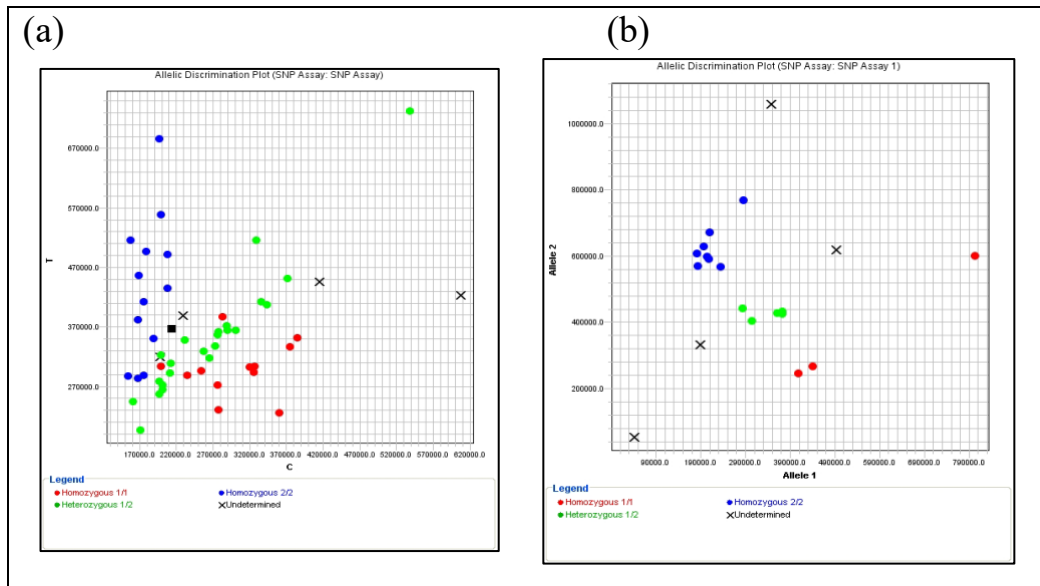
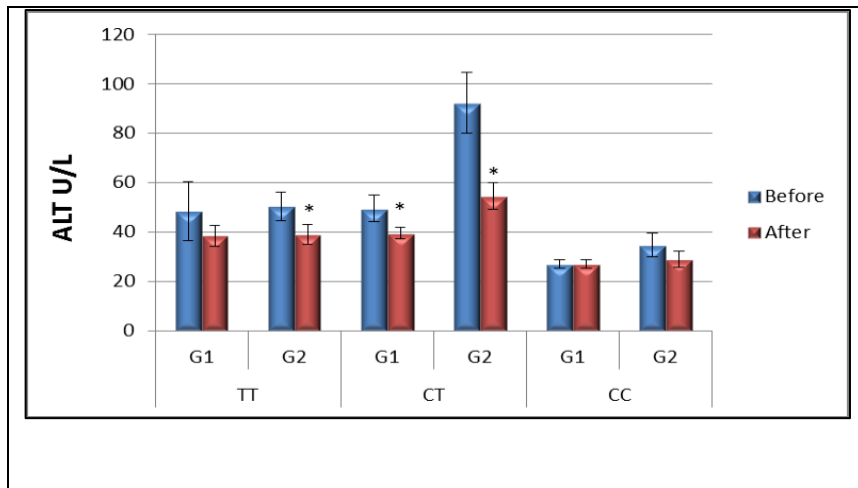


Figure (1):- Allelic discrimination plot for SNP rs2596542 C/T.



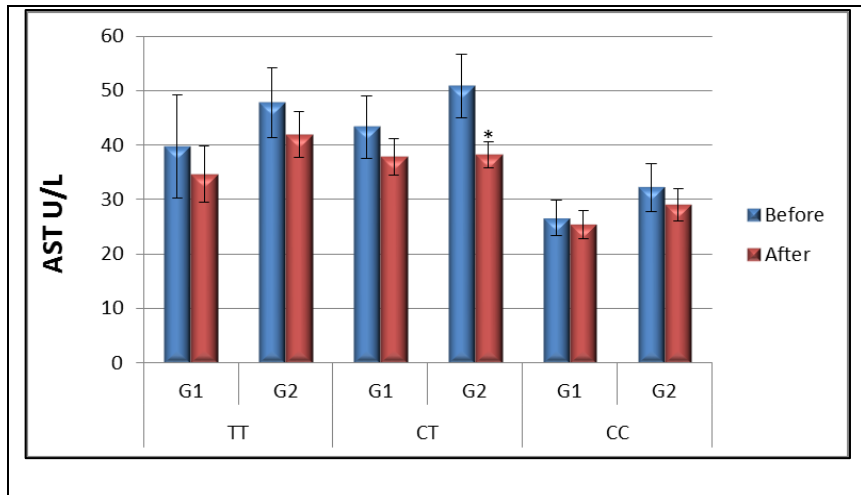


Figure (2):- Mean \pm SD of serum ALT and AST within the C/T SNP genotypes in the studied groups

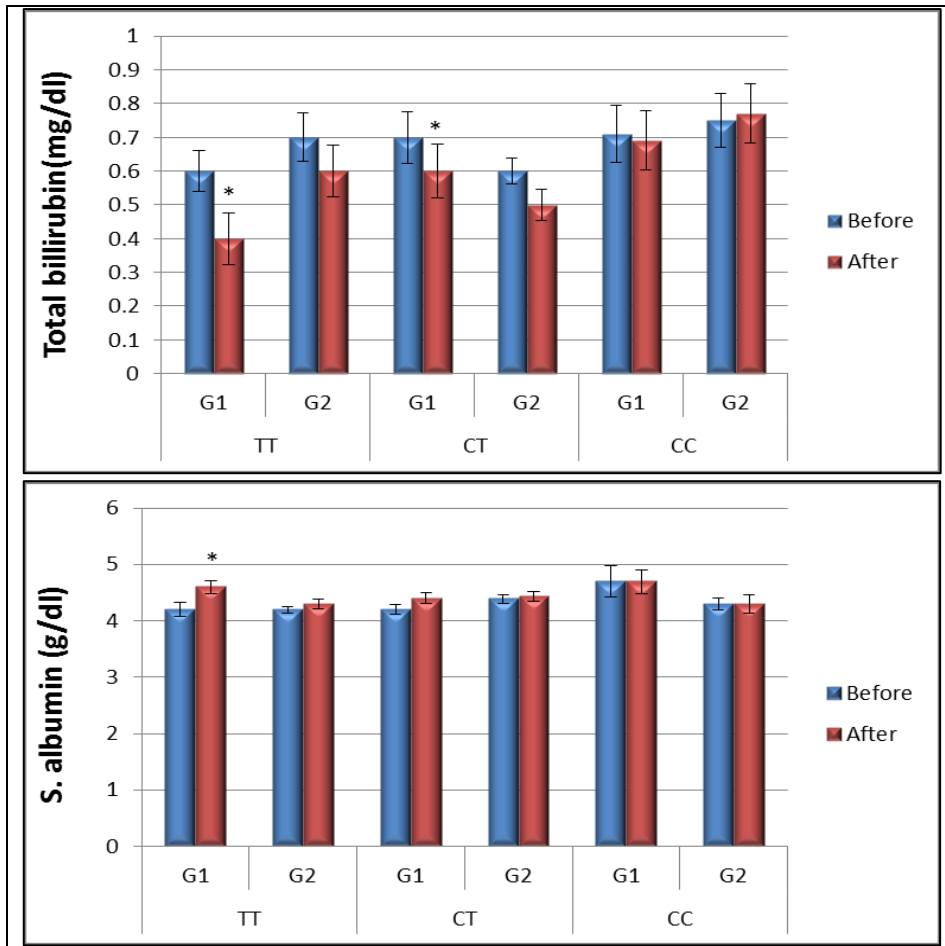


Figure (3):- Mean \pm SD of total bilirubin and serum albumin within the C/T SNP genotypes in the studied groups

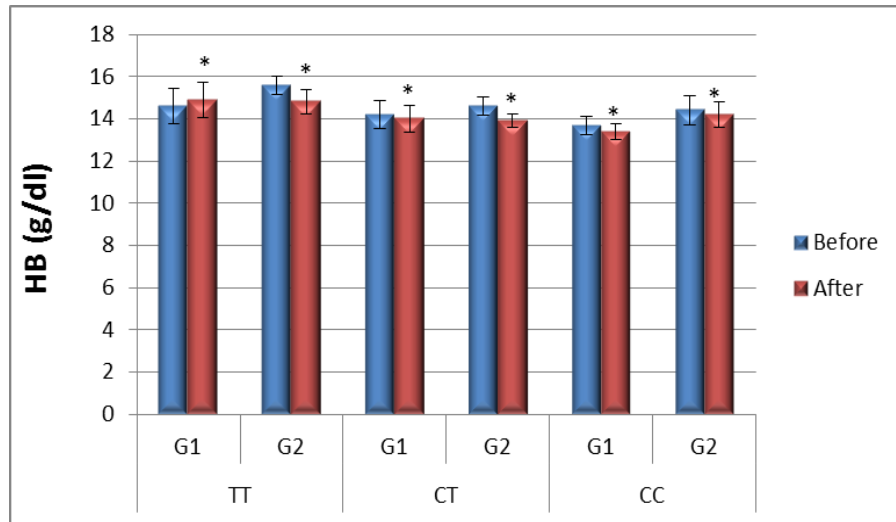


Figure (4):- Mean \pm SD of Hb within the C/T SNP genotypes in the studied groups.

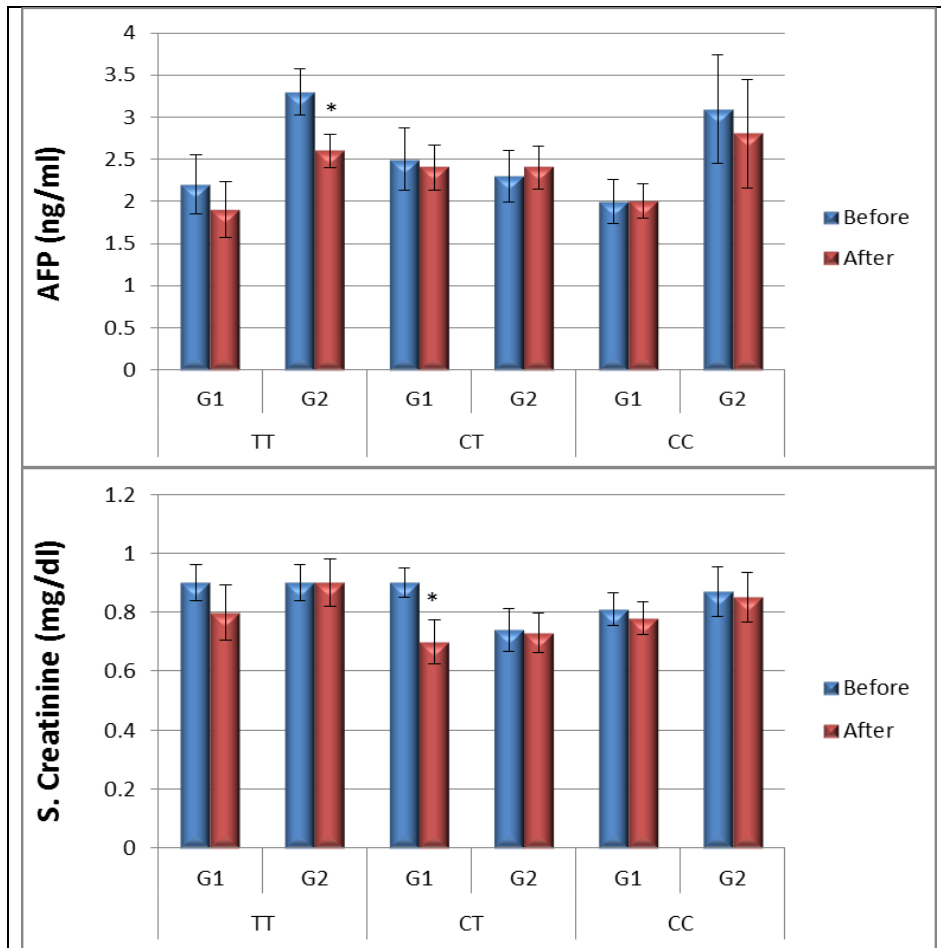


Figure (5):- Mean \pm SD of AFP and serum creatinine within the C/T SNP genotypes in the studied groups

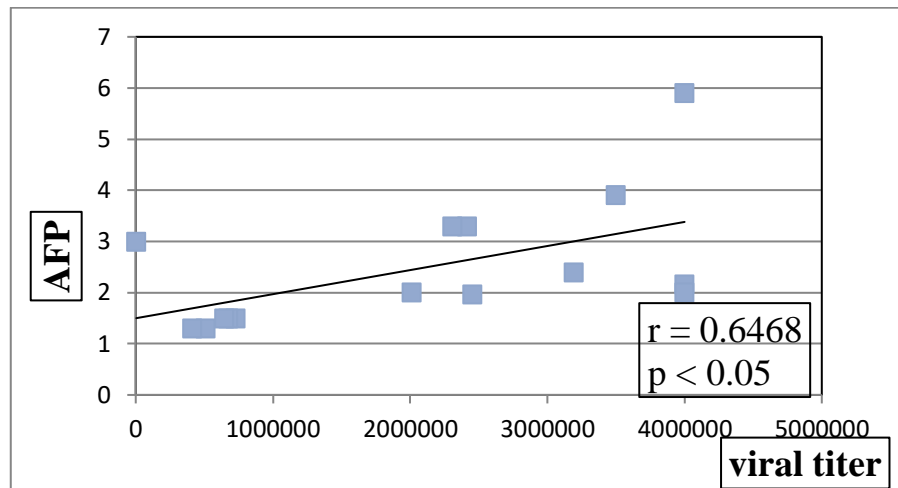


Figure (6): Correlation between AFP level and viral load of HCV in the CC genotype group.