RESEARCH ARTICLE

Association of *C-myc* and p53 Gene Expression and Polymorphisms with Hepatitis C (HCV) Chronic Infection, Cirrhosis and Hepatocellular Carcinoma (HCC) Stages in Egypt

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Abstract

The aim of this study is to investigate *c-myc* and p53 gene expression and polymorphisms in different stages of HCV infection,. Expression levels of *c-myc* and p53 were evaluated by RT-PCR and polymorphisms were determined by PCR-RFLP in 60 HCV patients classified into chronic infection, cirrhosis and HCC groups along with 30 controls. *c-myc* gene expression significant increased through the stages as compared to the control level (1.17, 1.82, 3.33 and 0.32, respectively), whereas p53 significantly declined (4,375, 3,842, 525 and 5,498, respectively). The *C-myc* CC genotype was predominant in the HCC group (90%) to a greater extent than in the cirrhosis, chronic infection and control cases (80%, 20% and 10%, respectively), while the GG genotype was predominant in controls (83%, as compared to 65%, 10% and 10%). The CG genotype was most common in chronic infection (15%). The p53 PP genotype predominated in controls (87%, with 15%, 10% and 20%, respectively, for the three stages) while the AA genotype demonstrated only slight increase to HCC (13%, 25% and 30%, respectively) and PA genotype was predominant in cirrhosis cases (90%). These findings reveal that *c-myc* and p53 gene expression and polymorphisms may be considered as promising sensitive genetic biomarkers for progression of HCV infection.

Keywords: *C-myc* - p53 - genetic polymorphism - HCV infection - qRT-PCR - PCR-RFLP

Asian Pac J Cancer Prev, **18 (8)**, 2049-2057

Introduction

Hepatitis C virus (HCV) infection is one of the main causes of chronic liver disease worldwide (Lavanchy, 2009), where, more than 170 millions are infected with HCV. Chronic hepatitis C (CHC) has a variable course with progression of 30% of patients to cirrhosis and consequent complications (Wiese et al., 2005; Massard et al., 2006). The highest incidence of HCV worldwide have been reported in Egypt as 15% of Egyptians were found to be seropositive for HCV antibodies with genotype 4 being responsible for not less than 90% of these cases (Mostafa et al., 2010). Currently, there are seven major HCV genotypes, which have different geographical distributions and susceptibilities to interferon-a treatment (Hayes et al., 2012). In Egypt, more than 90% of cases belong to HCV genotype 4 (Ray et al., 2000).

HCV encodes a single polyprotein (about 3,000 amino acids), which is processed to yield at least 10 structural and non-structural proteins (Houghton, 1996). Over the past decade, many reports have demonstrated that HCV infection mainly through its proteins regulates transcriptionally, cellular genes, particularly growth and apoptosis-related ones. A viral nucleocapsid protein (19-kDa) encoded by HCV, regulates either positively or negatively several cancer related genes including *c-myc* and c-fos. Also, it represses apoptosis induced by *c-myc*, fas antigen or tumor necrosis factor. Furthermore, transgenic mice expressing HCV core protein tend to develop HCC. Collectively, these findings suggest that HCV is involved, either directly or indirectly in HCV-mediated HCC (Moriya et al., 1998). Factors affecting viral clearance include age, gender, race, level of viremia, alcohol intake and HCV genotype. Genetic studies showed that single nucleotide polymorphisms of host genes affecting the outcome, progreesion of HCV infection and treatment response to antiviral therapy (Renda et al., 2011).

Myc family of proto-oncogenes has been considered to be one of the most studied oncogenes to date (Meyer and Penn, 2008). Crucial involvement of Myc proto-oncogene protein in the regulation of other gene's expression, cellular proliferation, growth, apoptosis, metabolic transformation and oncogenesis

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(Miller et al., 2012). *C-myc* protooncogene, the human cellular homologue of the v-myc oncogene of avian myelocytomatosis retrovirus MC29, which is located at chromosome 8 (8q24.12-q24.13), was found to act as a strong transcription factor, implicated in the control of cell differentiation and apoptosis. Induction of this transcription factor promotes cell proliferation and transformation by activating growth-promoting genes, including the ornithine decarboxylase (ODC1) and CDC25A genes and also the E2F1, E2F2 and E2F3 genes (Leone et al., 2001; Cowling et al., 2006).

SNPs of *c-myc* are likely to bring structural as well as functional changes in the protein. So, genetic variations in cisregulators of transcription at 8q24 can considerably change the germline expression levels of *c-Myc* thus contribute to cancer susceptibility, disease progression and treatment responses (De Baets et al., 2012; Noreen et al., 2015). The investigated Asn11Ser polymorphism is located within the N-terminal domain of *c-myc* and 35 amino acids away from the conserved *c-myc* Box. No functional studies of this polymorphism have been reported according to (Dugan et al., 2002). *C-myc* is activated in 70% of all human cancers, and recent studies have demonstrated the over-expression of *c-myc* in high-grade breast cancers (Blancato et al., 2004).

The p53 gene encompasses 20 kb of DNA with 11 exons which on transcription gives a 3.0 kb mRNA having 1179 bp open reading frame. On translation, this mRNA produces a 53 kDa protein (hence the name p53). The p53 gene mainly functions as a transcription factor and controls the expression of a wide range of genes with disparate functions (Foulkes, 2007). Either truncating mutation or point mutation of p53 gene may lead to the absence or malfunctioning of p53 (Van Dyke, 2007), induce the loss of apoptosis control, affect senescence, cell cycle-cycle regulation, repair of DNA damage, angiogenesis and regulation of oxidative stress; all are essential in carcinogenesis. Therefore, p53 gene is in a central position of many cancer related processes, and its mutations are frequently identified in cancer cells (Vousden and Lane, 2007).

P53 mutations have extensively been studied in human tumors and p53 has 11 exons and codes for a protein containing 393 amino acids (Goh et al., 2011; Marcel et al., 2011). Li et al., (2012) indicated that p53 has different functions such as DNA binding, cell cycle control, DNA restoration, differentiation, genomic plasticity, and programmed cell death (apoptosis). The codon 72 polymorphism of p53 results in Pro/Pro and Arg/Arg phenotypes, with the Pro/Pro phenotype having less apoptosis-inducing potential and higher cancer cell proliferation (Pim and Banks, 2004). p53 codon 72 polymorphisms are associated with risk of various cancers, including those of the liver, in addition, Pro/Pro phenotype is associated with increased risk of tumor progression and poor patient survival for non–small cell lung cancers and colorectal cancers (CRCs), particularly for (AAs) (Katkoori et al., 2009; Sumbul et al., 2012).

Numerous observations examined the role of p53 rs1042522 Arg/Pro polymorphism on hepatocellular carcinoma (HCC) and HCV-associated HCC incidences. Moreover, scientists suggested that there was a frequent loss of p53 rs1042522 Pro allele in HCV-positive carriers (Anzola et al., 2003; Leveri et al., 2004). Genetic polymorphism of *c-myc* intron 8 and p53 codon 72 were determined by different molecular techniques such as PCR-RFLP, multiplex PCR, RT-PCR, hybridization techniques and direct sequencing. In the present study, we aimed to detect the association between the genetic expression and genetic polymorphism of *c-myc* intron 8 and p53 codon 72 in different stages of HCV infection such as chronic, cirrhosis, HCC in Egyptian patients by using RT-PCR and PCR-RFLP.

Materials and Methods

Patients and control

In the current study, 95 patients with different liver diseases cases were attended to internal medicine department, Faculty of Medicine, Alexandria University, Alexandria, Egypt. After clinical, serological and radiological investigations, 35 patients were excluded due to HBV, HDV, Shistosomal infection, fatty liver, primary biliary cirrhosis, autoimmune hepatitis, continued alcohol abuse and other causes of liver diseases. Other 60 patients were positive only for HCV Ab and HCV RNA quantification by RT-PCR were classified into three main groups. First group: 20 patients with chronic HCV infection, second group: 20 patients with cirrhotic liver due to HCV infection and third group: 20 patients with hepatocellular carcinoma (HCC) due to HCV infection. Control healthy subjects (30 HCV negative individuals; 18 males and 12 females) were selected. All the procedures were carried out according to the principles of the faculty of Medicine guidelines and the study was approved by the Ethics Committee of Alexandria University of Medical Sciences, Egypt. A written informed consent was obtained from all subjects prior to recruitment and the study was started from March, 2015 to December, 2016.

Sample preparation

Under full aseptic conditions, 5 ml of blood from each patient and control were collected, left at room temperature for 60 min and then centrifuged at 8000 rpm for 5 min at 4ºC. Upper layer (serum) was separated, coded and stored at -20ºC.

Biochemical and serological tests

A serum for each sample was distributed into three aliquots. The first aliquot was used for full biochemical tests for liver including total protein level, serum albumin, alanine aminotransferase (ALT), aspartate aminotransferase (AST), gamma glutamyle transferase (GGT) and serum bilirubin level (Borax diagnostic, England). The second aliquot was used for serological tests for different serological markers, such as hepatitis C antibody (HCV Ab), hepatitis B surface antigen (HBsAg), hepatitis delta virus antibody (HDV Ab) and shistosomal antibody titres (Bio-Rad, France). The third sera aliquots were kept frozen at -80ºC for further molecular assays, such as genomic DNA extraction, HCV RNA extraction, quantitative HCV RNA titres, HCV genotyping and *c-myc*

and p53 genetic polymorphism.

Molecular tests

Genomic DNA, HCV RNA extraction from sera samples, quantification of HCV RNA and HCV genotyping

Genomic DNAs were extracted from 100 µl of sera samples using genomic DNA isolation kit (Qiagen, England) and DNAs samples were dissolved in 70 µl distilled deionized water and kept at -20ºC till polymerase chain reactions (PCR) and restriction fragment length polymorphism (RFLP) tests. HCV RNA was extracted from 100 µl of serum using a viral RNA TRIZOLE Kit (Bioline, England), according to manufacture instructions. HCV RNA samples were dissolved in 70 µl elusion buffer. According to (Vermehren et al., 2011), HCV RNA was quantified by a real-time polymerase chain reaction assay (detection limit: 50 IU/ml; Real-time HCV; Abbott Molecular, Des Plaines IL, USA). Assay protocols, cut-offs and result interpretations were carried out according to the manufacturer's instructions. The genotype of the amplified cDNA was determined by reverse hybridization using the line probe assay (INNO LiPA HCV II kit, Innogenetics Q2, Ghent, Belgium).

Quantification Real-Time PCR (q RT-PCR) of c-myc and p53 in different studied groups

To analyze the relative changes in *c-myc* and p53 genes expression, quantitative real-time PCR (qRT-PCR) was used. Primers sequences used for real-time PCR were as follows: *c-myc* sense: 5'-CAGTTGTCCCTGCCTTTTCT-3', antisense: 5'-CATTCCCAGCACCTCCTATT-3'; p53 sense: 5'-TGCGTGTGGAGTATTTGGATG-3', antisense: 5'-TGGTACAGTCAGAGCCAACCAG-3'; GAPDH sense: 5'-ACGCATTTGGTCGTATTGGG-3', antisense: 5'-TGATTTTGGAGGGATCTCGC-3'. Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) gene was used as the internal control for normalization of relative quantification. Real-time PCR was performed with SYBR Green I Light Cycler-based Real-Time PCR assay. Where, 20 µl reaction mixtures for the two genes were prepared using 4 µg Light Cycler Fast Start DNA master mixtures for SYBR Green I, 0.5 mM of each primer and 5 µl of cDNA. The amplification reaction was carried out under the following conditions: first 95ºC for 10 min, followed by 40 cycles at 95ºC for 5 sec and 60ºC for 10 sec and 72ºC for 20 sec. After PCR amplification, a melting curve was generated by holding the reaction at 95ºC for 60 sec, the gradient from 72 to 95ºC and raising by 1ºC for each step. The efficiency of amplification of the target genes (*c-myc* and p53), internal control (GAPDH) was examined using real-time PCR. Serial dilutions of cDNA were amplified by real-time PCR using specific primers for each gene. All reverse transcription- PCRs were performed in triplicate. Then, Δ CT was calculated for each cDNA dilution. The data were analyzed using least-squares linear regression. Relative quantification of *c-myc* and p53 expression was performed using 2-ΔΔ Ct method (Abdel-Hafiz, 2011).

Table 1. Primers Sequences and PCR Product

Primers	Primer sequence 5'→3'	Direction	PCR product size
c-myc (F)	CAGTTGTCCCTGCCTTTTCT	Sense	681 bp
c-myc (R)	CATTCCCAGCACCTCCTATT	Antisense	
P53 (F)	ATCTACAGTCCCCCTTGCCG	sense	296 bp
P53 (R)	GCAACTG ACCGTGCAAGTCA	Antisense	

Detection and genotyping of c-myc and p53 genes by specific PCR-RFLP

Primers for *c-myc* and p53 were designed and prepared according to Akbaş et al., (2012) and Oh et al., (2015), as shown in Table 1. Concerning to *c-myc* intron 2 region, A 681 bp fragment was amplified using the primer pairs *c-myc* (F and R). PCR assay was as follows: 12.5 µl of 2x PCR master mixture (Bioline, England), 2 µl from each primer pairs (20 nmol), 6 µl ddH2O (distilled deionized water) and 2.5 µl genomic DNA (50 nmol). PCR reaction included initial denaturation at 95ºC for 5 min, 35 cycles of amplification with denaturation at 94ºC for 40 sec, primer annealing at 60ºC for 40 sec, extension of primer at 72ºC for 40 sec and final extension at 72ºC for 10 min. The result of PCR amplicon was electrophoresed in 2% agarose gels in 0.5X Tris-borate-ethylene diaminetetraacetic acid (EDTA) buffer, stained with ethidium bromide (0.5 ng/ml) and photographed by the Syngene G: Box documentation system (Syngene, UK).

According to Oh et al., (2015), aliquot of the PCR product was digested with HhaI restriction enzyme (10u/µl). The digestion reaction included 10 µl amplified PCR product, 1 µl HhaI restriction, 2 µl 10X restriction enzyme digestion buffer (Fermentas Life Science, Germany) and 7 µl sterile ddH2O was spun at 500 rpm for 3 sec and incubated at 37ºC overnight. RFLP reaction was inhibited by keeping at 60ºC for 20 min, then the result of RFLP's digestion products were electrophoresed in 3% agarose gel in 0.5X Tris-borate-ethylene diaminetetraacetic acid (EDTA) buffer and stained with ethidium bromide. The images were captured using the Syngene G: Box documentation system (Syngene, UK).

For p53 codon 72 region, a 296 bp fragment was amplified using the primer pairs p53 (F and R). The PCR reaction mixture and PCR program conditions were detailed as *c-myc* gene, but with different annealing temperature (56ºC) (Akbaş et al., 2012). Aliquot of the PCR product was digested with Bsh1236I restriction enzyme (10u/µl, Fermentas Life Science, Germany). The digestion reaction and conditions the same *c-myc* gene.

Statistical analysis

Data are reported as means ± SD and statistical analyses were performed using the SPSS Statistical package, version 11.5 for Windows (SPSS. Inc., USA). The normally distributed data were analyzed by the Student t-test. Non-normally distributed data were analyzed by the chi-square test. All statistical tests were two-sided and a P value <0.05 was considered to be

Table 2. Demographic Data of the Four Studied Groups: Control, Chronic, Cirrhosis and HCC

Data	Control (30)	Chronic (20)	Cirrhosis (20)	HCC (20)	*p. value*
Age (year)					0.001
means±SD	46.3±10.8	38.2±12.6	41.6±11.2	51.3±8.6	
ranges	(34-58)	(27-52)	(30-52)	(44-61)	
Sex					
Male (n,%)	18/30 (60%)	12/20 (60%)	16/20 (80%)	14/20 (70%)	0.004
Female (n,%)	12/30 (40%)	8/20 (40%)	4/20 (20%)	6/20 (30%)	

p value ≤ 0.05 is significant; SD is Standard deviation and HCC is Hepatocellular carcinoma

Table 3. Serological Parameters of the Four Studied Groups

Parameter	Control (30)	Chronic (20)	Cirrhosis (20)	HCC (20)	*p.value*
HCV Ab (n,%)	0/30 (0.0 %)	20/20 (100 %)	20/20 (100 %)	20/20 (100 %)	0.004
HBsAg (n,%)	0/30 (0.0 %)	0/20 (0.0 %)	0/20 (0.0 %)	0/20 (0.0 %)	0.001
HDV Ab (n,%)	0/30 (0.0 %)	0/20 (0.0 %)	0/20 (0.0 %)	0/20 (0.0 %)	0.01
Schistosomal Ab	0/30 (0.0 %)	0/20 (0.0 %)		0/20 (0.0 %)	0.006
(n,%)			0/20 (0.0 %)		

p value ≤ 0.05 is significant; HCV Ab is hepatitis C virus antibody; HBsAg is hepatitis B virus antige and HDV Ab is hepatitis delta virus antibody

statistically significant.

Results

General characteristics of the studied populations

Four groups were included in the current study. The first group was control healthy group which contains 30 persons (60% males and 40% females), their ages ranged from 34 to 58 years, with a mean (SD) of 46.3 years (10.8 years). The second group was chronic HCV infection which contains 20 patients (60% males and 40% females), their ages ranged from 27 to 52 years, with a mean (SD) of 38.2 years (12.6 years). The third group was cirrhosis HCV infection which contains 20 patients (80% males and 20% females), their ages ranged from 30 to 52 years, with a mean (SD) of 41.6 years (11.2 years. The fourth group was HCC infection which contains 20 patients (70% males and 30% females), their ages ranged from 44 to 61 years, with a mean (SD) of 51.3 years (8.6 years). It should be noted that, there were significant differences in age and gender among the studied groups as shown in Table 2.

Biochemical parameters

The total protein levels varied with a mean (SD) of 6.12 (2.18), 4.63 (1.18), 3.88 (1.14) and 3.18 (2.11) in control, chronic, cirrhosis and HCC groups, respectively. The serum albumin levels varied with a mean (SD) of 3.38 (2.18), 2.16 (1.1), 2.11 (0.91) and 1.18 (1.33) in control, chronic, cirrhosis and HCC groups, respectively. The AST activities varied with a mean (SD) of 7.15 (3.61), 20.6 (6.48), 28.4 (11.6) and 23.7 (10.6) in control, chronic, cirrhosis and HCC groups, respectively. The ALT activities varied with a mean (SD) of 8.41 (3.61), 34.6 (10.8), 38.3 (12.6) and 26.9 (11.1) in control, chronic, cirrhosis and HCC groups, respectively. The total bilirubin levels varied with a mean (SD) of 0.81 (0.23), 2.11 (1.18), 2.46 (1.34) and 2.34 (0.91) in control, chronic, cirrhosis

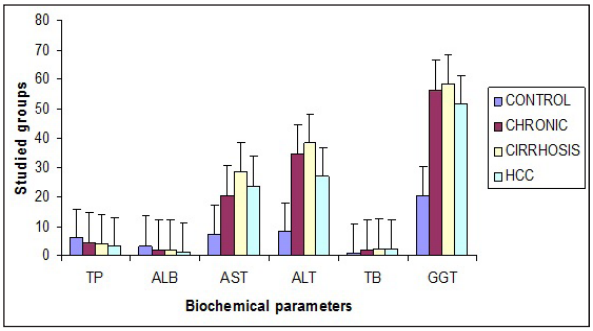

Figure 1. Biochemical Parameters in the Four Studied Groups

Table 4. HCV Quantification and Genotyping

Parameter	Chronic (20)	Cirrhosis (20)	HCC (20)	*p.value*
HCV RNA (IU/ml)				0.012
means±SD	$4.36X10^4$±$2.12X10^2$	$8.44X10^5$±$3.43\ X10^2$	$3.14X10^4$±$2.15X10^2$	
HCVgenotypes				0.08
G1(n,%)	0/20 (0.0%)	1/20 (5%)	0/20 (0.0%)	
G2 (n,%)	2/20 (10)	1/20 (5%)	1/20 (5%)	
G3 (n,%)	1/20 (5%)	0/20 (0.0%)	0/20 (0.0%)	
G4 (n,%)	17/20 (85%)	18/20 (90%)	19/20 (95%)	

p value≤ 0.05 is significant; G1 is Genotype 1; G2 is Genotype 2;G3 is Genotype 3; Genotype 4 and Genotype 4

Table 5. Genetic Polymorphisms of *c-myc* in Different Studied Groups

Studied groups	CC genotype (682 bp) (n,%)	GC genotype (681, 480 and 201 bp) (n,%)	GG genotype (480 and 201 bp) (n,%)	*p. value*
Control (30)	3/30 (10%)	2/30 (6.66%)	25/30 (83.33%)	0.018
Chronic (20)	4/20 (20%)	3/20 (15%)	13/20 (65%)	0.041
Cirrhosis (20)	16/20 (80%)	2/20 (10%)	2/20 (10%)	0.036
HCC (20)	18/20 (90%)	0/20 (0.0%)	2/20 (10%)	0.001

p value ≤ 0.05 is significant

Table 6. Genetic Polymorphisms of p53 in Different Studied Groups

Studied groups	PP genotype (296 bp) (n,%)	PA genotype (296, 169 and 127 bp) (n,%)	AA genotype (169 and 127 bp) (n,%)	*p. value*
Control (30)	26/30 (86.66%)	0/30 (0.0%)	4/30 (13.33%)	0.033
Chronic (20)	3/20 (15%)	12/20 (60%)	5/20 (25%)	0.021
Cirrhosis (20)	2/20 (10%)	18/20 (90%)	0/20 (0.0%)	0.001
HCC (20)	4/20 (20%)	10/20 (50%)	6/20 (30%)	0.091

p value ≤ 0.05 is significant

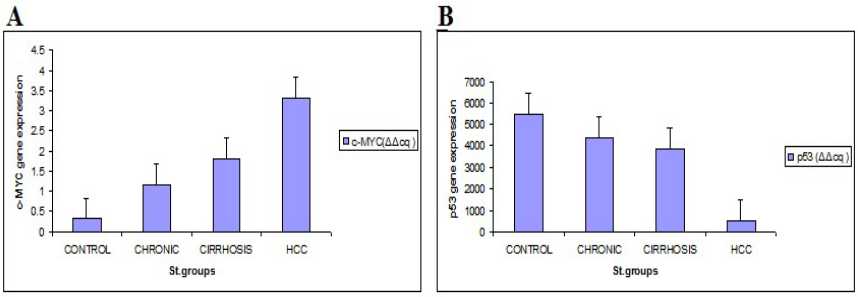

Figure 2. (A and B). A is *c-myc* and B is p53 Gene Expression in the Four Studied Groups

and HCC groups, respectively. Finally, the GGT activities varied with a mean (SD) of 20.4 (10.66), 56.4 (12.3), 58.2 (14.1) and 51.3 (6.3) in control, chronic, cirrhosis and HCC groups, respectively. All P values were ≤ 0.05 and were significant as shown in Figure 1.

Serological parameters

For serological markers, control group were negative for HCV Ab, HBSAg, HDV Ab and schistosomal Ab titres. While the other three groups: Chronic, cirrhosis and HCC groups were positive for HCV Ab and negative for HBSAg, HDV Ab and schistosomal Ab. All P values were ≤ 0.05 and were significant as shown in Table 3.

Molecular parameters

HCV quantification and genotyping

HCV RNA quantification titres (IU/ml) were increased in cirrhosis HCV group with a mean (SD) of $8.44X10^5$ ($3.34\ X10^2$) more than chronic HCV and HCC-HCV groups ($4.36X10^4$ ($2.12\ X10^2$, $3.14X10^4$ ($2.15\ X10^2$). On the other hand, HCV genotyping results showed that HCV genotype 4 was predominant in all studied groups more than G1, G2 and G3. All P values were ≤ 0.05 is significant as shown in Table 4.

Genetic expression of c-myc and p53 genes by qRT-PCR

For the c-myc gene expression, the results showed that ssignificant increasing in different studied groups (chronic, cirrhosis and HCC) compared to control group (1.17, 1.82, 3.33 and 0.32, respectively). On the contrary, p53 gene expression was decreased significantly in different studied groups (chronic, cirrhosis and HCC) compared to

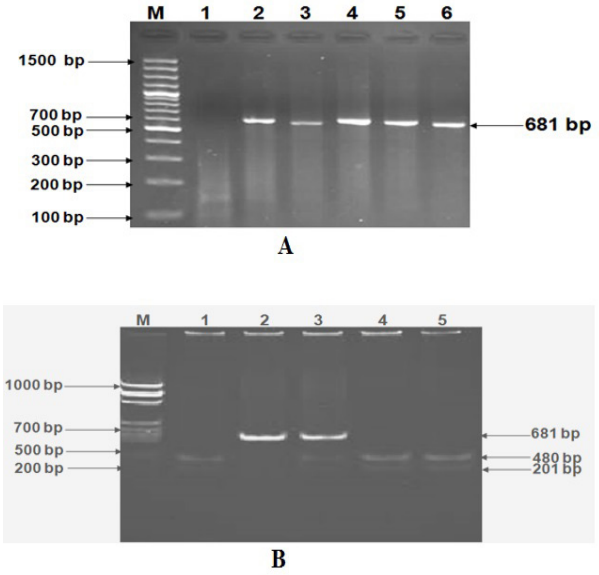

Figure 3. (A and B). A is Agarose gel electrophoresis of c-myc PCR products. Lane M: 1.5 Kb DNA ladder, lane 1: negative sample, lanes 2-6: *c-myc* PCR products (681 bp). B is Agarose gel electrophoresis of digested c-myc PCR with HhaI restriction endonuclease. Lane M: 1 kb DNA ladder, lane 2: CC genotype (681 bp), lane 3: CG genotype (681, 480 and 201 bp) and lanes 1, 4 and 5: GG genotype (480 and 201 bp)

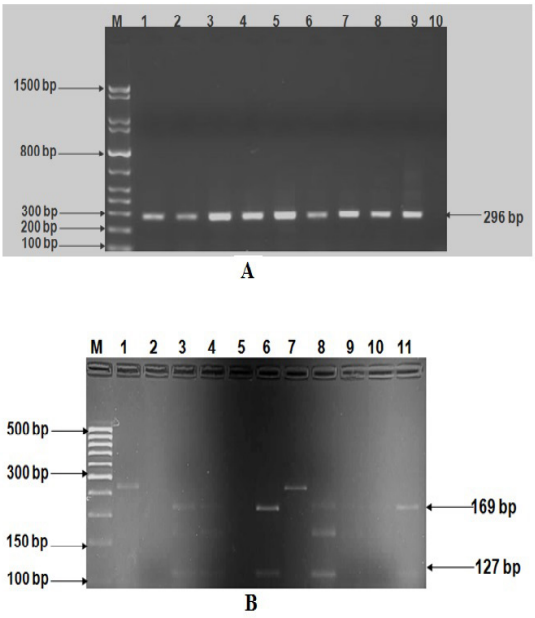

Figure 4. (A and B). A is Agarose gel electrophoresis of p53 PCR products. Lane M: 1.5 Kb DNA ladder, lanes 1-9: p53 PCR products (296 bp) and lane 10: negative samples. B is Agarose gel electrophoresis of digested p53 PCR with Bsh1236I restriction endonuclease. Lane M: 500 bp DNA ladder, lanes 1 and 7: PP genotype (296 bp), lanes 3, 4, 6, 8, 9 and 11: AA genotype (169 and 127 bp)

control group (4375, 3842, 525 and 5498, respectively). All P values were ≤ 0.05 is significant as shown Figure 2.

Distribution of c-myc and p53 genotypes of studied groups

Digestion of PCR products for *c-myc* gene (intron 8) with HhaI restriction enzyme generated three polymorphic alleles: Homozygous cystine genotype (CC= 681 bp), heterozygous glycine cystine genotype (CG= 681, 480 and 201 bp) and homozygous glycine genotype (GG= 480 and 201 bp) (Figures 3 A and B). Concerning digestion of PCR products for p53 (codon 72) with Bsh1236I restriction enzyme yielded three polymorphic alleles: Homozygous proline genotype (PP= 296 bp), heterozygous proline arginine genotype (PA= 296, 169 and 127 bp) and homozygous arginine genotype (AA= 169 and 127 bp) (Figures 4 A and B).

Frequencies of *c-myc* genotypes in the four studied groups, the results showed that CC genotype was predominant in HCC group (90%) more than cirrhosis, chronic and control groups (80%, 20% and 10 %, respectively). GG genotype was predominant in control group (83.33%) more than chronic, cirrhosis and HCC groups (65%, 10% and 10%, respectively). CG genotype was high in chronic group (15%) more than cirrhosis and control groups (10%, 6.66%, respectively) and absent in HCC group. On the other hand, the results showed that frequencies in p53 genotypes were, PP genotype was predominant in control group (86.66 %) more than chronic, cirrhosis and HCC groups (15%, 10% and 20%, respectively). AA genotype was varied in control, chronic and HCC groups (13.33%, 25% and 30%, respectively) and absent in cirrhosis group. PA genotype was predominant in cirrhosis group (90%) more than chronic and HCC groups (60% and 50%, respectively) and absent in control group. The results revealed significant differences in *c-myc* and p53 genotypes variants in studied groups, with P value ≤ 0.05 is significant as shown in Tables 5 and 6 and in Figure 5 (A and B).

Discussion

Demographic, clinical, virological, histological and molecular factors were studied among chronic, cirrhosis and HCC patients infected with HCV, moreover, the association between genetic polymorphisms of *c-myc* and p53 genes was investigated in the current study. The results revealed that there was a potential impact of *c-myc* and p53 genes (expressions and polymorphisms), indicating that these findings considered genetic markers for determining the progression of HCV infection.

In the present study, the results showed increasing significant enzymatic activity of AST, ALT, GGT and TB levels (P= 0.001, P= 0.03, P= 0.01and P= 0.024, respectively) in chronic, cirrhosis and HCC groups compared to the control group. On the contrary, the results showed significant decreasing of TP and ALB levels (P= 0.04 and P= 0.021, respectively) with progression of HCV infection. On the other hand, our results indicated that the HCV viremia level (VL) was increased in cirrhosis group more than both chronic and HCC groups. However, Roudot-Thoraval et al., (1997) and Taketa, (1998) found that the early normalization of abnormal ALT and AST levels of HCV infection may indicate response to antiviral treatment, which agree with our results. Also, Bruhn et al., (2015) found that the detailed knowledge of the VL distributions in each HCV infection stage was fundamental

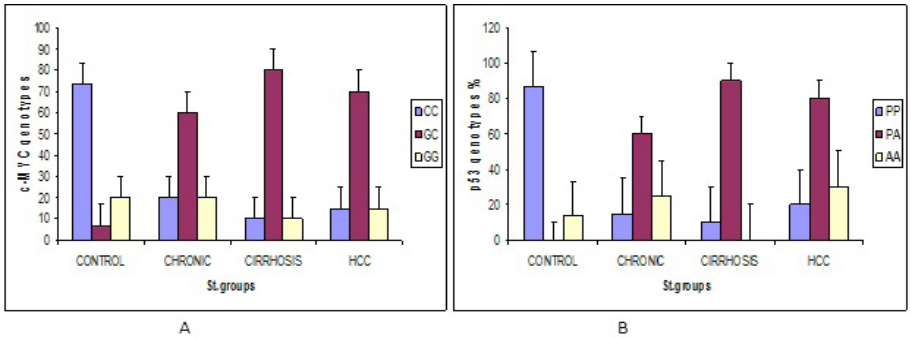

Figure 5 (A and B). A is Frequencies of *c-myc* Genotypes and B is p53 Genotypes in the Four Studied Groups

for understanding of the efficacy of different testing scenarios in removing transmission risk and progression of HCV infection.

In the present study also, our results showed that significant increasing of *c-myc* gene expression in chronic, cirrhosis and HCC groups compared to control group. Additionally, *c-myc* CC genotype was predominant in HCC group (90%) more than cirrhosis, chronic and control groups (80%, 20% and 10%, respectively). While, GG genotype was predominant in control group (83.33%) more than chronic, cirrhosis and HCC groups (65%, 10% and 10%, respectively). The last GC genotype was high in chronic group (15%) more than cirrhosis and control groups (10% and 6.66%, respectively), but absent in HCC group. These results agree with Higgs et al., (2013) and Quan et al., (2014) who found that *c-myc* expression was activated by HCV infection. Also, *c-myc* expression increases in hepatic tissues from HCV-infected patients, as well as, in HCV-transgenic mouse model. Where, *c-myc* plays a critical and important role in HCV-associated with HCC (Pelengaris et al., 2002; Nilsson and Cleveland, 2003). In a previous study to Robert et al., (2012), mutational analysis of the N-terminal domain have demonstrated the crucial role by recruiting nuclear cofactors to enable oncogenic and apoptotic activities of *c-myc* and this region is important for the biologic function of the protein. In another study, Park et al., (2002) found that the amino acid exchange form Asn to Ser might lead to an impaired function of *c-myc* activity.

Concerning p53 gene, the results showed that significant decreasing expression in chronic, cirrhosis and HCC groups compared to control group. In addition, PP genotype was predominant in control group (86.66%) more than chronic, cirrhosis and HCC groups (15%, 10% and 20%, respectively). While, AA genotype was varied in control, chronic and HCC groups (13.33%, 25% and 30%, respectively) and absent in cirrhosis group. Last genotype was PA was predominant in cirrhosis group (90%) more than chronic and HCC groups (60% and 50%, respectively) and absent in control group.

In some related previous studies, Matlashewski et al., (1987) found a new p53 single nucleotide polymorphism located at codon 72 (rs1042522) and associated with arginine to proline (Arg/Pro) substitution. Numerous observations examined the role of p53 rs1042522 Arg/Pro polymorphism on hepatocellular carcinoma (HCC) and HCV-associated HCC incidences (Anzola et al., 2003). The author also suggested that there was a frequent loss of p53 rs1042522 Pro allele in HCV-positive carriers. On the contrary, Leveri et al., (2004) found that there was no association between p53 rs1042522 genotypes and disease severity or hepatocarcinogenesis. Very recently, Eskander et al., (2014) investigated that p53 rs1042522 Pro/Pro and Arg/Arg genotypes may be potentially used as sensitive genetic markers for HCV genotype-4a susceptibility. While, Minouchi et al., (2002) found that no association between codon¶72 genotypes and HCV genotypes 2a and 2b infections. But, there was a significant correlation between male homozygotes for P53 72Pro with HCV type 1b infection. On the other hand, Huang et al., (2007) indicated that PP and AA genotypes may be potentially used as sensitive genetic markers for HCV genotype-4a susceptibility. Also, Okada et al., (2001) referred that p53 genetic polymorphisms were investigated frequently in patients with cirrhotic livers compared to patient with chronic hepatitis, suggesting that p53 polymorphisms at this stage may be a causative factor that may potentially leads to HCC. Moreover, Shaker et al., (2011) and Asselah et al., (2012) found that IL28B rs12979860 C/C genotype, p53 rs1042522 G/G genotype, CD95 rs1800682 A/A genotype and BCL2 rs1800477 A/A genotype were associated with better treatment response rates compared to the remaining HCV genotypes of the same polymorphic points.

Pro allele at the codon 72 of p53 gene might have a synergistic effect on HCC development for subjects with chronic HBV disease and family history of HCC in first-degree relatives (Zhu et al., 2005). Also, the subjects with both chronic liver disease and the Pro allele (Arg/Pro allele, Pro/Pro allele) had an increased risk of 7.60 (95% CI 2.28–25.31) to develop HCC (Yu et al., 1999). Comparing with Arg/Arg genotype, HBV-negative subjects with Arg/Pro and Pro/Pro genotypes have 1.97-fold and 3.36-fold increasing risk for HCC, respectively. While, the subjects with Pro allele and HCC family history yielded 11.81-fold increasing risk of HCC. Finally, Yoon et al., (2008) found that the increasing prevalence of HCC for p53 Pro/Pro over Arg/Arg was identified in big Korean cohorts (odds ratio 3.03, P= 0.006).

In conclusion, genetic expression and polymorphism of *c-myc* and p53 codon 72 may be used as genetic biomarkers for prediction and prognosis the different stages of HCV infection from chronic, cirrhosis and HCC stages among Egyptian population. For our knowledge, it can't be neglected that, there is the first study that investigate the relationship between genetic expression and polymorphism of *c-myc* and p53 codon 72 in different stages of HCV infection in Egypt. Hence, these finding can be used as an accurate diagnostic markers for different stages of HCV infection.

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