Egyptian Journal of Medical Microbiology Volume 29 / No.4 / October 2020 165-172 Online ISSN: 2537-0979

ORIGINAL ARTICLE

Study the Role of Tp53 in the Development of Hepatocellular Carcinoma in HCV Infected Patients

[1]Nahed F. Fahmy*, [2]Nagwa S. Ahmed, [3]Ghada Galal, [2]Mohamed A. Abdel Elmoaty, [3]Ahmed Abudeif, [2]Zeinab M. Kadry, [1]Nesma A. Mohamed

[1]Microbiology & Immunology Department, Faculty of Medicine, Sohag University, Egypt
[2]Biochemistry Department, Faculty of Medicine, Sohag University, Egypt
[3]Tropical Medicine Department, Faculty of Medicine, Sohag University, Egypt

Key words:
Tumor protein 53; Hepatocellular carcinoma; Hepatitis C virus

Corresponding Author:
Nahed Fathallah Fahmy Mohamed
Microbiology & Immunology Department, Faculty of Medicine, Sohag University, Egypt
Tel.: 01142283865
01017558587
nanafahmy_783@yahoo.com
nahed_fahmy@med.sohag.edu.eg

ABSTRACT

Background: *Hepatocellular carcinoma (HCC) is considered as the primary malignancy of the liver that usually occurs on top of chronic viral hepatitis. The TP53 (tumor protein 53) is a tumor suppressor gene which is the key in tumor development and progression and the single nucleotide polymorphism (SNP) of the p53 gene codon 72 (p53Arg/Pro) changes the structure of the protein.* ***Objectives****: to determine the association of the TP53 Arg72Pro polymorphism with the risk of HCC development in Hepatitis C virus (HCV) infected Egyptian patients.* ***Methodology:*** *This is a case control study conducted in Sohag University Hospital on 100 participants (20 HCC, 20 CHCV, 40 LC and 20 control). TP53 gene polymorphism was identified by Polymerase chain reaction-Restriction fragment length polymorphism (PCR-RFLP), serum level of TP53 was measured by quantitative ELISA.* ***Results****: The GG genotype / G allele has the highest frequency in the controls, while the CC genotype/ C allele was more frequently found in the HCC patients. Carriage of TP53 CC genotype and TP53 serum level have a statistical significant association with HCC development.* ***Conclusion****: the CC genotype and the Pro (C) allele of TP53, codon 72 are risk factors for HCC in patients infected with HCV and the serum TP53 level is overexpressed in HCC patients.*

INTRODUCTION

HCC "the king of cancer" is the primary malignancy of liver that usually occurs on top of chronic liver disease due to Hepatitis viruses infection. HCC develops in HCV-related liver cirrhosis with a yearly rate around 3%[1]. The World Health Organization estimates that 130-170 million people are infected with HCV worldwide and that more than 350000 people die of HCV-related liver diseases each year[2]. The tumor suppressor gene TP53 is located on chromosome 17, encodes a 53-kDa nuclear phosphoprotein which can activates the expression of p21 and affects the cell cycle G1 phase checkpoints, regulates the cells entering the S phase and manages the repair of damaged cells. When the repair fails, apoptosis is induced to prevent carcinogenesis [3]. Mutations of the TP53 gene have been widely associated with cancer. Besides mutations, a common G/C single nucleotide polymorphism occurs in p53 gene, exon 4, codon 72, at the second position resulting in substitution of guanine (CGC) with cytosine (CCC) nucleotide, and subsequently affecting the amino acid product of the affected codon, producing proline residue (Pro) instead of arginine residue (Arg), this SNP known as (p53 Arg72 Pro) which affects TP53 function[4]. Each individual inherits 2 alleles of TP53 gene, one allele from each parent that determines the p53 genotype which can be heterozygous polymorphism (Arg/Pro) or homozygous with no polymorphism (Arg/Arg) or homozygous polymorphism (Pro/Pro). The two polymorphic types of TP53 have structural differences with variable characters[5]. The aim of our study to determine the association between the TP53 gene polymorphism (Arg-72-Pro / G-C) and the risk of HCC in HCV-infected patients and to detect the relation between the TP53 gene polymorphism and the serum TP53 levels in HCC patients.

METHODOLOGY

This is a case-control study conducted in Sohag University Hospital during the period from May 2017 to February 2019 on twenty-patients with chronic hepatitis C virus infection (CHCV), forty patients with HCV-related liver cirrhosis (LC), twenty- patients with HCV-related liver cirrhosis with HCC on top and twenty healthy adults matched for age and sex with the patients group as controls. Each patient was subjected to complete medical history taking and clinical examination. Liver function tests, hepatitis markers, Quantitative HCV PCR, level of Alpha fetoprotein (AFP) and radiological examination; abdominal ultrasound was performed for all patients. Patients with hepatic focal lesion were subjected to triphasic

DOI: https://doi.org/10.51429/EJMM29421

Abdominal CT or MRI to confirm the diagnosis of HCC. Ethical approval was obtained from the ethical committee of Faculty of Medicine, Sohag University.

Sample collection:

Blood samples were collected from the patients and controls in EDTA and plain tubes. The Whole blood in EDTA tubes was used for DNA extraction, while blood samples in plain tubes were centrifuged for separation of serum. Serum was used for measurement of the level of TP53 by ELISA.

Molecular detection of TP53 gene polymorphism:

DNA extraction:

Genomic DNA was extracted from Buffy coat which was collected from EDTA blood by using Thermo Scientific Gene JET Whole Blood Genomic DNA Purification Mini Kit according to manufactures' instruction. The extracted DNA was stored at -20^{o}C to be used for amplification of the target gene by PCR.

Polymerase chain reaction (PCR):

PCR assay was performed by using 200 ng of the extracted DNA in a total reaction volume of 50 µL. The PCR mix consists of 25 µL of Master mix (MyTaq Red Mix, Bioline, England), 2.5 µL of each primer 'forward"(5'-TGAGGACCTGGTCCTCTG ACT-3') and "reverse" (5'-AAGAGGAATCCCAAAGTTCCA-3') [6]. Thirty five cycles of amplification were performed in thermal cycler (T Gradient-Biometra). After initial denaturation of DNA at 95^{o}C for 7 min, each cycle included 3 steps; denaturation at 94^{o}C for 60 seconds, primer annealing at 50^{o}C for 60 seconds, extension at 72^{o}C for 45 seconds and a final extension at 72^{o}C for 10 min following the last cycle. The amplified products were visualized on 2% agarose gel stained with ethidium bromide. The stained gels were visualized and documented with a gel documentation system and analyzed visually to determine the size of PCR amplicon of the target gene 424 bp.

Restriction fragment length polymorphism (RFLP) Assay:

The amplicons were digested with BstUI restriction endonuclease. For each restriction digestion reaction, 21.5 µL of the amplified PCR product were digested with 1 µl of the BstUI restriction enzyme and 2.5 µL of restriction enzyme buffer "1 X", the reaction mixture (25 µL) was incubated at 37°C for 1 hour, then electrophoresed on 2% agarose gel, stained with ethidium bromide and visualized under UV illumination using gel documentation system. The size of DNA fragments was determined directly by comparison with 100 bp DNA ladder (Jena Bioscience, Germany),. The restriction fragments generated after digestion of the target gene by BstUI restriction endonuclease are shown in table 1.

Quantitative measurement of TP53 by ELISA:

Blood samples centrifuged for separation of serum. Human TP53 was measured in serum samples using ELISA Kit (Sinogeneclon Biotech, catalog No. SG-10350) by double antibody technique according to manufacturer's instructions. The absorbance of samples was measured at a wavelength of 450 nm. The concentration of TP53 in the serum samples was determined by comparing the O.D. of the samples to the standard curve.

Table 1: showing size of amplicon, restriction fragments, genotypes and alleles of TP53.

Size of PCR amplicon	Size of restriction fragments	Type of polymorphism	Genotype / Allele	
424 bp	424 bp	(Arg/Arg) No polymorphism	GG	G allele
	263, 161 bp	(Pro/Pro) homozygous polymorphism	CC	C allele
	424, 263, 161 bp	(Arg/Pro) heterozygous polymorphism	GC	G allele /C allele

Statistical analysis:

Data were analyzed using IBM SPSS Statistics for Windows, version 25. Quantitative data were expressed as mean±standard deviation, median and range. Qualitative data were expressed as number and percentage. Independent Samples *t*-test and One-Way ANOVA test were used for normally distributed data. Chi-square (χ^2) test and Fisher's Exact Test were used for comparison of qualitative variables as appropriate. Univariate and multivariate binary logistic regression analysis were used to determine predictor variables of HCC. A 5% level was chosen as a level of significance in all statistical tests used in the study.

RESULTS

This study was conducted on 80 patients (46 males and 34 females). Among them, 40 patients had HCV-related liver cirrhosis, 20 patients had HCC on top of HCV-related liver cirrhosis and 20 patients had chronic HCV without liver cirrhosis. Twenty healthy controls matched for age and sex with the patients' groups were included.

According to the results of PCR-RFLP (figure 1,2,3), the distribution of the TP53 genotypes among the study groups is listed in (table 2). The GG (wild type) was the common genotype detected in the LC group

(55%), CHCV group (45%) and the controls (55%). The CC genotype was found with high frequency in the HCC group (40%) followed by the LC group (22.5%). By comparing the distribution of CC genotype in the four study groups, it was found that patients with HCC had a significant high frequency of CC (pro/pro) genotype in comparison with other groups.

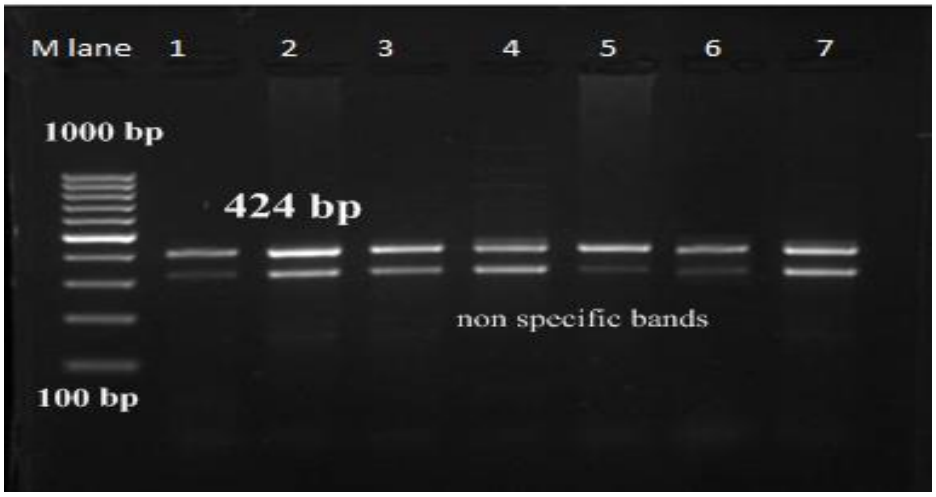

Fig. 1: Showing the TP53 gene after amplification by PCR; M lane= molecular marker (100-1000 bp), L1,2,3,4,5,6,7= TP53 gene (424 bp).

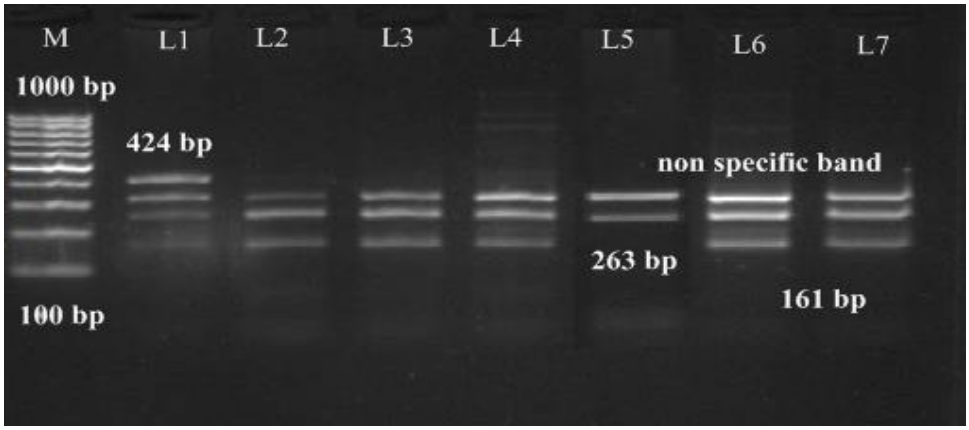

Fig. 2: Showing the genotypes of TP53 by PCR-RFLP. M lane= molecular marker (100-1000 bp), L1; heterozygous polymorphism GC= 424, 263 and 161 bp. L2-L7; homozygous polymorphism CC= 263 bp and 161 bp.

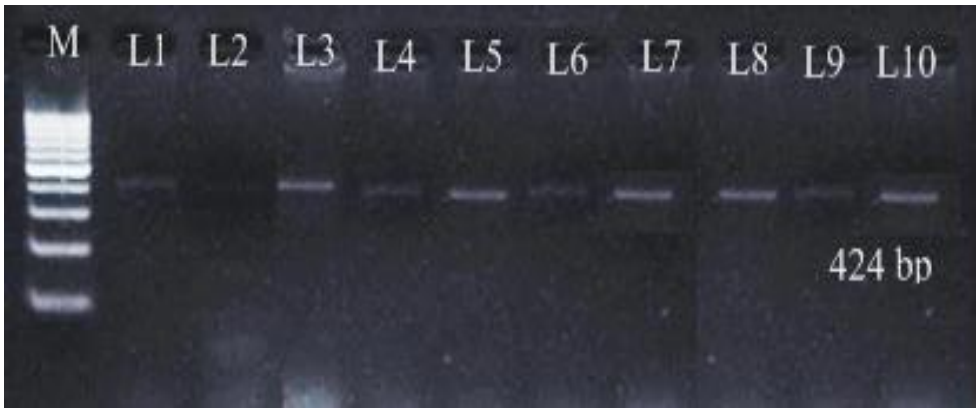

Fig. 3: Showing the genotypes of TP53 by PCR-RFLP. M lane= molecular marker (100-1000 bp), L1-L10; no polymorphism GG=424 bp.

The frequency of alleles in our study among the 4 groups is listed in table 2. The G allele was detected more frequently in the controls (72.5%), followed by the CHCV group (67.5%) and LC group (66.3%). While the C allele was common in the HCC group (55%). So, the allelic frequencies in the HCC group were significantly different from those in other study groups (P 0.002). The GG genotype / G allele has the highest frequency in the control group, while the CC genotype/ C allele was more frequently detected in the HCC group.

Table 2: TP53 genotypes distribution among four studied groups

Parameters		Groups				
		HCC (n=20)	**LC (n=40)**	**CHCV (n=20)**	**Control (n=20)**	**P-value**
Distribution of Genotypes	**Homozygotes (Wild type) (arg/arg) GG**	6 (30%)	22 (55%)	9 (45%)	11 (55%)	P1 0.001 P2 0.003 P3 0.001 P4 0.065 P5 0.416
	Heterozygotes (pro/arg) GC	6 (30%)	9 (22.5%)	9 (45%)	7 (35%)	
	Homozygotes (pro/pro) CC	8 (40%)	9 (22.5%)	2 5%)	2 (10%)	
Frequency of alleles	**G allele**	18 (45%)	53 (66.3%)	27 (67.5%)	29 (72.5%)	P-value 0.002
	C allele	22 (55%)	27 (33.7%)	13 (32.5%)	11 (27.5)	

P-value compared the four groups.
P1 compared between HCC group and LC group; P2 compared between HCC group and CHCV group
P3 compared between HCC group and control group; P4 compared n between LC group and CHCV group
P5 compared n between LC group and control group

Comparison between the four groups as regard TP53 serum level is illustrated in table 3. HCC patients had a significant increase in TP53 serum where mean±SD is (17.286±7.9) compared to LC patients, CHCV patients and normal control (p-value= 0.0001, 0.001, 0.0001 respectively). Moreover, LC patients had a statistically significant increase in TP53 serum compered to CHCV patients and normal control (p-value= 0.0001, 0.001 respectively). The relation between plasma TP53 levels and TP53 genotypes in all study groups is summarized in table 4. In HCC group high levels of TP53 are more significantly associated with CC genotypes as (p-value=0.036)

Table 3: Comparison between four groups as regard TP53 serum level.

parameters	HCC (n=20)	LC (n=40)	CHCV (n=20)	Control (n=20)	p-value	P1	P2	P3	P4	P5
TP53 ug/ml Mean± SD Median (Range)	17.286±7.9 19 (1.6-30.9)	7.19±6.33 4 (1.3-19.5)	1.519±0.811 1.2 (0.4-3.5)	1.2±0.507 1.3 (0.4-1.8)	0.0001	0.001	0.0001	0.000 1	0.000 1	0.001

p-value compared four groups
P1 compared between HCC group and LC group
P2 compared between HCC group and CHCV group; P3 compared between HCC group and control group
P4 compared n between LC group and CHCV group; P5 compared n between LC group and control group

Table 4: Relation between plasma TP53 levels and TP53 codon 72 genotypes in all study groups.

Groups		Genotypes			Anova P-value
		GG	GC	CC	
HCC	**N**	6	6	8	
	Mean	21.51	17.99	10.95	0.036*
	SD	5.871	7.56	7.47	
LC	**N**	22	9	9	
	Mean	4.26	5.81	7.95	0.318
	SD	3.3	6.33	7.16	
CHCV	**N**	9	9	2	
	Mean	1.588	1.54	1.5	0.834
	SD	0.819	0.919	0.567	
Control	**N**	11	7	2	
	Mean	1.055	1.29	1.7	0.226
	SD	0.47	0.55	0.14	

Independent predictors for development of HCC in patients with HCV-related liver cirrhosis is summarized in table 5. Univariate binary logistic regression analysis showed that age, carriage of TP53 CC genotype and TP53 serum level were the factors that have a significant association with HCC development. Multivariate binary logistic regression analysis confirmed that carriage of CC genotype (odds ratio (OR): 2.658, 95% confidence interval (CI 95%): 0.435-16.24, P <0.01) and TP53 serum level (OR): 0.801,95% (CI 95%): 0.713-0.900, P<0.0001) are significantly associated with HCC development.

Table 5: Univariate and multivariate binary logistic regression analysis of predictor variables of HCC in patients with HCV-related liver cirrhosis

Characteristics	Univariate analysis		Multivariate analysis	
	OR (CI 95%)	*P*-value	OR (CI 95%)	*P*-value
Age (years) -LC: Range 33-75 ys Mean 56.45±9.18 -HCC: Range 42-75 ys Mean 59.75±8.36 -CHCV: Range 22-60 ys Mean 39.1±11.2	0.935 (0.883–0.991)	0.024*	0.940 (0.853-1.037)	0.216
Sex Male Female	 2.042(0.692-6.027) 1	 0.196		
Smoking No Yes	 1 0.329(.110-1.429)	 0.157		
Hypertension No Yes	 1.227(0.442-3.408) 1	 0.694		
Child class A B C	 2 1(0.0001-0.0001) 1(0.0001-0.0001)	 0.998		
MELD score	0.97(0.895-1.046)	0.406		
P53 genotype GG (arg/arg) GC (pro/arg) CC (pro/pro)	 1 1.3(.327-5.434) 3.259(0.877-12.106)	 0.688 0.01*	 1 0.783(0.106-5.78) 2.658(0.435-16.24)	 0.810 0.01
P53 level ug/ml	0.804(0.733-0.883)	0.0001*	0.801(0.713-0.900)	0.0001

- Child class; used to assess the prognosis of chronic liver disease, mainly cirrhosis.
- MELD score: Model for End-Stage Liver Disease is a scoring system for assessing the severity of chronic liver disease.

The relation between TP53 serum level and AFP among HCC patients was summarized in table 6. By statistical analysis, there was no significant correlation (P-value=0.203)

Table 6: showing the correlation between TP53 serum level and AFP in HCC patients

Enzyme	AFP (ng/mL)	*P-value*
TP53	*r =0.233*	***0.203****

r = Spearman's correlation coefficient.
*Statistically non-significant correlation

DISCUSSION

The prevalence of HCC is considered to be the fifth common tumor and the third cause of mortality due to cancer worldwide [7]. The etiology of HCC is due to multiple factors, but resulting mainly from interaction of environmental factors possibly HBV, HCV infection which lead to chronic hepatitis, liver cirrhosis and finally HCC, with genetic factors due to TP53 polymorphism.[8,9]

Genetic polymorphisms that affect gene expression and gene function contributing to hepatocarcinogenesis, can determine susceptibility of patients for HCC development. Thus, detection of these single nucleotide polymorphisms (SNPS) is important because it can identify patients at risk for HCC and study the pathophysiologic mechanisms contributing to HCC. Also, it can identify genetic biomarkers of HCC which can be used together with the traditional diagnostic methods for staging and detect the prognosis of the tumor. Moreover, identification of these SNPs may reduce HCC mortality through early diagnosis, patients care and personalized therapy [10]. Therefore, genetic risk factor to cancer should be a research focus in scientific community

Damage of genomic DNA due to internal or external threats, results in activation of TP53, which induce apoptosis. The Arg allele can bind to mitochondrial membrane, stimulates release of cytochrome C, resulting in activation of Caspases enzymes ending in physiological cell death, The Pro allele binds with low affinity to the regulatory proteins and partly associated with apoptosis. So, the Arg allele is capable of inducing apoptosis more effectively than the Pro allele, thus preventing development of cancer in cells[11]. This difference explains why the TP53-Pro variant may increase the risk to HCC. The results of our study are consistent with these theories, multivariate binary logistic regression analysis which confirmed that carriage of CC genotype "Pro/Pro" has a significant association with HCC development (OR): 2.658, (CI) 95% : 0.435-16.24, P <0.01).

According to the results of PCR-RFLP in our study, the distribution of the three genotypes in all study groups were as follows: Arg/Arg 55%, Arg/Pro 35% and Pro/Pro 10% in controls. Arg/Arg 45%, Arg/Pro 45% and Pro/Pro 5 % in CHCV. Arg/Arg 55%, Arg/Pro 22.5% and Pro/Pro 22.5% in LC cases Arg/Arg 30%, Arg/Pro 30% and Pro/Pro 40% in HCC patients respectively. Genotype distributions in HCC cases in comparison with LC, controls, CHCV were statistically significant. Our study results are consistent with study done by Neamatallah et al.[6]. Another research in Turkey, showed results in line with other finding, in comparison with the Arg/Arg genotype, patients carrying the CC genotype (2 Pro allele) had a 3.2-fold increase in the risk for HCC development (95% CI=1.24-8.22, P=0.02)[12]. In contrast to our result, Cai et al.,[13] conducted a study on Chinese patients, to investigate the association between the p53 polymorphism and HCC, but they found that there was no significance (Arg/Pro: OR, 1.00 and 95% CI, 0.66-1.50; Pro/Pro: OR, 1.06 and 95% CI, 0.69-1.64; Arg/Pro + Pro/Pro: OR, 1.02 and 95% CI, 0.70-1.50).

In our study, the serum TP53 level was significantly higher in the HCC patients when compared with the LC group or CHCV or healthy controls. In contrast to our results, Neamatallah et al.[6] found that the plasma TP53 level was significantly lower in the HCC patients in comparison with the LC group or with the controls, so, in HCC group, the plasma TP53 level was down regulated as a result of mutation and functional inactivation of TP53 gene. Another study performed by Liu et al., [14] who found that P53 is one of the changing factors in advanced HCC as TP53 has higher expression level in early and advanced stages of HCC, in comparison with CHCV group and cirrhosis group. The contradictory results of these researches explained by variation in the expression of TP53 according to the stage of the tumor, in HCC early stages, the TP53 is upregulated and overexpressed resulting in increase in the plasma level of TP53, while in HCC late stages, the TP53 is downregulated resulting in decrease in the plasma level of TP53. Overexpression of TP53 occurs due to expression of TP53 from ectopic sites or mutant TP53 reactivation in tumor cells. Further studies should be performed using larger sample size of HCC patients with different stages.

Our study showed a relation between plasma TP53 levels and TP53 codon 72 genotypes in HCC patients, high levels of TP53 are more significantly associated with CC genotypes as (p-value=0.036).

Various studies have attempted to define the association between p53 polymorphism and HCC susceptibility; however, the findings were controversial and contradictory with the different ethnic backgrounds of the study population. In this study, we investigated the independent predictors for development of HCC in

patients infected with HCV. The univariate binary logistic regression analysis revealed that age, carriage of CC genotype in TP53 gene and TP53 serum level were the factors significantly associated with HCC development. Multivariate binary logistic regression analysis confirmed that carriage of CC genotype and TP53 serum level are significantly associated with development of HCC. Our study is in line with a study done by Ezzikouri et al.[15] which indicated that p53 polymorphism is associated with a significant elevated risk of HCC in the study individuals and Sümbül et al.[12] suggested the Pro/Pro homozygote polymorphism may be a genetic susceptibility factor for HCC in Turkish population. In addition, , Neamatallah et al.[6] observed that Pro/Pro (C/C) genotype and Pro (C) allele of the TP53 codon 72 were associated with a high risk of HCC in HCV-infected Egyptian patients.

In contrast to our results, Di Vuolo et al.[16], proposed that TP53 polymorphism was not a significant risk factor for HCC in Italian patients. Also, Cai et al.[13] found no significant association between the p53 codon 72 polymorphism and susceptibility to HCC. The discrepancies among these studies may be due to the geographic location of the study subjects, the sample size, confounding factors and genotyping assays. For example, differences in ethnicity may affect the distribution of alleles, in addition to the temperature and intensity or UV rays[17].

In our study there was no significant differences among the studied groups except significant difference between age of HCC group and CHCV group where *p*-value = 0.0001). The incidence of HCC increases with age. The development of HCC is uncommon before 40 years of age. Our result also showed increasing significant enzymatic activity of AST, ALT and total bilirubin (p= 0.0001 and p= 0.0001) in liver cirrhosis and HCC groups compared to the controls. On the contrary, the results showed significant decreasing of platelets and albumin levels (P= 0.0001 and P= 0.0001 respectively) with progression of HCV infection.

AFP may be useful in the diagnosis and follow-up of cases of HCC, for HCC screening in patients with chronic liver disease (CLD), although increased levels are associated with malignancies other than primary HCC[18]. Studies suggest that in clinically HCC diagnosed patients, high levels of AFP levels more than 400 ng/ml strongly confirm the presence of HCC by a histopathological diagnosis. AFP has higher values with the stage of cancer and it is a prognostic indicator to follow up efficacy of treatment[19]. While, in another study done in Egypt, the mean serum AFP levels had no significant change in HCC tumors with multiple focal lesions and larger size of tumor (>3cm) [20].

In our study, the levels of AFP in the serum of patients with HCC have a mean+SD 810.8± 364.7, by comparing the mean values of AFP with the different TP53 genotype among the HCC group, there was no statistically significant difference (P-value= 0.135). Also, we investigate the relation between TP53 serum level and AFP among HCC patients, but there was no statistically significant correlation (r =0.233, P-value= 0.203). In contrast to our findings, Carr et al.,[21] found a high percentage of negative or low AFP HCC patients representing 42% concluding that AFP has been a poor screening tool, especially for small size HCCs. Also, large HCCs occurred in absent or low range AFP patients. El- Shennawy[22] studied the predictive factor associated with HCC in HCV infected patients, by using univariate logistic regression analysis, he found that AFP was not a successful predictor for HCC diagnosis which is consistent with our results.

Our study have some limitations which should be taken in consideration while interpreting the results. First, the sample size is considered relatively small, further studies based on a larger number of subjects are required to confirm these findings. Second, this was a hospital-based case-control study and less representative of HCC patients in the general population.

CONCLUSION

The TP53 CC genotype is associated with an increased risk of HCC in HCV-infected Egyptian patients and the overexpression of serum TP53 is a significant predictor for HCC and it is considered as an additional tumor marker to AFP to increase the diagnostic potential of AFP in HCC patients.

Conflicts of interest:
The authors declare that they have no financial or non financial conflicts of interest related to the work done in the manuscript.

- Each author listed in the manuscript had seen and approved the submission of this version of the manuscript and takes full responsibility for it.
- This article had not been published anywhere and is not currently under consideration by another journal or a publisher.

REFERENCES

1. De Re V, Tornesello LM, Zorzi MD, Caggiari L. Clinical Significance of Polymorphisms in Immune Response Genes in Hepatitis C-Related Hepatocellular Carcinoma. Front. Microbiol. 2019; 10 (475); 1-10.
2. Coppola N, Pisaturo M, Sagnelli C, Onorato L, and Sagnelli E. Role of genetic polymorphisms in hepatitis C virus chronic infection. World J Clin Cases. 2015; 16; 3(9): 807–822.
3. Insinga A, Cicalese A, Faretta M, Gallo B, Albano L, Ronzoni S, Furia L, Viale A and Pelicci PG. DNA damage in stem cells activates p21, inhibits

p53, and induces symmetric self-renewing divisions. Proc Natl Acad Sci USA.2013; 110: 3931-3936.

4. Buchman VL, Chumakov PM, Ninkina NN, Samarina OP, Georgiev GP. A variation in the structure of the proteincoding region of the human p53 gene. Gene. 1988; 70:245–252. doi: 10.1016/0378-1119(88)90196-5.
5. Siddique M, Sabapathy K. Trp53-dependent DNA-repair is affected by the codon 72 polymorphism. Oncogene. 2006; 25:3489–3500. doi:10.1038 /sj.onc.1209405.
6. Neamatallah MA, El-Missiry MA, Said MMA, Elbendary M, Othman AI, Ammar OA. TP53 polymorphism as a risk factor for hepatocellular carcinoma in hepatitis C virus-infected Egyptian patients. Egyptian Journal of Basic and Applied Sciences. 2014; 1(1), 9–15.
7. Jemal A, Bray F, Center MM, Ferlay J, Ward E, Forman D. Global cancer statistics. CA: A Cancer Journal for Clinicians. 2011; 61(2), 69–90.
8. Vijayaraman KP, Veluchamy M, Murugesan P, Shanmugiah KP, Kasi P D. p53 exon 4 (codon 72) polymorphism and exon 7 (codon 249) mutation in breast cancer patients in southern region (Madurai) of Tamil Nadu. Asian Pacific Journal of Cancer Prevention. 2012; 13(2), 511–516.
9. Lin YJ, Lee MH, Yang HI, Jen CL, You SL, Wang LY, Lu SN, Liu J, Chen CJ. Predictability of liver-related seromarkers for the risk of hepatocellular carcinoma in chronic hepatitis B patients. PLoS One. 2013; 8(4).
10. Ludwig JA and Weinstein JN. Biomarkers in cancer staging, prognosis and treat-ment selection. Nat Rev Cancer. 2005; 5:845–856. doi:10.1038/nrc1739.
11. Gogada R, Prabhu V, Amadori M, Scott R, Hashmi S,Chandra D. Resveratrol induces p53-independent, X-linked inhibitor of apoptosis protein (XIAP)-mediated Bax protein oligomerization on mitochondria to initiate cytochrome c release and caspase activation. J Biol Chem. 2011; 286:28749e60.
12. Sümbül AT, Akkız H, Bayram S, Bekar A, Akgöllü E and Sandıkçı M. p53 codon 72 polymorphism is associated with susceptibility to hepatocellular carcinoma in the Turkish population: a case–control study. Molecular biology reports. 2012; 39(2), pp.1639-1647.
13. Cai j, Cai y, Ma Q, Chang f, Xu L, Zhang G. Association of p53 codon 72 polymorphism with susceptibility to hepatocellular carcinoma in a Chinese population from northeast Sichuan. BIOMEDICAL REPORTS. 2017; 6: 217-222.
14. Liu M, Miao N, Li J, Wang Z, Wang Z, Li M, Sang W, Su L, Li Q, Shi X. Correlation of HSP70, P53 and Bmi-1 in hepatitis, cirrhosis and hepatocellular carcinoma. Int J Clin Exp Pathol. 2016; 9(2), 473–480.
15. Ezzikouri S, El Feydi AE, Chafik A, Benazzouz M, El Kihal L, Afifi R, Hassar M, Pineau P, Benjelloun S. The Pro variant of the p53 codon 72 polymorphism is associated with hepatocellular carcinoma in Moroccan population. Hepatol Res. 2007; 37:748–754.
16. Di Vuolo, V., Buonaguro, L., Izzo, F., Losito, S., Botti, G., Buonaguro, F.M. and Tornesello, M.L. TP 53 and MDM2 gene polymorphisms and risk of hepatocellular carcinoma among Italian patients. Infectious agents and cancer. 2011; 6(1), p.13.
17. Lu SQ, Jia ST, Luo Y. Recent advances in mutant p53 and novel personalized strategies for cancer therapy. Yi Chuan= Hereditas. 2011; 33(6), 539–548.
18. Jia HL, Ye QH, Qin LX, Budhu A, Forgues M, Chen Y, Liu YK, Sun HC, Wang L, Lu HZ. Gene expression profiling reveals potential biomarkers of human hepatocellular carcinoma. Clinical Cancer Research. 2007; 13(4), 1133–1139.
19. Murugavel KG, Mathews S, Jayanthi V, Shankar EM, Hari R, Surendran R, Vengatesan R. Alpha-fetoprotein as a tumor marker in hepatocellular carcinoma: investigations in south Indian subjects with hepato-tropic virus and aflatoxin etiologies. International Journal of Infectious Dis-eases. 2008; 12, 71—76.
20. Fathy WM, Elbassal F, Farouk S, Azzam A, Elfert A, Soliman S, Elaskary SA, Gomaa A. Evaluation of Circulating MicroRNA 483-5p as a Useful Diagnostic Tool of Hepatocellular Carcinoma in Egyptian Patients. Egyptian Journal of Medical Microbiology. 2018; 27 (3): 45-52.
21. Carr B, Akkiz H, Üsküdar O, Yalçın K, Guerra V, Kuran S, Karaoğullarından U, Altıntaş E. HCC with low- and normal-serum alpha-fetoprotein levels. Clin Pract. 2018; 15 (1): 453–464.
22. El- Shennawy SM, El-Masry EA, Mohamad EA and Awad ET. Clinical Significance of AFP, HGF & Micro RNA-122 Levels in Patients with Liver Cirrhosis and Hepatocellular Carcinoma. Egyptian Journal of Medical Microbiology. 2017; 26 (4): 103-112.