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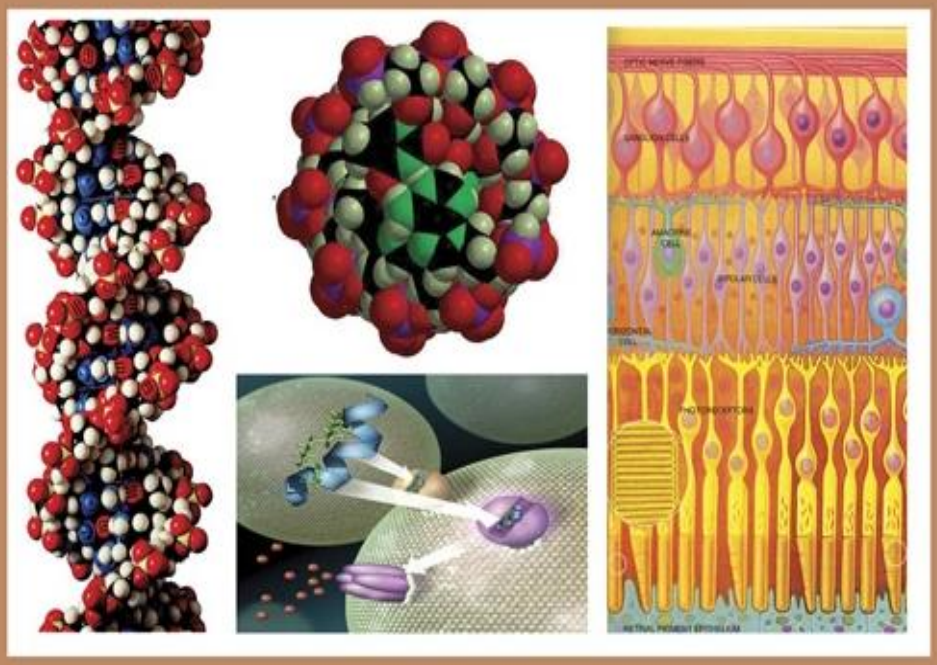

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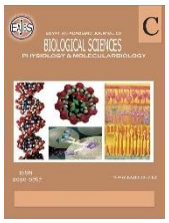

The Association between Catalase Gene rs1001179 and rs769217 Polymorphisms and The Hepatocellular Carcinoma Risk in the Egyptian Patients

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ABSTRACT

Using a thorough case-control design, we evaluated whether CAT SNPs were hepatocellular carcinoma (HCC) risk factors in the current study. Employing PCR-RFLP analysis, the genotypes of CAT rs1001179 and rs769217 were determined in 50 HCC-diagnosed patients and 50 healthy controls. CAT rs1001179 distributions revealed considerable variations between both groups, either in terms of allelic or genotypic distribution, since TT genotypes and the T allele differed significantly between the examined groups. Regarding the CAT rs769217, there were also significant differences in TT genotypes and T allele between the studied groups In Conclusion, the collected information characterized the CAT gene in HCC patients in Egypt for the first time and associations between CAT gene rs1001179 (262C/T) and CAT rs769217 (389C/T) and the propensity to HCC.

INTRODUCTION

Currently, liver cancer is the most prevalent illness in the world and the fourth leading cause of cancer-related death [Bray *et al*., 2018, Akinyemiju *et al*., 2017]. It affected 841,000 individuals and caused 782,000 fatalities in 2018 [Bray *et al*., 2018]. In 2015, the American Cancer Society predicted that 35,660 new instances of liver cancer will be identified and that 24,550 liver cancer-related deaths will occur in the United States (Siegel *et al*., 2015). Hepatocellular carcinoma (HCC) is considered one of the extremely prevalent illnesses and the main cause of cancer-related mortality across the globe (Siegel *et al.,* 2015). The geographic distribution of HCC varies greatly across the globe, with China accounting for an estimated fifty percent of all cases (WHO 2015). In regions where hepatitis B and C are prevalent, including sub-Saharan Africa and Asia, liver cancer is more prevalent [El-Zayadi *et al*., 2005]. Diagnosis is more prevalent among persons aged 55 to 65. Egypt has the second-highest rate of HCC in the world, which can be ascribed to the presence and effects of the hepatitis C virus (HCV). Active surveillance for HCC, HCV screening programmers, and early commencement of antiviral medication with new powerful direct-acting antivirals (DAAs) for individuals with chronic HCV infection is the most significant measures to lower the risk of HCC in Egypt (Fatmaa *et al*., 2020; Wafaa *et al*., 2020; and Abudeif *et al*., 2019).

HCC is the most prevalent form of liver cancer, the third greatest cause of cancer-related deaths globally, and the sixth most prevalent cancer in Egypt among both men and women. The increased incidence of HCC in Egypt is mostly attributable to the high frequency of viral hepatitis C and B and their associated consequences [Lehman *et al*., 2008; GBD 2015]. HCC carcinogenesis is a multifaceted and intricate method, and its cause is mostly unknown. Currently, smoking, chronic viral hepatitis, aflatoxin exposure, alcohol use, and liver cirrhosis are established risk factors for HCC (Chuang *et al*., 2009; Ha *et al*., 2012; Bruix *et al*., 2015). However, only a small proportion of individuals with proven risk factors develop the HCC, implying that the other environmental and genetic mediators may be implicated in the development of HCC (Chen *et al*., 2009). People with chronic liver disease who are at risk for HCC should be examined with ultrasound imaging every six months (Mantovani *et al*., 2017).

Conditions of therapy depend on the stage of the malignancy, the availability of treatment resources, liver function, and therapeutic skill [Chen *et al*., 2006]. Despite improvements in therapy, the overall survival rate of HCC patients has not improved [Marrero 2013]. Therefore, early identification of HCC is crucial for guiding treatment options. The identification of biomarkers for early-stage detection will aid in enhancing treatment options and ensuring that more patients receive the appropriate care [Stoller *et al*., 1993].

Reactive oxygen species (ROS) are extremely reactive chemicals; in larger concentrations, they may damage the cellular structure, including DNA, and change cellular function, which is a key risk factor for the development of cancer (Birben *et al*., 2012; Marnett 2000). Aerobic organisms contain anti-oxidant systems consisting of enzymatic and non-enzymatic antioxidants to inhibit the cellular damage initiated by ROS. Superoxide dismutase (SOD), one of the two basic systems of enzymatic antioxidants, transforms superoxide into hydrogen peroxide, whereas catalase (CAT) lowers this H_2O_2 to water (Zelko *et al*., 2002; Kirkman 1999); therefore, protecting against ROS-induced damage. A single nucleotide polymorphism (SNP) in the SOD or CAT genes may influence the stability and activity of the enzyme's antioxidant activity, resulting in decreased activity of the functional protein for ROS detoxification. Various cancer types are related to transitions in multiple SNPs of the point mutation of A to T in codon 326 of Ex7 in the promoter area of CAT gene and C262T polymorphism in 262 regions of the promoter region of CAT gene (Tefik *et al*., 2013; Geybels *et al*., 2015; Su *et al*., 2015; Kakkoura *et al*., 2016; Jamhiri *et al*., 2017). Recent studies have shown links between the CAT C262T polymorphism and the risk of numerous malignancies, including prostate cancer (Karunasighe *et al*., 2012), and breast cancer (Kakkoura *et al*., 2016), hepatocellular (Su *et al*., 2015; Suhail *et al*., 2018).

This work was performed to inspect the connection between CAT rs1001179 (262C/T) and CAT rs769217 (389C/T) gene polymorphisms with HCC risk in the Egyptian cohort.

MATERIALS AND METHODS

Study Subjects:

In the current case-control research, 50 histologically proven HCC patients and 50 healthy age- and sex-matched controls were recruited among Egyptians at the National Liver Institute Hospital, Minufiya University, Egypt. During 2019-2020, all studies were conducted in compliance with the requirements of the Health and Human Ethical Clearance Committee for Clinical Research (Minufiya University), and informed permission was acquired from all individuals. The group involves 50 HCC patients divided into 40 males and 10 females (Mean: 60.34 ± 8.05 years). Fifty HCC cases were distributed according to HCV (21 Negative and 29 Positive) and

range age (43-80 years). All patients who have positive serum HCV Ab identified by serology were confirmed by qualitative PCR to detect HCV-RNA. The HCC patients were investigated by ultrasonography and computed tomography (CT) scan. All control 50 healthy volunteers with unrelated healthy blood donors, free of any chronic diseases; they were 22 males and 28 females. The demographic data, including sex, age, location of residence, socioeconomic position, family history, dietary habits, tobacco and alcohol usage status, and other confounding risk variables, were gathered by structured personal interviews. Each subject's permission was acquired with knowledge of the risks involved.

Blood samples were obtained only from patients who gave informed consent for Haemogram analysis and Biochemical investigations and AFP. A full history was taken for all patients and control. Peripheral blood samples were collected in two tubes, the first for routine workup and another one for DNA extraction.

Catalase Gene Genotyping Assays:

Genomic DNA was extracted from whole blood samples obtained under EDTA-sterile conditions from both patients and controls. Using ABIOpure™ complete DNA extraction kit, DNA was insulated from peripheral blood (Bothell, WA 98021 USA)

SNPs in CAT rs1001179 (262C/T) and CAT rs769217 (389C/T) genes were investigated using the PCR-RFLP technique. The fragment was amplified in the 25 ul reaction volume using 2 x PCR master mixes (Thermo Fisher Scientific Inc. USA) with a set of primers shown in table (1). The primers (forward 5′- AGAGCCTCG CCCC GCCGGACCG-3′ and reverse 5′- TAAGAG CTGAGAAAGCATAGCT-3′). Under the following circumstances (95° C for 5 minutes, followed by 35 cycles of 95° C for 30 s, 56° C for 30 s, 72° C for 45 s, and a final step of extension at 72° C for 10 minutes), the elongation temperature was 72° C for 10 minutes. 10 µl of the amplified 185-bp product was digested with 1 unit of *SmaI* for 1 h at 37 °C with 1 unit of *SmaI* (New England Biolabs, Schwalbach, Germany). After digestion, GG genotype polymorphism creates two bands with 155-bp and 30-bp and TT genotype creates only one band with 185 bp in size where, whereas the heterozygous genotype CT produced 185, 155, and 30-bp bands. Whereas the CAT rs769217 (389C/T) polymorphism was done by RFLP technique by using the primer pairs (forward, 5’- GCCGCCTTTTT GCCTA TCCT -3’; reverse, 5’- TCCCGCCCAT CTGCTCCAC-3’). The PCR conditions consist of an initial denaturation phase at 94 °C for 10 minutes, followed by 35 cycles at 95 °C for 30 seconds, 60 °C for 30 seconds, and 72 °C for 45 seconds, and a final extension step at 72 °C for 5 minutes. The PCR products were digested for 1 hour at 55 C with 1 U of *BstXI* rapid digest (New England Biolabs, Beverly, MA, USA). *BstXI* site was found in the TT genotype but not in the CC genotype. Two fragments of 108-bp and 94-bp were generated with the TT genotype, while the CC genotype produces only a single 202-bp band. Three bands (202, 108 and 94-bp) were generated in individuals with the heterozygous CT genotype. Following overnight incubation, 30 minutes of electrophoresis at 100 V on a 3% agarose gel stained with ethidium bromide were employed to monitor the digested products using Gel-Doc imaging equipment (E-Box VILBER, France).

Statistical Analysis:

The data were analyzed utilizing SPSS version 20.0 (SPSS Inc., Chicago, IL, USA). A chi-square test was used to examine the genotype frequencies of CAT rs1001179 (262C/T) and CAT rs769217 (389C/T) gene polymorphisms between HCC patients and controls. Using standard deviation and the mean, quantitative data analysis was done. A statistical study's power and sample size were calculated to get a value of 0.8. A p-value less than or equal to <0.05 was considered statistically significant when detected.

RESULTS

Characteristics of the Study Population:

The case and control individuals' characteristics are shown in detail (Table 1). There were no significant gender or age differences between the control group and HCC patients. Moreover, patients with HCC had substantially higher levels of AFP, AST, TBIL, and ALT than healthy persons (P<0.001). Hematological examinations revealed that the quantities of WBCs, RBCs, hemoglobin (Hb), prothrombin, and platelets are much lower in HCC than in control groups (P<0.001). Creatinine and total bilirubin levels were comparable across the two groups (P>0.05).

Table 1. Selected clinical and demographic characteristics of patients and controls.

Variables	Patients (n =50) Mean ± SD	Control (n = 50) Mean ± SD	Test of sig.	p
Demographic data				
Age, years	60.34 ± 8.05	59.88 ± 9.86	t=0.256	.0799
Sex, n (%)	M, 40(80%)-F, 10(20%)	M, 38 (76%)-F, 12 (24%)	χ^2= 13.752	0.067
HCV carriers, n (%)	29(58%)	0(0%)	-----	
Hematological profile				
HB	12.52 ± 1.61	13.16 ± 1.21	t=2.237*	0.028*
RBCs.	4.35 ± 0.64	4.59 ± 0.36	t=2.322*	0.023*
TLC	5.19 ± 1.89	6.91 ± 1.50	t=5.041*	<0.001*
PLT	114.08 ± 52.97	260.16 ± 70.63	U=114.50*	<0.001*
Pro. Conc.	70.58 ± 14.60	95.99 ± 4.67	t=11.719*	<0.001*
INR	1.29 ± 0.20	1.03 ± 0.04	U=151.0*	<0.001*
Biochemical parameters				
Creatinine	0.95 ± 0.23	0.99 ± 0.16	t=0.978	0.331
AST	57.88 ± 35.58	32.78 ± 5.72	U=420.50*	<0.001*
ALT	47.52 ± 37.58	26.16 ± 6.60	U=571.50*	<0.001*
Total bilirubin	1.40 ± 0.71	1.08 ± 0.12	U=1067.0	0.204
Direct bilirubin	0.58 ± 0.44	0.19 ± 0.08	U=396.50*	<0.001*
Albumin	3.23 ± 0.61	4.27 ± 0.61	t=8.551*	<0.001*
AFP	1058.99 ± 2482.84	5.99 ± 0.99	U=296.0*	<0.001*
Child pugh score				
A	56.0%			
B	42%			
C	2%			
Focal lesion				
Size (cm)	5.03 ± 3.13			
Number				
1	74%			
2	16%			
3	2%			
>3	8%			

t: Student t-test. - **U:** Mann Whitney test- ***:** Statistically significant at $p \leq 0.05$.

Genotypic and Allelic Frequencies of Catalase SNPs in the Studied Groups:

The primary objective was to investigate the frequency of CAT polymorphisms in Egyptian HCC patients. To gain information on the distribution of CAT SNPs among the HCC patients, the genomic DNA taken from 50 control volunteers was studied. 60% of the CAT rs1001179 genotypes in this control group were CC, 36% were CT, and 4% were TT, whereas 64% of the CAT rs769217 genotypes were CC, 32% were CT, and 4% were TT (Fig. 1 and Table 2). In the control group, the SNPs CAT rs769217 and

rs1001179 exhibited Hardy-Weinberg equilibrium ($\chi 2$ tests, $p \geq 0.05$), allowing for the genotype distribution of HCC patients to continue.

Subsequently, the distribution of CAT polymorphisms among a group of 50 Egyptian HCC patients was determined. The CAT rs1001179 genotypes distribution was found to be 36% CC, 54% CT, and 10% TT, whereas the distributions for the CAT rs769217 genotype distributions were 30 CC, 60 CT, and 10% TT, respectively (Fig. 1b and Table 2). Figure 1b depicts the genotype frequencies for CAT rs769217 (CC, TT, CT) among the HCC and control cohorts demonstrating that there was a significant difference in CT genotype and the dominant genotypes (CT+TT) and the moreover allelic significance was recorded with T allele between the patient and the control group, as presented in Table 2. Moreover, the same analysis was accomplished for CAT rs1001179; there was a significant difference in CT genotype and the dominant genotypes (CT+TT) and allelic significance was recorded with the T allele between the patient and the control group (Fig. 1a and Table 2).

To explore the significance of the CAT rs1001179 and CAT rs769217 gene polymorphisms in the clinicopathological status of HCC patients, the connection between clinical characteristics and distribution of CAT SNPs in HCC patients was investigated and evaluated. This examination included size and number of focal lesions, HCV infection (anti-HCV), child grade, and common HCC clinical-pathological characteristics including AST, ALT, and AFP. There was no association between CAT rs1001179 and CAT rs769217 gene polymorphisms and any clinicopathologic state or indicators (Tables 3 &4).

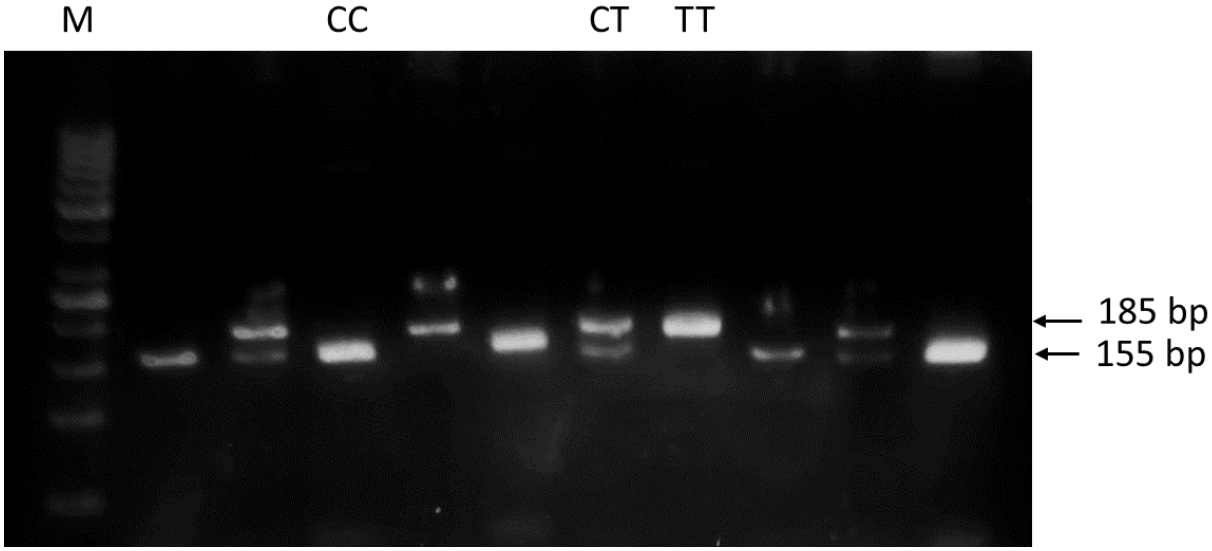

Fig. 1a. PCR-RFLP analysis of CAT rs1001179 (262C/T) mutation. *SmaI* restriction enzyme cannot cleave the TT genotype with a single band with a fragment length of 185 bp. While the C allele is cleaved by *SmaI* and yields two fragments (155 bp and 30 bp). The heterozygote generates three bands (185 bp, 155 bp, 30 bp). M = 50 bp ladder DNA marker.

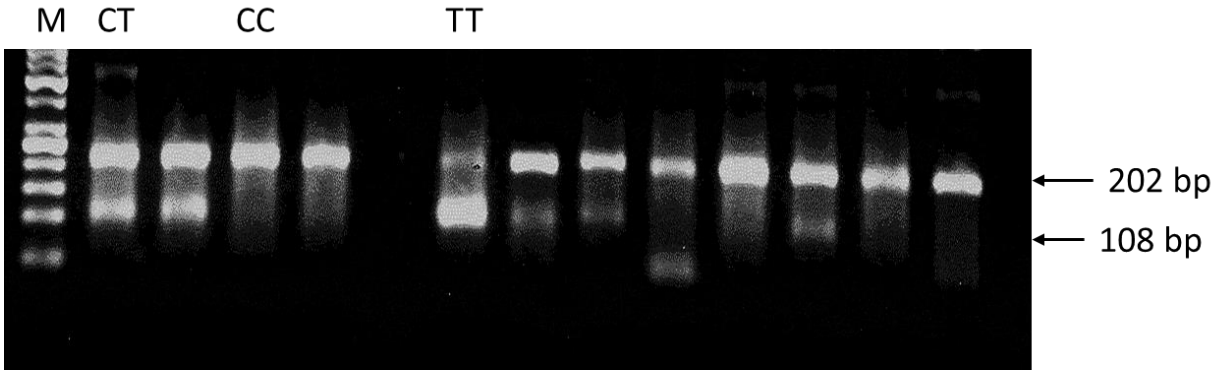

Fig. 1b. PCR-RFLP analysis of CAT rs769217 (389C/T) mutation. *BstXI* restriction enzyme cannot cleave the CC genotype with a single band with a fragment length of 202 bp. While the TT genotype is cleaved by *BstXI* and yields two fragments (108 bp and 94 bp). The heterozygote generates three bands (202 bp, 198 bp, 94 bp). M = 50 bp ladder DNA marker.

Table 2. CAT gene genotyping among the studied groups

Variables	HCC (n = 50)		Control (n = 50)		OR	p	95% CI.
	No.	%	No.	%			
CAT rs1001179							
CC®	18	36.0	30	60.0			
CT	27	54.0	18	36.0	2.500	0.031*	1.085 – 5.762
TT	5	10.0	2	4.0	4.167	0.108	0.731 – 23.759
CT +TT	32	64.0	20	40.0	2.667	0.017*	1.188 – 5.985
Allele							
C®	63	63.0	78	78.0			
T	37	37.0	22	22.0	2.082	0.021*	1.116 – 3.884
CAT rs769217							
CC®	15	30.0	32	64.0			
CT	30	60.0	16	32.0	4.0	0.002*	1.688 – 9.478
TT	5	10.0	2	4.0	5.333	0.061	0.926 – 30.715
CT+ TT	35	70.0	18	36.0	4.148	0.001*	1.798 – 9.573
Allele							
C®	60	60.0	80	80.0			
T	40	40.0	20	20.0	2.667	0.002*	1.417 – 5.010

OR: Odds ratio for Patients

CI: Confidence interval LL: Lower limit UL: Upper Limit

*: Statistically significant at $p \leq 0.05$.

Table 3. Relation between CAT rs1001179 and biochemical parameters in patients group (n = 50)

Variables	CAT rs1001179						Test of Sig.	p
	CC® (n =18)		CT (n =27)		TT (n = 5)			
	No.	%	No.	%	No.	%		
Creatinine								
Min. – Max.	0.70 – 1.30		0.44 – 1.70		0.50 – 1.40		F=0.101	0.904
Mean ± SD.	0.96 ± 0.15		0.95 ± 0.26		0.91 ± 0.33			
AST								
Min. – Max.	22.0 – 210.0		13.0 – 200.0		40.0 – 77.0		H=0.891	0.640
Mean ± SD.	59.06 ± 41.51		56.63 ± 34.89		60.40 ± 15.69			
ALT								
Min. – Max.	11.0 – 239.0		14.0 – 145.0		15.0 – 60.0		H=0.470	0.790
Mean ± SD.	53.06 ± 50.92		44.41 ± 29.67		44.40 ± 18.56			
Total bilirubin								
Min. – Max.	0.60 – 3.50		0.52 – 2.20		0.80 – 3.10		H=0.510	0.775
Mean ± SD.	1.48 ± 0.93		1.30 ± 0.45		1.66 ± 0.96			
Direct bilirubin								
Min. – Max.	0.15 – 2.10		0.11 – 1.38		0.30 – 1.20		H=1.015	0.602
Mean ± SD.	0.63 ± 0.59		0.53 ± 0.33		0.68 ± 0.43			
Albumin								
Min. – Max.	2.50 – 4.40		2.10 – 4.30		2.50 – 4.30		F=2.824	0.069
Mean ± SD.	3.45 ± 0.56		3.05 ± 0.58		3.42 ± 0.70			
AFP								
Min. – Max.	2.91 – 9709.0		2.40 – 9902.0		69.05 – 1330.0		H=2.933	0.231
Mean ± SD.	1292.42 ± 2808.17		987.63 ± 2519.0		604.01 ± 466.16			
Child Pugh score								
A	11	61.1	14	51.9	3	60.0	x^2= 1.916	^{MC}p= 0.953
B	7	38.9	12	44.4	2	40.0		
C	0	0.0	1	3.7	0	0.0		
Focal lesion								
Number								
1	13	72.2	21	77.8	3	60.0	x^2= 7.706	^{MC}p= 0.215
2	2	11.1	5	18.5	1	20.0		
3	0	0.0	1	3.7	0	0.0		
>3	3	16.7	0	0.0	1	20.0		
Size								
<2	0	0.0	1	3.7	0	0.0	x^2= 2.493	^{MC}p= 0.849
2 – 5	14	77.8	18	66.7	3	60.0		
>5	4	22.2	8	29.6	2	40.0		
Min. – Max.	2.50 – 14.0		1.50 – 13.0		2.50 – 12.50		H=0.713	0.700
Mean ± SD.	5.39 ± 3.70		4.56 ± 2.52		6.30 ± 4.04			
Median	4.0		4.0		5.0			

Table 4. Relation between CAT rs769217 and biochemical parameters in patients group (n = 50)

Variables	CAT2						Test of Sig.	p
	CC® (n =15)		CT (n =30)		TT(n = 5)			
	No.	%	No.	%	No.	%		
Creatinine								
Min. – Max.	0.50 – 1.20		0.44 – 1.70		0.70 – 1.02		F=0.894	0.416
Mean ± SD.	0.90 ± 0.20		0.99 ± 0.26		0.88 ± 0.12			
AST								
Min. – Max.	29.0 – 84.0		22.0 – 210.0		13.0 – 54.0		H=3.527	0.171
Mean ± SD.	55.33 ± 17.63		62.77 ± 42.91		36.20 ± 16.72			
ALT								
Min. – Max.	15.0 – 60.0		11.0 – 239.0		14.0 – 101.0		H=0.724	0.696
Mean ± SD.	37.27 ± 13.04		52.50 ± 45.30		48.40 ± 35.33			
Total bilirubin								
Min. – Max.	0.70 – 3.50		0.52 – 3.10		0.90 – 1.94		H=1.915	0.384
Mean ± SD.	1.62 ± 0.79		1.33 ± 0.69		1.20 ± 0.46			
Direct bilirubin								
Min. – Max.	0.12 – 2.10		0.11 – 1.80		0.20 – 1.38		H=1.271	0.530
Mean ± SD.	0.68 ± 0.51		0.53 ± 0.40		0.56 ± 0.53			
Albumin								
Min. – Max.	2.40 – 4.30		2.10 – 4.40		2.40 – 3.70		F=0.355	0.703
Mean ± SD.	3.31 ± 0.67		3.22 ± 0.59		3.04 ± 0.57			
AFP								
Min. – Max.	2.40 – 9902.0		2.80 – 9709.0		7.60 – 949.0		H=2.029	0.363
Mean ± SD.	871.33 ± 2525.10		1279.48 ± 2673.22		299.06 ± 413.26			
Child Pugh score								
A	9	60.0	17	56.7	2	40.0	χ^2= 3.743	^{MC}p= 0.522
B	5	33.3	13	43.3	3	60.0		
C	1	6.7	0	0.0	0	0.0		
Focal lesion								
Number								
1	12	80.0	20	66.7	5	100.0	χ^2= 3.084	^{MC}p= 0.929
2	2	13.3	6	20.0	0	0.0		
3	0	0.0	1	3.3	0	0.0		
>3	1	6.7	3	10.0	0	0.0		
Size								
<2	0	0.0	1	3.3	0	0.0	χ^2= 1.721	^{MC}p= 1.000
2 – 5	11	73.3	20	66.7	4	80.0		
>5	4	26.7	9	30.0	1	20.0		
Min. – Max.	2.0 – 11.0		1.50 – 14.0		2.50 – 14.0		H=0.801	0.670
Mean ± SD.	4.31 ± 2.22		5.27 ± 3.27		5.80 ± 4.70			
Median	4.0		4.25		4.50			

DISCUSSION

SNPs, also known as single nucleotide base substitutions, are the most common mutations in the sequence of the human genome. SNP is a nucleotide sequence variation that occurs in more than 1% of at least one population (Chanock, 2001). Combinations of SNPs within the same gene or across separate genes may collectively result in disease development. SNPs alter the phenotypic expression of an identified gene, hence increasing an individual's susceptibility to a certain illness. As such, genetic association studies have begun to examine the influence of SNPs on disease outcomes (Chanock, 2003).

Cirrhosis of the liver, chronic viral hepatitis, aflatoxin exposure, alcohol drinking, and smoking are current risk factors for HCC (Ha *et al*., 2012; Schütte *et*

al., 2009). However, a tiny proportion of individuals with established risk factors develop HCC, indicating that environmental and genetic mediators may be implicated in HCC development. Examining the prevalence of CAT rs1001179 and CAT rs769217 in the HCC patients and healthy controls was thus of significant interest, especially in light of the lack of information regarding the genotype distribution of CAT SNPs in the HCC patients.

In the current study, it was found that a statistically significant correlation was found between the CAT rs1001179 (TT genotype and T allele) and the risk of HCC in the overall samples. The Allele A in CAT rs1001179 represent 37.0 % in HCC group (n = 50) but represent 22.0 % in control group so according to this percentage the Allele A is significant (p= 0.002) statistically.

In CAT polymorphism *-262C/T* and CAT activity, it was discovered that CC homozygotes exhibited more CAT activity than individuals with the CT or TT genotype (Nadif *et al*., 2005; Ahn *et al*., 2005, 2006b; Perianayagam *et al*., 2007). In contrast, Mak *et al*. (2007) could not find any correlation between the CAT *-262C/T* polymorphism and CAT activity. The presence of the -262 T allele protects against neurodegeneration and diminished physical fitness and has been linked to enhanced cognitive performance (Christiansen *et al*., 2004; Galecki *et al*., 2009a). It was discovered that CC genotype carriers were the most common (Christiansen *et al.,* 2004). The *-262C/T* CAT polymorphism was not associated with HCC in cirrhosis or adult brain tumors (Rajaraman *et al.,* 2008), the appearance of precancerous changes in the gastric mucosa (Steenport *et al.,* 2007), and negative results in acute renal failure (Perianayagam *et al.,* 2007), chronic renal disease and its development (Crawford *et al*., 2012b), or depressive disorder (Galecki *et al*., 2009a, b). Contrary, Ezzikouri *et al.,* (2010) discovered that patients with the CAT TT genotype had a 3.41-fold greater risk of HCC and lower CAT activity.

Moreover, in the present study, there was a substantial positive correlation between CAT rs769217 TT genotype and T allele and the risk of HCC in the overall samples. The Allele T in CAT rs769217 represent 40.0 % in HCC group (n = 50) while represent 20.0 % in control group so according to this percentage the Allele T is significant (p= 0.002) statistically.

The 389C>T mutation decreases the interaction between DNA and protein and suppresses CAT gene transcription (Yang *et al*., 2015). CAT mutations with the T allele are related to an increased incidence of liver fibrosis and HCC (Sousa *et al*., 2016). Liu et al. stated that the CAT rs769217 T allele enhanced the risk of HBV-related chronic hepatitis, cirrhosis, and liver cancer (Liu *et al*., 2015). The T allele at CAT rs769217 reduced the incidence of VPA-mediated AST abnormalities in this investigation. Moreover, according to Liu *et al*., 2015, the CAT rs769217 T allele is related to an elevated risk of CHB, HBV-HCC, and HBV-LC in the Guangxi community.

In a hospital-based study, our HCC patients were picked from hospitals, but the controls were obtained from a community group, therefore selection bias cannot be ruled out totally. Potentially confounding variables might have been reduced by additional data analysis adjustments and classification.

Conclusions

In this case-control research, in the Egyptian population, the association between CAT gene polymorphisms and HCC risk was studied, and it was concluded that CAT gene polymorphisms have a great effect and are considered a risk factor in HCC disease and the CAT rs1001179 TT genotype and (T) allele of the CAT gene are linked to an elevated risk of HCC in HCV-infected individuals. Moreover, our findings suggest that the TT genotype and T allele of CAT rs769217 may be a genetic risk factor for HCC. To verify the association between these SNPs and HCC risk, however, impartial studies with a large number of patients would be necessary. However, there

are still constraints that must be considered before interpreting these results. First: sample size and ethnic composition. Second, a distinction in HCC etiology. Third: is a deficiency of vital information, including environmental variables and gene-environment interactions.

Ethics Approval: The local Ethics Committee of (GEBRI), University of Sadat City, has approved our study.

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