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Genetic variations in DNA-repair genes (XRCC1, 3, and 7) and the susceptibility to hepatocellular carcinoma in a cohort of Egyptians

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Abstract

Chronic hepatitis C (CHC) is a worldwide etiology of chronic hepatic insult particularly in Egypt. DNA-repair systems are responsible for maintaining genomic integrity by countering threats posed by DNA lesions. Deficiency in the repair capacity due to genetic alterations in DNA-repair genes can lead to genomic instability and increased risk of cancer development. The present work aimed at studying the possible association between XRCC1-G28152A (rs25487), XRCC3-C18067T (rs861539), and XRCC7-G6721T (rs7003908) single nucleotide polymorphisms (SNPs) and the susceptibility to hepatocellular carcinoma (HCC) in Egyptian population. The study was conducted on 100 newly diagnosed HCC patients and 100 age- and sex-matched healthy controls. Laboratory workup revealed that all HCC patients have chronic hepatitis C viral infection. Genotyping of the studied SNPs was performed by real-time PCR. The heteromutant genotype of XRCC1 (GA) conferred an almost two-fold increased risk of HCC (OR , 2.35; 95% CI, 1.33-4.04). Regarding XRCC7, the heteromutant (TG) genotype conferred a two-fold increased risk of HCC (OR , 2.17; 95% CI, 1.23-3.82). Coinheritance of the polymorphic genotypes of XRCC1 and 7 was significantly higher in HCC cases than controls and was associated with an 11-fold increased risk of HCC (OR , 11.66; 95% CI, 2.77-49.13). The frequency of XRCC3 polymorphic genotypes in HCC patients was close to that of the controls.

KEYWORDS

Egypt, HCC, rs25487, rs7003908, rs861539, XRCC1, XRCC3, XRCC7

1 | INTRODUCTION

Hepatocellular carcinoma (HCC) is the most common primary cancer of the liver, accounting for 75% to 85% of liver cancer cases.[1] In Egypt, HCC accounts for 70.48% of all liver tumors. It is the second most cancer among men and the sixth among women.[2] Egypt has high incidence of HCC about 21% in cirrhotic Egyptian patients. HCC rising incidence in Egypt is mostly due to high prevalence of viral hepatitis and its complications.[3] Hepatitis C virus (HCV) is a worldwide etiology of chronic hepatic insult particularly in Egypt where genotype-4 is the principal genotype being carried by over 90% of infected patients, and the remaining infections are due to genotype-1.[4] Chronic hepatitis C is the leading cause of liver cirrhosis (93%)[5] and consequently Hepatocellular carcinoma (HCC) globally particularly in Egypt.[6]

DNA-repair genes play a major role in maintaining genomic stability through different repair pathways. A wide variety of DNA damage may be induced by endogenous metabolic products or by

environmental carcinogens. If the rate of DNA damage exceeds the capacity of the cell to repair it, the accumulation of errors can overwhelm the cell and result in early senescence, apoptosis, or cancer.[7] There are four major DNA-repair pathways in human cells: nucleotide excision repair (NER), base excision repair (BER), mismatch repair (MMR), and double-strand break repair (DSBR).[8] The NER pathway mainly removes bulky DNA adducts typically generated by exposure to polycyclic aromatic hydrocarbons in tobacco smoke. The MMR pathway corrects incorrectly paired bases during DNA replication errors. The BER pathway is responsible for the removal of oxidized DNA bases that may arise from endogenous or exogenous agents. The DSBR pathway is responsible for repairing double-strand breaks caused by a variety of exposures as ionizing radiation and free radicals.[9]

Base excision repair (BER) is the predominant DNA damage repair pathway. The X-ray repair cross-complementing group 1 (XRCC1) protein encoded by XRCC1 gene is a key player in the multistep BER pathway.[10] DNA single-strand breaks (SSBs) arise directly from damage to the deoxyribose moieties or indirectly as intermediates of DNA base excision repair (BER).[11] If left unrepaired, SSBs are a major threat to genetic stability and cell survival, accelerating mutation rates and increasing levels of chromosomal aberrations.[12] XRCC1 is devoid of any enzymatic activity, but it is thought to act as a scaffolding protein for other repair factors such as DNA ligase III, DNA polymerase, AP (apurinic/apyrimidinic) endonuclease and poly (ADP ribose) polymerase-1 and polymerase-2 (PARP 1,2).[11,12] There are two different pathways involved in double-strand break (DSB) repair: homologous recombination (HR) and nonhomologous end joining (NHEJ). In the DSB-HR pathway, XRCC3 protein participates in homologous recombination, maintaining chromosome stability and participating in DNA repair.[13] XRCC7 gene is an NHEJ DSBs repair gene. XRCC7 encodes the catalytic subunit of a nuclear DNA-dependent serine/threonine protein kinase (DNA-PK). The protein participates in the recognition and repair of double-strand breaks via NHEJ repair pathway.[14,15] DSBs are the most detrimental form, because they may lead to both chromosomal breakage and rearrangement and ultimately lead to tumorigenesis of cancers such as HCC.[16]

Genetic variations affecting DNA-repair pathways might make these lesions unrepaired or incorrectly repaired which may contribute to carcinogenesis. The aim of the present study was to clarify the possible association between XRCC1 (rs25487), XRCC3 (rs861539), and XRCC7 (rs7003908) genetic polymorphisms and the risk for Hepatocellular carcinoma (HCC) in a cohort of an Egyptian population.

2 | MATERIALS AND METHODS

2.1 | Study population

The current case-control study was conducted on 200 participants; 100 HCC patients on top of HCV-related liver cirrhosis and 100 healthy controls. The research protocol was approved by the Research Ethics Committee of the Departments of Clinical Pathology, Cairo University, and Theodor Bilharz Research Institute. All procedures performed were in accordance with the recommendation of the Declaration of Helsinki 1964 and its later amendments or comparable ethical standards. Informed written consents were obtained from all participants before enrollment in the study. HCC patients were recruited from Multidisciplinary HCC Clinic, Kasr Al Ainy Hospital, Cairo University and Theodor Bilharz Research Institute. They were 69 males and 31 females and their ages ranged between 29 to 70 years with a mean age of 54.94 ± 8.65 years. Our inclusion criteria were Egyptians, adults >18 years old and both sexes. The exclusion criteria were patients with hepatitis B virus infection and all other causes of liver cirrhosis, Liver cancers other than HCC.

Diagnosis and management of HCC was based on the updated guidelines of the American Association for the study of liver diseases (AASLD),[17] the European Association for the study of the liver (EASL) guidelines,[18] and Barcelona Clinic of Liver Cancer (BCLC) guidelines.[19] Participants were subjected to history taking, clinical examination and laboratory evaluation that included complete blood count, Liver biochemical testing (alanine and aspartate transaminases [ALT and AST]), alkaline phosphatase, serum bilirubin, albumin, urea and creatinine and α-fetoprotein (AFP). Based on serum bilirubin and albumin levels, INR, presence of ascites or hepatic encephalopathy, patients were stratified according to Child-Pugh scoring system into class A, B, and C. Testing for HCV antibodies and HBsAg was performed by immunoassay (ADVIA Centaur CP, Germany). The control subjects are age-sex matched, non-relative healthy blood donors with no underlying liver diseases, normal liver ultrasonography, normal liver and kidney function tests and seronegative for HCV and HBV infections. The demographic, clinical and laboratory data of HCC patients is presented in Tables 1 and 2.

2.2 | Genotyping of XRCC1 (rs25487), XRCC3 (rs861539), and XRCC7 (rs7003908)

DNA extraction from peripheral blood leukocytes was done with the GeneJET Whole Blood Genomic DNA Purification Mini Kit Purification (Fermentas Life Sciences, Mini kit#K0781, Lithuania) according to the manufacturer's instructions and stored at −20°C until use. Genotyping of the studied single nucleotide polymorphisms (SNPs) was performed by TaqMan allelic discrimination assay on Real-Time PCR system (Applied Biosystem, Foster, CA) containing probes for both alleles labeled with either FAM or VIC dyes according to manufacturer's recommendations and as previously described.[20]

2.3 | Statistical analysis

Data were coded and entered using the statistical package SPSS (Statistical Package for the Social Sciences) version 24. Data were

TABLE 1 Clinical data of HCC patients at diagnosis

Item	HCC patients (n = 100) no. (%)
Sex, male/female	69 (69%)/31 (31%)
Family history of HCC	39 (39%)
Smoking	35 (35%)
Bilharziasis	42 (42%)
Diabetes mellitus	34 (34%)
Jaundice	67 (67%)
Lower limb edema	35 (35%)
Hepatic cirrhosis	100 (100%)
Splenic size Enlarged/normal	68 (68%)/32 (32%)
Ascitis	40 (40%)
Child-Pugh classification A	68 (68%)
B	24 (24%)
C	8 (8%)
Number of hepatic lesions	
Mean ± SD	1.54 ± 0.7
Median (range)	(1) 1-4
Size of hepatic lesions, cm	
Mean ± SD	1.84 ± 0.53

Abbreviations: HCC, hepatocellular carcinoma; SD, standard deviation.

TABLE 2 Laboratory data of HCC patients at diagnosis

Item	HCC patients (n = 100)
Hb level, gm/dL	
Range	7.2-16
Mean ± SD (median)	11.18 ± 1.6 (6.4)
TLC ($\times 10^3/cm^3$)	
Range	2.6-17.3
Mean ± SD (median)	7.3 ± 3.5 (6.4)
Platelet count ($\times 10^3/cm^3$)	
Range	58-413
Mean ± SD (median)	149.1 ± 60.16 (140)
ALT, IU/L	
Range	8-120
Mean ± SD (median)	38 ± 22.34 (34)
AST, IU/L	
Range	10-120
Mean ± SD (median)	74.1 ± 46.5 (63)
ALP, IU/L	
Range	30-195
Mean ± SD (median)	93.1 ± 36.69 (90)
Bilirubin (mg/dL)	
Range	0.7-7.22
Mean ± SD (median)	2.3 ± 1.4 (2)
AFP, mg/dL	
Range	1.9-2502
Mean ± SD (median)	215 ± 65 (443.5)
Total protein, g/dL	
Range	2.6-8.5
Mean ± SD (median)	6.48 ± 1.12 (6.75)
Albumin, gm/dL	
Range	1.6-4.3
Mean ± SD (median)	3 ± 0.6 (3.1)
Urea, mg/dL	
Range	18-150
Mean ± SD (median)	46.34 ± 28.96 (36.2)
Creatinine, mg/dL	
Range	0.46-4.13
Mean ± SD (median)	1.1 ± 0.62 (0.96)

Abbreviations: AFP, alfafetoprotein; ALP, alkaline phosphatase; ALT, alanine aminotransferase; AST, aspartamaminotransferase; Hb, hemoglobin; HCC, hepatocellular carcinoma; Plts, platelets; SD, standard deviation; TLC, total leukocytic count.

summarized using mean, standard deviation, median, minimum and maximum in quantitative data and using frequency (count) and relative frequency (percentage) for categorical data. Comparisons between quantitative variables were done using the non-parametric Kruskal-Wallis and Mann-Whitney tests. For comparing categorical data, $\chi 2$ test was performed. Exact test was used instead when the expected frequency was less than 5. Genotype and allele frequencies were compared between the disease and the control groups using binary logistic regression. Adjusted odds ratio (OR) and 95% confidence interval (CI) were calculated for risk estimation. *P* values less than .05 were considered as statistically significant. Sample size calculation: based on an expected frequency for XRCC1, 3, and 7 SNPs in the control group and HCC patients,[3,11] almost 200 participants (100 controls and about 100 HCC patients) were found to yield a power of 85% at a *P* value of .05. Genotypes frequencies were tested for consistency with Hardy-Weinberg equilibrium using an exact test.

3 | RESULTS

The genotypic and allelic frequencies of XRCC1-G2152A (rs25487), XRCC3-C18067T (rs861539), and XRCC7-G6721T (rs7003908) SNPs in HCC patients and controls are presented in Tables 3 and 4. Statistical analysis showed that XRCC1-G28152A (rs25487) heteromutant genotype (GA) was significantly higher in HCC patients than controls and conferred an almost three-fold increased risk of HCC (OR, 2.35; 95% CI, 1.33-4.04). The homomutant genotype (AA) was higher in HCC patient than control but the difference was statistically insignificant. The variant, A allele conferred almost two-fold increased risk of HCC (OR, 1.88; 95% CI, 1.22-2.89). As for XRCC3-C18067T (rs861539) SNP, there was no statistical difference in the distribution of the polymorphic genotypes between HCC cases and

TABLE 3 Genotypic and allelic frequencies of XRCC1 (rs25487), XRCC3 (rs861539), and XRCC7 (rs7003908) SNPs in HCC patients and controls

Genotypes and alleles	Controls no. (%)	HCC patients no. (%)	OR	95% CI		*P* value
XRCC1-G28152A (rs25487)						
GG	57/100 (57%)	33/100 (33%)	Reference			
GA	36/100 (36%)	57/100 (57%)	2.35	1.33	4.16	.003
AA	7/100 (7%)	10/100 (10%)	1.4	.540	4.04	.09
GA + AA	43/100 (43%)	67/100 (67%)	2.69	1.56	4.8	.001
Allele G	0.75	0.61	Reference			
Allele A	0.25	0.39	1.88	1.22	2.89	.004
XRCC3-C18067T (rs861539)						
CC	27/100 (27%)	35/100 (35%)	Reference			
CT	56/100 (56%)	50/100 (50%)	0.79	0.45	1.57	.39
TT	17/100 (17%)	15/100 (15%)	0.86	0.29	1.6	.38
CT + TT	73/100 (73%)	65/100 (65%)	0.69	0.4	1.84	.7
Allele C	0.55	0.6	Reference			
Allele T	0.45	0.4	0.82	0.55	1.21	.31
XRCC7-G6721T (rs7003908)						
GG	32/100 (32%)	20/100 (20%)	Reference			
TG	37/100 (37%)	56/100 (56%)	2.17	1.23	3.82	.007
TT	31/100 (31%)	24/100 (24%)	0.7	0.38	1.31	.27
TG + GG	68/100 (68%)	80/100 (80%)	1.88	0.99	3.59	.053
Allele G	0.51	0.48	Reference			
Allele T	0.49	0.52	1.11	0.75	1.63	.62

Abbreviations: CI, confidence interval; HCC, hepatocellular carcinoma; OR, odds ratio; SNP, single nucleotide polymorphism.
P < .05 = significant.

controls. The heteromutant genotype (TG) of XRCC7-G6721T (rs7003908) was significantly higher in HCC patients than controls and conferred two-fold increased risk of HCC (OR, 2.17; 95% CI, 1.2-3.82). Although the variant (*T*) allele was higher in HCC patients than controls the difference was statistically insignificant. Combined genotypes analysis revealed that coinheritance of the polymorphic genotypes of XRCC1 and 7 was associated with 11-fold increased risk of HCC (OR, 11.66; 95% CI, 2.77-49.13). Statistical comparison between HCC patients harboring the wild and the polymorphic genotypes of XRCC1 revealed that ALT level was significantly higher in patients having the polymorphic genotypes. Regarding XRCC3, Child-Pugh score A was more prominent among patients having the

TABLE 4 Combined genotypes distribution of the studied SNPs in HCC patients and controls

Combined genotypes	Controls no. (%)	HCC patients no. (%)	OR	95% CI		*P* value
XRCC (1 + 3)						
GG + CC	9 (9%)	9 (9%)	Reference			1
GA + AA/CT + TT	10 (10%)	10 (10%)	1	0.28	3.57	
XRCC (1 + 7)						
GG + GG	14 (14%)	6 (6%)	Reference			**< .001**
GA + AA /TG + TT	6 (6%)	20 (20%)	**11.66**	2.77	49.13	
XRCC (3 + 7)						
CC + GG	5 (5%)	3 (3%)	Reference			1
CT + TT/TG + TT	30 (27%)	17 (17%)	0.94	0.2	4.45	
XRCC (1 + 3 + 7)						
GG + CC + GG	5 (5%)	2 (2%)	Reference			**.13**
GA + AA /CT + TT/TG + TT	21 (21%)	33 (33%)	**3.92**	0.7	22.13	

Abbreviations: CI, confidence interval; HCC, hepatocellular carcinoma; OR, odds ratio; SNP, single nucleotide polymorphism.
P < .05 = significant.

variant genotypes, while score B was more frequent in those having the wild genotype. As for XRCC7, the number of hepatic focal lesions was higher in those with the wild genotype.

4 | DISCUSSION

HCC frequently arises in the context of chronic injury and inflammation that promotes DNA damage and chromosomal aberrations. DNA damage may be repaired by enzymes encoded by the DNA-repair pathways.[21] The fundamental pathogenic event in carcinogenesis is the accumulation of DNA damage and errors in DNA repair. Polymorphisms in genes involved in DNA repair are likely to play an important role in the prognosis of HCC and are useful factors for determining the risk of cancer progression or recurrence. Various genetic factors are predicted to affect treatment efficiency and prognosis in patients with HCC, such as X-ray cross-complementing group 1 (*XRCC1*), group 3 (*XRCC3*), and group 7 (XRCC7).

In the current work, laboratory investigations revealed that all HCC patients were chronic viral hepatitis C patients (HCV positive/HBV negative). Hepatitis C virus (HCV) infection is a global health problem being the second most common chronic viral infection in the world.[22] The Egyptian Demographic Health Survey, a cross-sectional survey including hepatitis C virus (HCV) biomarkers, estimated HCV prevalence among the 15 to 59 years age group to be 10%. Accordingly, Egypt has the highest HCV prevalence in the world.[23]

Chronic hepatitis C (CHC) is a worldwide etiology of chronic hepatic insult particularly in Egypt where genotype-4 is the principal genotype. CHC disease progression is variable among ethnic groups and viral genotypes.[4-6] The carcinogenesis of HCV-associated HCC is proposed to be a multistep process involving upregulation of inflammatory cytokines and induction of oxidative stress from chronic hepatitis, fibrosis, liver regeneration, and, ultimately, the development of cirrhosis.[24] Moreover, HCV may play a direct role in hepatic carcinogenesis through involvement of viral gene products in inducing liver cell proliferation.[25] Coexistence of chronic HCV infection and liver cirrhosis with genetic risk factors could induce or participate in hepatic carcinogenesis.

Mutations of XRCC1 may increase the risk of cancers by impairing the interaction of XRCC1 with other enzymatic proteins, altering DNA-repair activity and subsequently induce the carcinogenesis.[10] In the present work, the frequency of XRCC1-G28152A (rs25487) polymorphic genotypes in HCC patients was 57% for the heteromutant (GA) genotype and 10% for the homomutant genotype (AA). The frequency of the heteromutant (GA) genotype was higher than that reported in French and Chinese populations being 37.5% and 40.9%, while it was near to those reported for the homomutant (AA) genotype being 15% and 6.8%, respectively.[20,26] Lower frequencies were reported in Gambia being 17.5% and 2% for the GA and AA genotypes.[27] Higher frequency of the AA genotype was reported in Pakistanian population being 34%, while the GA genotype was 28%.[12] This could be referred to as ethnicity and the sample size enrolled in these studies together with the method applied for genotyping. The heteromutant genotype (GA) was significantly higher in HCC patients than controls (57% vs 36%) and conferred two-fold increased risk of HCC (OR, 2.35; 95% CI, 1.33-4.16). The homomutant genotype (AA) was higher in HCC patient than control (10% vs 7%) but the difference was statistically insignificant. This goes with the meta-analysis performed by Qi et al[28] which included fifteen studies and covered a total of 2554 cases and 3320 controls to examine the association between XRCC1-G28152A polymorphism and the risk of HCC. The results of their subgroup analysis by ethnicity indicated that the XRCC1-G28152A polymorphism was associated with an increased risk of HCC in Asian populations.[28] Moreover, the study of Yao et al[20] showed that HCC patients with XRCC1-GA and AA genotypes faced a significantly increased risk of HCC in Chinese population (OR, 2.16; 95% CI, 1.86-2.5) for GA genotype; OR, 4.77; 95% CI, 3.26-6.98 for AA genotype).

In contrast, the meta-analysis of Wu et al[29] of HCV-related HCC in China showed that there was no association between XRCC1 polymorphism and risk of HCV-related HCC (OR, 0.92; 95% CI, 0.79-1.07). Surprisingly, Zeng et al[30] and Gulnaz et al[12] reported that the polymorphic genotypes of XRCC1were higher in controls than HCC patients. Gulnaz et al[12] reported that the GA genotype was found to be protective against the development of HCC (OR, 0.52; 95% CI, 0.28-0.92). This could be attributed to the ethnic differences between the studied cohorts in addition to the sample size as their study was conducted on 46 HCC cases and 46 controls.

In an attempt to study the association between the studied SNP and the clinic-pathological feature of the disease, statistical comparison between HCC patients harboring the wild and the polymorphic genotypes revealed that there was no statistically significant differences between the two groups regarding their age at diagnosis, gender, clinical, radiological or laboratory characteristics except for alanine transaminase (ALT) level which was significantly higher in patients having the polymorphic genotypes. The study of Yu et al[31] reported that patients having the variant gentypes were significantly associated with a high risk of vascular and regional invasion and eventually unfavorable prognosis.

The frequency of XRCC1 G28152A (rs25487) polymorphic genotypes in our Egyptian controls was 36% for the heteromutant genotype (GA) and 7% for the homomutant genotype (AA). These were close to that previously reported in Egyptians being 40% and 42.6% for the GA genotype and 9.4% and 8% for the AA genotype.[32,33] It was also close to that of Taiwanese, Chinese and Koreans being 38.6%, 35% and 36% for the GA genotype and 9.1%, 8%, and 4% for the AA genotype, respectively.[31,34,35] Higher frequencies were reported in controls of European descent (Brazilians, North Americans and Italians) as the GA genotype ranged from 41% to 50% and the AA genotype ranged from 10% to 17%.[36-40] On the contrary, Zhai et al[41] reported that the frequency of GA and AA genotypes in Chinese controls were 9.7% and 38.8%, respectively. This could be attributed to the ethnic difference between the studied populations. Furthermore, discrepancies between the frequencies of the polymorphic genotypes were noticed among Chinese controls according to the area of the study whether Jiangsu, Guangxi, or Beijing.

The most commonly investigated SNP in XRCC3 gene is T241M (rs861539), resulting in an amino acid substitution from Thr to Met at codon 241. This may affect the function of the encoded protein and consequently alter its DNA-repair capacity. Positive associations between XRCC3-Ther241Met and malignancies have been reported in different types of cancers as bladder cancer, lung cancer, cervical cancer, and melanomas.[42]

Genotypic analysis revealed that the polymorphic genotypes of XRCC3-C18067T (rs861539) in HCC patients were 50% for the heteromutant (CT) and 15% for the homomutant (TT) genotypes. The frequency of the TT genotypes in Pakistani HCC patients was close to that reported in our Egyptian patients being 14%, while the CT genotype was lower being 36%.[12]

In the current study, the distribution of the polymorphic genotypes in HCC patients was close to that of controls. To the contrary, Yao et al [20] reported that the XRCC3 polymorphism conferred three and six-fold increased risk of HCC for the heteromutant and homomutant genotypes, respectively (OR, 3.3; 95% CI, 2.85-3.87 for the CT and OR, 5.8; 95% CI, 3.91-13.75 for the TT). Similar results were reported by Liu and colleagues.[43] Furthermore, Ji et al[44] constituted the first meta-analysis investigating the association between XRCC3-C18067T polymorphism and HCC risk in Chinese population. The analysis showed that XRCC3-C18067T polymorphism might be associated with increased risk of HCC in the Chinese population.

Statistical comparison between HCC patients having the wild and the polymorphic genotypes revealed that there was no statistically significant difference between the two patients' groups except for Child-Pugh scoring as score A was more prominent among patients having the variant genotypes, while score B was more frequent in those having the wild genotype. On comparing the laboratory data, Hb level was significantly higher in those harboring the polymorphic genotypes ($P = .038$), while total and direct billirubin and blood urea were higher in those having the wild genotype ($P = .006$, .026, and .049, respectively).

The frequency of XRCC3-C18067T (rs861539) polymorphic genotypes in Egyptian controls was 56% and 17% for the CT and TT genotypes, respectively. These frequencies were close to that reported in Italians, North Americans and Brazilians from either African or European descent where the heteromutant genotype ranged between 42% and 52%, while the homomutant genotype ranged between 14% and 22%.[36] Lower frequencies of the polymorphic genotypes were reported in Pakistani population being 42% and 5% for CT and TT genotypes, respectively.[12]

XRCC7 gene encodes the catalytic subunit of DNA-activated protein kinase (DNA-PK), involved in NHEJ repair pathway. It is speculated that XRCC7-G672T (rs7003908) polymorphism may regulate splicing and cause mRNA instability.[45] In our HCC patients, 56% have the heteromutant (TG) genotype, while 20% have the homomutant (TT) genotype. The TG genotype was significantly higher in HCC patients than control (56% vs 37%) and conferred two-fold increased risk of HCC (OR, 2.17; 95% CI, 1.23-3.82). Long et al[46] and Yao et al,[20] reported that the TG genotype was associated with increased HCC risk among Chinese population (OR, 2.6; 95% CI, 1.79-3.8 and OR, 2.6; 95% CI, 2.9-4.03, respectively). However, Long et al[46] attributed HCC risk to the G allele not the T allele as revealed by our results. They reported that individuals with the wild type (G) allele of XRCC7 (GG or TG) faced an increased risk of HCC being five-fold for the GG genotype (OR, 5.04; 95% CI, 3.28-7.76) and three-fold for the TG genotype (OR, 3.45; 95% CI, 2.4-4.94) in Chinese population. In contrast to our findings, Hsieh et al[47] in their hospital-based case-control study reported that the heteromutant (TG) genotype had a protective effect on HCC susceptibility which was obvious among males and alcoholics, but not females. This could be attributed to the different descents of the studied groups.

Statistical comparison between HCC patients harboring the wild and the polymorphic genotypes of XRCC7 -G6721T SNP regarding their clinical and laboratory characteristics revealed that the number of hepatic focal lesions was higher in those with the wild genotype.

The frequency of XRCC7 G6721T polymorphic genotypes in our Egyptian controls was 37% for the heteromutant (TG) genotype and 31% for the homomutant (TT) genotype. Higher frequencies were reported in the Far East; China and Taiwan being 58.1% and 30%,[46] 57.3% and 30.3%,[20] 44.7% and 38.9% and 48.7% and 43.3%[15] for the TG and TT genotypes, respectively. The discrepancies between these studies could be attributed to the ethnic differences between the studied populations. In Iranian and Indian studies, the TG genotype was higher in their controls but the TT genotype was close to that reported in our study being 48.2% and 28.5%[48] and 46.4% and 32%[49] for the TG and TT genotypes, respectively. Combined genotypes analysis showed that coinheritance of the polymorphic genotypes of XRCC1 and XRCC7 was associated with an 11-fold increased risk of HCC (OR, 11.66; 95% CI, 2.77-49.13), while the risk decreased to be almost four-fold when the polymorphic genotypes of the trio XRCC1, XRCC3, and XRCC7 were coinherited. This might be referred to the effect of XRCC3 as the polymorphic genotypes of XRCC3 SNP were higher in controls than HCC patients.

In conclusion, our study showed that XRCC1 G28152A (rs25487) and XRCC7 G6721T (rs7003908) genetic polymorphisms could be considered as molecular risk factors for HCC in Egyptians. The search for genetic factors that could help to select high-risk populations and thus to modulate the indications of screening procedures is necessary. Moreover, the identification of predictive factors could lead to a better diagnosis and planning of new preventive strategies for high-risk individuals.

4.1 | Study limitations

The relatively small sample size of this study is a limitation of the present work. Larger sample size is recommended to validate our results regarding the role of the studied SNPs as molecular risk factors for HCC.

CONFLICT OF INTERESTS

All authors declare that they have no conflict of interests.

AUTHOR CONTRIBUTIONS

The study design was set by AAE, HAR and MK. Patients' selection, clinical assessment and revision of patients' data before statistical analysis was carried out by AA-A and HS. Sample collection, data tabulation and genotyping of the studied SNPs were carried out by AES. HAR and MK were responsible for monitoring the genotypic analysis, data presentation and analysis and writing the manuscript. IAAK and NZ were responsible for revising the review of the literature and follow the tasks assigned to AES. All authors revised and approved the final form of the manuscript.

ETHICS STATEMENT

The research protocol was approved by the Research Ethics Committee of the Departments of Clinical Pathology and Tropical Medicine, Cairo University, and all procedures were performed in accordance with the 1964 Helsinki Declaration. Informed written consent was obtained from all participants before enrollment in the study.

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