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X-ray Repair Cross-Complementing group 1 Single-Nucleotide Polymorphism (rs25487): a Risk Factor for Hepatocellular Carcinoma in the Egyptian Population

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ABSTRACT

Chronic hepatitis C virus (HCV) infection is a worldwide etiology of hepatocellular carcinoma (HCC) particularly in Egypt. DNA-repair systems are responsible for maintaining genomic integrity. Base excision repair (BER) is one of the major DNA-repair pathways. The X-ray repair cross-complementing group 1 (XRCC1) protein encoded by XRCC1 gene is a key player in the multistep BER pathway. Deficiency in the repair capacity due to genetic alterations in DNA-repair genes can lead to genomic instability and increased risk of cancer development. Our study aimed to investigate the association between XRCC1 gene polymorphism (rs25487) (c.28152A>G) and HCC with chronic HCV infection in Egyptian patients. The study was conducted on 35 patients with HCC and 15 patients with HCV related cirrhosis serving as pathological control group. Detection of XRCC1 gene polymorphism (rs25487) (c.28152A>G) was performed by real time polymerase chain reaction (RT-PCR). The study showed higher frequency of XRCC1 (GG, GA) genotypes and increased (G) (allele) frequency in patients with HCC compared to patients with HCV related cirrhosis. However, the difference was statistically non-significant. Further studies on larger samples size are required, with the need to perform DNA sequencing from tissue samples to confirm the association between XRCC1 gene polymorphism (rs25487) and HCV related HCC in Egyptian population.

INTRODUCTION

HCC is a worldwide health problem. In Egypt, HCC is considered as the most challenging health problem as its incidence is increasing (Rashed *et al.*, 2020). Chronic hepatitis B virus (HBV) and HCV infection are considered ones of the major risk factors to develop HCC (Hang and Wedd, 2021). Chronic unresolved liver inflammation is associated with repair and tissue remodeling processes which often leads to fibrosis and subsequent progression to cirrhosis and initiation of hepatocarcinogenesis (Zanotti *et al.*, 2022).

HCC is a complex multistep process involving progressive accumulation of somatic genomic alterations pinpointing different cellular and molecular events (Alqahtani *et al.*, 2019). DNA damage due to endogenous or exogenous carcinogens may be repaired by enzymes encoded by the DNA repair pathways (Ghaderi-Zefrehi *et al.*, 2021).

Highly conserved DNA repair systems are settled to process DNA damage and maintain genomic integrity. Among these repair systems, XRCC1 which is responsible for repairing of oxidative DNA damage and single-strand breaks (London, 2015). XRCC1 acts as scaffolding protein which interacts with multiple repair enzymes allowing them to carry out their enzymatic steps in repairing DNA (Xu *et al.*, 2015).

XRCC1 (rs25487) single nucleotide polymorphism (SNP) is the substitution of A to G that leads to (p. Arg399Glu) (exon ten). Polymorphisms in DNA repair genes may influence the DNA repair capacity, which is crucial for preventing genomic instability and subsequently, may be associated with higher risk of malignancy (El-Garawani., 2020).

This study aimed to investigate the association between XRCC1 gene polymorphism (rs25487) (c.28152A>G) and HCC in a group of Egyptian patients with chronic HCV infection.

MATERIALS AND METHODS

Subjects: This study is a cross sectional study which was held from October 2021 till October 2022 on 35 patients who had HCC on top of HCV and 15 patients who had HCV related liver cirrhosis. They were recruited from Tropical Medicine Department of Ain Shams University Hospitals. An informed consent was taken from all participants. The study protocol was approved by the Research Ethics Committee at Ain Shams University, Faculty of Medicine, Ain Shams University.

Subjects Were Classified Into Two Groups:

Group I: HCC Group (n = 35):

This group included thirty-five (35) patients who had HCV related HCC diagnosed by triphasic abdominal CT. They were recruited from Tropical Medicine Department of Ain Shams University Hospitals. They were 24 males and 11 females with age ranging from 42 to 76 years.

Group II: HCV related Liver Cirrhosis Group (n = 15):

This group included fifteen (15) patients who had HCV related liver cirrhosis who were age and sex matched serving as pathological control group. They were 6 males and 9 females with age ranging from 40 to 70 years.

Subjects with any of the following conditions were excluded from our study: Chronic HBV infection, fatty liver diseases, metabolic liver diseases, autoimmune liver diseases and alcoholic liver diseases.

All participants in our study were subjected to the following: full history taking, thorough clinical examination especially abdominal examination, radiological investigations including abdominal ultrasound, triphasic abdominal CT for diagnosis of HCC and dynamic MRI whenever needed, bone scan and chest CT for staging. Laboratory investigations including: liver function tests including: total and direct bilirubin, alanine transaminase (ALT), aspartate transaminase (AST), total protein, albumin, serum alpha fetoprotein level (AFP), viral hepatitis markers (HbsAg, and HCV Ab), complete blood count (CBC), prothrombin time (PT), partial thromboplastin time (PTT), international normalised ratio (INR) and XRCC1 SNP genotyping by RT- PCR.

Sampling:

Ten milliliters (10 mL) of venous blood were collected under aseptic precautions from all subjects. Three mL blood were delivered into EDTA vacutainer and were stored at -20°C to be used for subsequent detection of XRCC1 G28152A (rs25487) gene polymorphism by real-time quantitative polymerase chain reaction (RT-PCR).

The remaining 7 mL blood were distributed between citrate tube, gel separating tube and EDTA vacutainers for the rest of investigations. Hemolysed samples were discarded. Repeated freezing/thawing of samples was avoided.

Methods:

a) Assay of Liver Function Tests :

Serum ALT, AST, total protein, albumin, total bilirubin and direct bilirubin were performed on AU 680 chemistry analyzer (Beckman Instruments Inc., Scientific Instruments Division, Inc. 250 S. Kraemer Blvd. Brea, CA92634-3100, USA**).**

b) Assay of viral hepatitis markers and serum AFP:

Anti-HCV antibody, HBsAg and AFP were analyzed on cobas e 411 immunoassay autoanalyzer using kits supplied by Roche Diagnostics (Roche

Diagnostics GmbH, Sandhofer Strasse 116, D-68305 Mannheim). The assay principle is based on an electrochemiluminescence immunoassay (ECLIA) technique.

c) Assay of CBC:

Assay of CBC was performed using XN-1000 six-part differential hematology analyzer, provided by Sysmex, Bornbarch, Germany**.**

d) Assay of PTT, PT and INR:

Assay of PTT, PT and INR was performed using Stago max compact analyzer provided by Diagnostic STAGO incorporation, New Jersey, US**.**

e) Assay of XRCC1 polymorphism by RT-PCR:

Detection of XRCC1 polymorphism (rs25487) was performed by TaqMan real time PCR kit supplied by Thermo scientific (Third Avenue, Waltham, MA, USA). The technical steps included: Extraction of genomic DNA from peripheral blood leucocytes in EDTA tubes by The GeneJET™ Whole Blood Genomic DNA Purification Mini Kit. The kit utilizes silica-based membrane technology in the form of a convenient spin column. Samples are digested with Proteinase K in the supplied Lysis Solution. The lysate is then mixed with ethanol and loaded onto the purification column, where the DNA binds to the silica membrane. Impurities are effectively removed by washing the column with the prepared wash buffers. Genomic DNA is then eluted under low ionic strength conditions with the elution buffer followed by amplification of the extracted DNA and allelic discrimination by RT-PCR.

Principle:

TaqMan genotyping assays genotype SNPs using the 5′ nuclease assay for amplifying and detecting specific SNP alleles in DNA samples. Each TaqMan genotyping assay contains two primers for amplifying the sequence of interest and two TaqMan probes for detecting alleles Table (1). The presence of two probe pairs in each reaction allows genotyping of the two possible variant alleles at the SNP site in a DNA target sequence. The genotyping assay determines the presence or absence of a SNP based on the change in fluorescence of the dyes associated with the probes.

Table 1: Probes sequences used in the PCR reaction:

Mutant probe	5'-VIC-AAGCAACTATTTCATAACTT-MGB-3'
Wild type probe	5'-FAM-CCCACCCTTCACACTATTAC-MGB-3'

Result Interpretation:

A substantial increase in FAM dye fluorescence only indicates homozygous wild type (allele 1), substantial increase in VIC dye fluorescence only indicates homozygous mutant type (allele 2), and substantial increase in both VIC and FAM dye fluorescence indicates allele 1 allele 2 heterozygosity.

Statistical Methods:

Data were collected, revised, coded and entered to the Statistical Package for Social Science (V.23_IBMcorp_USA_2015) for data analysis. Mean ± Standard deviation (SD) are applied to express quantitative parametric data. Median and inter-quartile range are applied to quantitative non parametric data. Categorical data are expressed as number (n) and percentage (%). The comparison between groups regarding qualitative data was done by using Chi-square test. Student t-Test is used to assess the statistical significance of the difference between two study group means for parametric data. Mann-Whitney U-test is applied for statistical comparison between two independent sets of data if one or both of them have a skewed distribution. P-value <0.05 was considered significant, p-value <0.01 was considered highly significant and p-value >0.05 was considered non-significant.

RESULTS

Descriptive and comparative

statistics of demographic data, clinical parameters and Child-Pugh score in HCC patients' group and HCV related liver cirrhosis control group revealed that there is no statistically significant difference between both groups as shown in Table (2).

Table 2: Descriptive and comparative statistics of the demographic data, clinical parameters and child-Pugh score in the HCC patients group and HCV related liver cirrhosis control group:

Parameters		HCC patients' group	HCV related Liver cirrhosis control group	t*/χ2	P-value	Significance
		N= 35	N= 15			
Age	**Mean ± SD year**	59.0 ±10.12	55.07 ± 11.92	1.1938*	>0.05	NS
Sex	**Female n (%)**	11 (31.4%)	9 (60.0%)	3.571	>0.05	NS
	Male n (%)	24 (68.6%)	6 (40.0%)			
Body mass index (BMI)	**Mean ± SD**	24.19 ± 3.19	25.77 ± 3.36	1.576*	>0.05	NS
Ascitis	**None n (%)**	13 (37.1%)	4 (26.7%)	1.996	>0.05	NS
	Mild n (%)	3 (8.6%)	3 (20.0%)	3.401	>0.05	NS
	Moderate n (%)	9 (25.7%)	5 (33.3%)	3.453	>0.05	NS
	Massive n (%)	10 (28.6%)	3 (20.0%)	4.452	>0.05	NS
Encephalopathy	**None n (%)**	31 (88.6%)	11 (73.3%)	1.996	>0.05	NS
	Grade I n (%)	1 (2.9%)	2 (13.3%)	3.401	>0.05	NS
	Grade II n (%)	2 (5.7%)	2 (13.3%)	3.453	>0.05	NS
	Grade III n (%)	1 (2.9%)	0 (0.0%)	4.452	>0.05	NS
Performance status (PS)	**Grade 0 n (%)**	16 (45.7%)	3 (20.0%)	1.996	>0.05	NS
	Grade 1 n (%)	2 (5.7%)	1 (6.7%)	3.401	>0.05	NS
	Grade 2 n (%)	10 (28.6%)	7 (46.7%)	3.453	>0.05	NS
	Grade 3 n (%)	4 (11.4%)	3 (20.0%)	4.452	>0.05	NS
	Grade 4 n (%)	3 (8.6%)	1 (6.7%)	1.996	>0.05	NS
Child-Pugh score	**Child A n (%)**	7 (20.0%)	2 (13.3%)	3.401	>0.05	NS
	Child B n (%)	18 (51.4%)	4 (26.7%)	3.453	>0.05	NS
	Child C n (%)	10 (28.6%)	9 (60.0%)	4.452	>0.05	NS

t: Student t-test, χ2: Chi-Square test, SD: standard deviation. P-value >0.05: Non significant (NS).*

A highly statistically significant difference was found between HCC patients' group and HCV related liver cirrhosis control group regarding PT and INR values being higher in control group, while no statistically significant difference was found between both groups regarding PTT, CBC and liver function test as shown in Table (3).

A highly statistically significant difference was found between HCC patients' group and HCV related liver cirrhosis control group regarding serum AFP being higher in HCC patients' group. The median of serum AFP in HCC patients' group was 18 ng/mL with Q1-Q3(12.1- 81) and the median of serum AFP in HCV related liver cirrhosis control group was 1.53 ng/mL with Q1-Q3(1.28-2) as shown in Figure (1).

The XRCC1 (rs25487) was genotyped in all studied subjects; the descriptive and comparative statistics of the genotype and allele frequency of XRCC1 (rs25487) polymorphism are illustrated in Table (4) and Figure (2). In HCC patients' group, 15 patients (42.8%) had the homozygous GG genotype, 17 patients (48.6%) had the heterozygous GA genotype, and 3 patients (8.6%) had the wild AA genotype. On the other hand, in HCV related liver cirrhosis control group, 7 patients (46.7%) had the homozygous GG genotype, 6 patients (40%) had the heterozygous GA

genotype, and 2 patients (13.3%) had the wild AA genotype. The G allele was present in 67.2% of HCC patients group and in 66.7% of the HCV related liver cirrhosis control group. The A allele was present in 32.8% of the HCC patients' group and in 33.3% in HCV related liver cirrhosis control group. There was no statistically significant difference between the HCC patients' group and HCV related liver cirrhosis control group regarding the genotype and allele frequency.

No significant difference was found between XRCC1 (rs25487) genotypes regarding Milan criteria and BCLC staging as shown in Table (5).

Table 3: Descriptive and comparative statistics of the laboratory parameters between HCC patients' group and the HCV related liver cirrhosis control group:

Parameters		HCC patients' group	HCV related Liver cirrhosis control group	*t*•/z	P-value	Significance
		No. = 35	No. = 15			
(PTT/seconds)	**Mean ± SD**	42.97 ± 7.04	48.00 ± 10.65	1.973•	>0.05	NS
(PT/seconds)	**Mean ± SD**	18.84 ± 3.34	22.94 ± 7.26	2.757•	<0.01	**HS**
INR	**Mean ± SD**	1.49 ± 0.26	1.81 ± 0.57	2.764•	<0.01	**HS**
Hemoglobin (g/dL)	**Mean ± SD**	10.38 ± 1.87	10.54 ± 1.44	0.290•	>0.05	NS
Platelets (10^3/uL)	**Median (Q1-Q3)**	141 (111-226)	125 (71-164)	-1.037	>0.05	NS
WBCS (10^3/uL)	**Mean ± SD**	9.81 ± 5.19	9.32 ± 4.38	-0.320•	>0.05	NS
Total protein (g/dl)	**Mean ± SD**	6.29 ± 0.97	6.38 ± 0.89	0.311•	>0.05	NS
Albumin (g/dl)	**Mean ± SD**	2.75 ± 0.59	2.73 ± 0.51	0.125•	>0.05	NS
ALT (IU/L)	**Median (Q1-Q3)**	24 (18 - 45)	20 (14- 26)	-1.388	>0.05	NS
AST (IU/L)	**Median (Q1-Q3)**	40 (26 -93)	37 (25-58)	-1.006	>0.05	NS
Total bilirubin (mg/dl)	**Median (Q1-Q3)**	2.5 (1.3 - 5.3)	2.7 (1.5- 3.9)	-0.222	>0.05	NS
Direct bilirubin (mg/dl)	**Median (Q1-Q3)**	1.7 (0.5 - 3.6)	1.7 (0.8-2.3)	-0.350	>0.05	NS
Total protein (g/dl)	**Mean ± SD**	6.29 ± 0.97	6.38 ± 0.89	0.311•	>0.05	NS

t: Student t-test, P-value< 0.01: highly significant (HS), P-value >0.05: Non significant (NS). SD: standard deviation, (Q1-Q3): interquartile range, Z: Mann Whitney test.

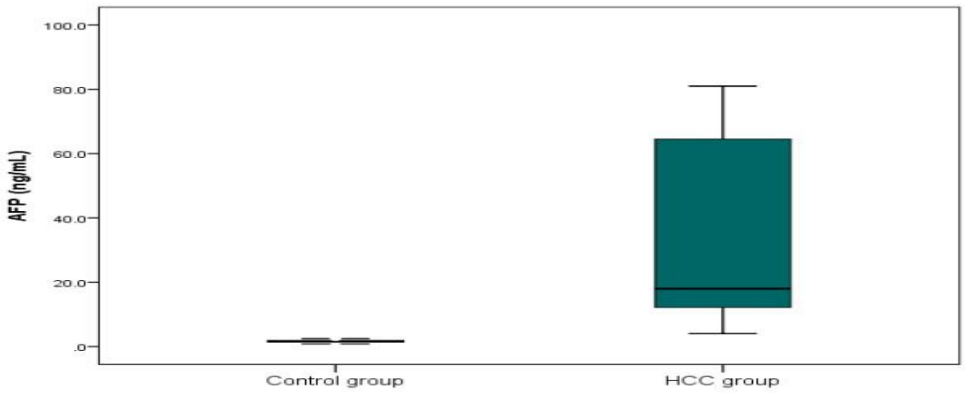

Fig. 1: Box-blot showing comparison of median levels of serum AFP between HCC patients group and HCV related liver cirrhosis control group.

Table 4: Descriptive and comparative statistics between HCC patients group and HCV related liver cirrhosis control group regarding the XRCC1 (rs25487) genotype frequency and (G/A) allele frequency:

XRCC1 (rs25487)	HCC patient group		HCV related Liver cirrhosis control group		χ2	P-value	Significance
	N	%	N	%			
AA genotype	3	8.6%	2	13.3%	0.440	>0.05	NS
GA genotype	17	48.6%	6	40.0%			
GG genotype	15	42.8%	7	46.7%			
A allele	23	32.8%	10	33.3%	0.002	>0.05	NS
G allele	47	67.2%	20	66.7%			

χ2: Chi-Square test.
P-value >0.05: Non significant (NS).
AA: wild genotype.
GA:Heterozygous mutant genotype. GG: Homozygous mutant genotype.

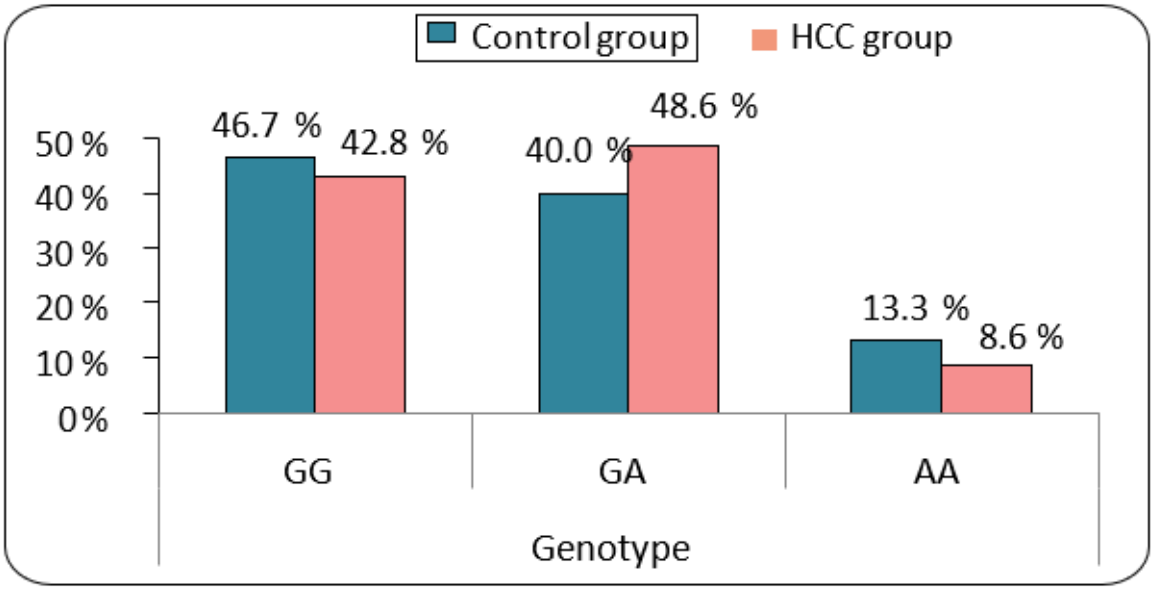

Fig. 2: Bar chart showing XRCC1 genotype frequency in HCC patients' group and HCV related Liver cirrhosis control group.
AA: wild genotype. GA: Heterozygous mutant genotype. GG: Homozygous mutant genotype.

Table 5: Descriptive and comparative statistics of XRCC1(rs25487) genotypes regarding Milan criteria and BCLC staging:

		XRCC1 genotypes						χ2	P-value	Significance
		AA		GA		GG				
		N	%	N	%	N	%			
Milan criteria	**Within Milan**	1	33.3%	3	17.6%	6	40%	1.982	>0.05	NS
	Beyond Milan	2	66.7%	14	82.4%	9	60%			
Ascitis	**None**	2	66.7%	7	41.1%	4	26.7%	2.091	>0.05	NS
	Mild	0	0.0%	1	5.9%	2	13.3%			
	Moderate	0	0.0%	4	23.5%	5	33.3%			
	Massive	1	33.3%	5	29.5%	4	26.7%			
Encephalopathy	**None**	2	66.7%	15	88.2%	14	93.3%	6.773	>0.05	NS
	Grade I	1	33.3%	0	0.0%	1	6.7%			
	Grade II	0	0.0%	1	5.9%	0	0.0%			
	Grade III	0	0.0%	1	5.9%	0	0.0%			
Performance status (PS)	**Grade 0**	2	66.7%	5	29.5%	9	60%	7.890	>0.05	NS
	Grade 1	0	0.0%	1	5.9%	1	6.7%			
	Grade 2	0	0.0%	8	47.1%	2	13.3%			
	Grade 3	0	0.0%	2	11.6%	2	13.3%			
	Grade 4	1	33.3%	1	5.9%	1	6.7%			
Child-Pugh score	**Child A**	1	33.3%	2	11.6%	4	26.7%	6.962	> 0.05	NS
	Child B	1	33.3%	7	41.3%	10	66.7%			
	Child C	1	33.4%	8	47.1%	1	6.6%			
BCLC Staging	**Stage 0**	0	0.0%	1	5.9%	1	6.7%	8.162	>0.05	NS
	Stage A	2	66.7%	1	5.9%	3	20%			
	Stage B	0	0.0%	3	17.6%	7	46.7%			
	Stage C	0	0.0%	5	29.5%	2	13.3%			
	Stage D	1	33.3%	7	41.1%	2	13.3%			

χ2: Chi-Square test.
P-value >0.05: Non significant (NS).
AA: wild genotype, GA: Heterozygous mutant genotype, GG:Homozygous mutant genotype.

DISCUSSION

Hepatocellular carcinoma is a consequence of several risk factors, such as chronic HCV and HBV infection, aspergillus flavus toxin, and long-term alcohol consumption (Siegel *et al.*, 2018). However, not all individuals exposed to these factors develops HCC. Increasing evidence suggests that HCC is not predisposed only by external factors but also by genetic factors (Yang *et al.*, 2023).

XRCC1 gene plays a crucial role in base excision repair (BER) in DNA repair pathways (Abou-Alfa *et al.,* 2018). Several polymorphisms in the XRCC1 gene have been described that are significantly associated with higher levels of DNA adducts (segment of DNA bound to a cancer-causing chemical), sister chromatid exchanges, somatic mutations, and chromosomal damage (Fu *et al.,* 2017. These variations can alter XRCC1 function leading to diminishing its repair kinetics and resulting in altered efficiency of XRCC1. As a result, a possible induction of malignancy development may occur. Several studies have explored the impact of XRCC1 polymorphism in various malignancies (De Jonge *et al.,* 2017).

In the XRCC1 gene, several SNPs have been identified among which three common polymorphisms have been widely investigated: the XRCC1 rs25487, rs25489 and rs1799782 (Merchant *et al.,* 2023). Each polymorphism is named according to the

restriction site that was initially used to identify it. Our SNP of interest, rs25487 (G/A) is located in exon 10 region of the XRCC1 gene and contains a single G to A base transition at position 399 (Shi *et al.*, 2016). Association between XRCC1 polymorphisms and HCC susceptibility has attracted extensive attention. Studies have showed that genetic mutations of XRCC1 may affect the risk and progression of HCC. However, due to the differences in ethnicity, country and experimental techniques, the results of the studies investigating the relation between XRCC1 polymorphisms and HCC have been controversial (Bazgir *et al.*, 2018).

Based upon the above data, our study aimed to investigate the clinical utility of the XRCC1 gene polymorphism (G/A) (rs25487) in Egyptian HCC patients in order to explore their association with HCC disease susceptibility. To our knowledge, few studies have been made to assess XRCC1 (rs25487) polymorphism in Egyptian patients with HCC and results are still controversial.

Our study was conducted on 50 subjects classified into 35 HCC patients diagnosed according to clinical examination, laboratory investigations in addition to triphasic CT and 15 patients who had HCV related liver cirrhosis who were age and sex matched serving as control group. To achieve our goal, genotyping for the polymorphism of the XRCC1 gene rs25487 (G/A) was performed by real time PCR.

Our results revealed that the genotypic and allelic frequencies of XRCC1 gene polymorphism rs25487 showed no statistically significant difference between HCC patients' group and HCV related liver cirrhosis control group. These results may be attributed to heterogeneity of HCC, possibly due to the gene–variant associations varying among different kinds of cancer according to the different mechanisms of carcinogenesis and there are many XRCC1 haplotypes that tend to always occur together to elicit specific disease like (XRCC1: rs25487, rs25489, rs1799782). It is thought that identifying the associations of these specific haplotypes sequence can facilitate identifying all polymorphic sites that are nearby on the chromosome.

Our results are in agreement with those of Gulnaz *et al.* 2013 who studied the association between XRCC1 gene polymorphism and HCC in Pakistanian population and reported that XRCC1 gene polymorphism did not show a significant role in the development of HCC by being present in 66% (165/250) of HCC patients versus 70% (158/225) in the HCV related liver cirrhosis control group. Our results also agreed with results of Yao *et al.* 2014, who studied the association between XRCC1 gene polymorphism and HCC in French population and also no significant role was observed in the development of HCC by being present in 62.5% (187/300) of HCC patients versus 64.5% (193/300) in the HCV related liver cirrhosis control group. Moreover, results of Elgharably *et al.*, 2016 study reported that XRCC1 polymorphism did not find a significant role in the development of HCC by being present in 80% (80/100) of HCC patients versus 85% (85/100) in the HCV related liver cirrhosis control group.

On the contrary to our study, the meta-analysis done by Qi *et al.*, 2014 was the first to report the association between XRCC1 gene polymorphism and HCC in Chinese population and observed a significant association between XRCC1 gene polymorphism and HCC being present in 16.1% (34/210) of HCC patients versus 6.1% (13/210) in the liver cirrhosis control group. However, another study on Chinese population performed by Wu *et al.*, 2013 also showed no significant difference between XRCC1 gene polymorphism and HCV-related HCC being present in 7.12% (19/266) of HCC patients versus 12.5% (25/ 200) in HCV related liver cirrhosis control group.

On the other hand, an Egyptian study performed by Mahmoud *et al.*, 2019 concluded that AA genotype and A-allele of XRCC1 rs25487 G/A showed significant increase in the patients with HCC compared

to HCV related liver cirrhosis control group. This study attributed their results to the fact that XRCC1 gene encodes one of the major repair factors involved in BER, as it fixes base damage and DNA single strand breaks. Strikingly, another Egyptian study performed by Elkhoudary *et al.,* 2021 demonstrated a strong significant association between both allelic and genotyping distributions of GA genotype and G-allele XRCC1 rs25487 G/A with HCC.

Moreover, regarding the allelic frequency, Duan *et al.,* 2012 results showed that G allele (mutant type) more frequent in their study and they showed significant association with HCC. These results may attribute to interactions among the genotypes of XRCC1 gene polymorphism rs25487 and DNA adducts.

Another discrepancy was given by Gulnaz *et al.,* 2013 who observed that the GA genotype was found to be protective against the development of HCC.

The discrepancies in the results between our study and those of the mentioned researchers may be attributed not only to different study designs and sample sizes but also to the ethnic variations. In addition, different techniques have been used in different studies, e.g. Mahmoud *et al.,* 2019 used the confronting two-pair primer method for PCR (PCR-CTPP). Moreover, it became evident that single-locus effects cannot explain multifactorial diseases. Thus, when the single polymorphism effect is not present alone or is not strong enough, it is important to characterize the other gene polymorphisms related to the disease susceptibility.

The present study didn't find any significant association between XRCC1 rs25487 (G/A) gene polymorphism genotypes and alleles with the clinical characteristics of HCC patients including age, sex, BMI and laboratory data including CBC, AFP, coagulation profile (PT, INR), liver function test (total protein, albumin, AST, total and direct bilirubin), BCLC and Milan criteria. However, a statistically significant difference was revealed between genotypes as regards PTT being higher in GA genotype and ALT being higher in AA genotype. These findings were comparable with Li *et al.,* 2012 who did not find any association between XRCCI polymorphism and clinical parameters of the disease.

However, Sumbul *et al.,* 2012 noted an association between XRCC1 gene polymorphism (rs24587) and clinico-pathological parameters of the disease regarding both ALT and AST and attributed these results to the claim that this polymorphism leads to increase damage of liver cells. Injured liver cells leak higher than normal amounts of certain chemicals including liver enzymes into the blood stream.

Conclusion

In conclusion, our study had observed higher frequency of XRCC1 (GG, GA) genotypes and increased (G) (mutant allele) frequency in patients with HCC compared to patients with HCV related cirrhosis, however, values did not reach statistically significant association in the sample of Egyptian patients studied. One reason for our observed negative results could be attributed to the small sample size in our study, thus, needing further studies with homogeneous samples, with larger sample size to clarify the statistical significance of the association of the G allele and the GG genotype of the XRCC1 with HCC. Additional studies are required to evaluate other XRCC1 haplotypes and other gene polymorphisms such as XRCC3 and XRCC7 associated with HCC development and conduction of more studies to investigate the impact of the XRCC1 gene polymorphism (rs 25487) on prognosis and outcome of HCC. Furthermore, the comparison between other studies that use different techniques like DNA sequencing from tissue samples of the same patients to confirm the absence of mutations or their presence with low frequencies is recommended. Thus, although our study revealed no significant results, yet, our study highlights the urgency to study this SNP with comparable techniques to clarify the association of this world- known SNP with

our ethnic group as published results are still highly controversial.

Declarations:

Ethical Approval: The study protocol was approved by the Research Ethics Committee at Ain Shams University, Faculty of Medicine, Ain Shams University, reference number: FMASU MS 516/2021.

Conflict of interests: The authors declare no conflicts of interest.

Authors Contributions: Menat Allah Ali Shaaban contributed to writing the manuscript and made the final version of the manuscript. Wessam El-Sayed Saad, Menat Allah Ali Shaaban, Marwa Elazab Mahmoud and Nada Atef Abdelhamed, contributed to laboratory work and analysis of data. Maha Mohsen Mohamed Kamal El-Din and Nada Atef Abdelhamed assisted in the collection of samples and patients' data. All authors contributed significantly to the study's conception, design, and final approval of the manuscript.

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Availability of Data and Materials: The datasets used and/or analyzed during the current study are available from the corresponding author on request.

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