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Research Article

Xeroderma Pigmentosum Complementation Group D (XPD) Codon 751 and Exonuclease 1 Glu 589 Lysgene Polymorphismsare Associated with Hepatocellular Carcinoma in Egyptian Patients with HCV (Genotype-4)

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Abstract

Aim: Xeroderma Pigmentosum Complementation Group D (XPD) is a major DNA repair gene in Nucleotide Excision repair system. Exonuclease 1 (Exo 1) is an important modulator in mismatch repair system. Our aim is to investigate the association of XPD/ERCC2 Lys751Gln (A/C) (rs 13181) and Exo- 1 Glu 589 Lys (G/A) (K589E) polymorphisms with Hepatocellular Carcinoma (HCC) in Egyptian patients.

Methods: Fifty HCC patients and 50 healthy controls were included. Genotyping for XPD/ERCC2 Lys751Gln was performed by Real-Time PCR and for Exo- 1 (K589E) Glu/Lys by PCR- RFLP.

Results: Patients with XPD Lys751Gln combined (AC + CC) genotypes had an increased risk of HCC compared to the wild AA genotype (p = 0.027, OR = 2.47). The mutant C allele frequency was higher in HCC than in controls, (p = 0.035). Mean serum albumin level was lower in patients carrying (AC + CC) genotypes than in AA genotype (p = 0.027). Median serum levels of ALT, AFP and creatinine were higher in AA genotype than in combined genotypes (p = 0.011, 0.007, 0.045 respectively). Exo- 1 Glu 589 Lys (AA) mutant genotype was associated with increased risk of HCC (p = 0.031).The frequency of the mutant A allele was higher in HCC compared to controls, (p = 0.044). Serum creatinine in wild type was higher than in other genotypes (p = 0.034).

Conclusion: XPD Lys751Gln and Exo- 1 Glu589 Lys gene polymorphisms are important HCC modulators in Egyptian patients with HCV genotype 4.

Keywords: HCC; XPD Lys751Gln (A/C); Exo- 1 Glu 589 Lys (G/A); RT-PCR; RFLP

Introduction

Globally, Hepatocellular Carcinoma (HCC) is one of the most common malignancies ranking the sixth [1] and it is the fastest growing cause of death in cancer patients in Asia [2]. In Egypt, HCC is the principal cancer among men; with the main risk factors are Hepatitis C Virus (HCV) and Hepatitis B Virus (HBV) [3]. Being a complex process with multiple etiologies, the pathogenesis of HCC is not fully understood and thus requires urgent studies [2]. DNA response to cell damage during the early stages of carcinogenesis has been reported in many types of cancer [4]. Genomic instability preceding HCC has been accused for malignant transformation [5]. The genomic integrity is seriously threatened by DNA break. Inactivation of DNA repair results in uncontrolled cell proliferation and hence increases cancer risk [6]. Xeroderma Pigmentosum group D (XPD) is one of the major players in Nucleotide Excission Repair (NER) pathway [7]. The XPD Lys751Gln (rs13181) polymorphism has been shown to have an effect on DNA repair capacity, possibly by altering the amino acid sequence of the protein [8], leading to reduced repair capacity and increased cancer susceptibility [9]. In HCC, the XPD Lys751Gln was strongly related to HCC risk in Chinese population [10]. Human DNA repair mechanisms are thought to prevent or delay genetic instability, thus acting as a barrier against cancer development [11,12]. The Mismatch Repair (MMR) is one of the major DNA repair pathways in human cells, which maintains the stability of genome, mediates DNA recombination, and modulates cell cycle arrest [13]. The gene Exonuclease 1 (Exo 1) belongs to the MMR system, A guanine (G)/adenine (A) common single nucleotide polymorphism at first position of codon 589 in exon 13 of Exo 1 gene, results in the substitution of glutamic acid (Glu, E) residue (GAG) bylysine (Lys, K) residue (AAG) (Exo 1 K589E) and has been suggested to influence the products of Exo 1 mRNA [14]. Several studies have investigated the relation between Exo- 1 (K589E) polymorphism and risk of cancer such as; lung cancer [15], breast cancer [16], gastric cancer [17] and gliomas [18]. Accordingly, we hypothesized that K589E polymorphism in Exo 1 gene may act as a genetic modifier in the susceptibility to HCC. We aimed to study the relationship of XPD/ERCC2 Lys751Gln (A/C) and Exo- 1 Glu 589 Lys (G/A) genes and HCC.

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Methods

This is a case control study carried out on 100 subjects divided into two groups:

Group I: included 50 patients with HCC, selected from Endemic medicine and hepatogastroentrology department at Kasr al Ainy Hospital, Cairo University. Patients with liver masses discovered on surveillance by ultrasonography were further investigated by serum Alpha Fetoprotein Level (AFP) and Computed Tomography (CT) scan or Magnetic Resonance (MRI). HCC was diagnosed according to the American Association for the Study of Liver Diseases (AASLD) updated practice guidelines [19].

Group II: Included 50 apparently healthy participants with no evidence of liver disease as a control group.

Before the start of enrolling our participants, we obtained a written informed consent from all of them. Our study protocol and consent were approved by ethics committee of Cairo University Hospital. All procedures performed in our study were in accordance with the ethical standards of the ethics committee of Cairo University Hospital and with the 1964 Helsinki declaration and its later amendments.

HCC patients were subjected to:

• Full history taking focusing on previous hepatic disorders and symptoms related to Chronic Liver Disease (CLD).

• Complete Clinical examination and Calculation of Child-Pugh score [20].

• Calculation of the Model of End-stage Liver Disease (MELD) score: using the following formula: 0.957 x Log (creatinine mg/dl) + 0.378 x Log (bilirubin mg/dl) + 1.120 x Log (INR) [21,22] which stratifies patients according to the severity of the disease in an objective and continuous ranking scale.

Laboratory investigations

All laboratory tests were performed in Chemical Pathology department, Cairo university hospital.

Specimen: Ten milliliters (10 ml) venous blood were collected and divided as follows:

• 4 ml in a plain sterile vacutainer left to clot for 10 minutes at room temperature and then centrifuged at 3000 rpm for 5 minutes. The serum was then separated into two separate aliquots, the first aliquot was used for laboratory analysis of the liver and kidney function tests and the other one was immediately frozen at –20°C, used for the assay of AFP and hepatitis markers (HBsAg& HCV Ab).

• Two milliliters (2 ml) were put into a sterile Ethylene Diamine Tetra-Acetic Acid (EDTA) vacutainer tube for CBC.

• Two milliliters (2 ml) were put into a sterile citrate vacutainer tube for PT, PC and INR.

• Two milliliters (2 ml) were collected into a sterile EDTA vacutainer tube and stored at –20°C to be used for the genotyping technique.

i. Routine liver & kidney function tests including serum Total Bilirubin, Direct Bilirubin, Alanine Aminotranseferase (ALT), Aspartate Aminotranseferase (AST), Total Protein, serum Albumin, Alkaline Phosphatase (ALP), serum urea and creatinine. They were assayed on Beckman Coulter AU 680 auto-analyzer.

ii. Complete blood picture (including haemoglobin level, total leukocytic count and platelet count) was done on Beckman Coulter LH 750 auto-analyzer.

iii. Prothrombin Time (PT), Prothrombin Concentration (PC) and International Normalized Ratio (INR) were assayed by the automated StagoDiagnoticaSTart 4 coagulometer system.

iv. Serum AFP level was assayed using Enzyme Linked Immuno-Sorbent Assay (ELISA) using kit purchased from Human# (Cat. no. 52010) [23].

v. Serum HCV Ab was assayed using ELISA method using kit purchased from Human# (Cat. no. 51275) [24].

vi. Serum HBsAg was assayed using ELISA method using kit purchased from Human# (Cat. no. 51048) [24].

The control group was subjected to: full history taking and laboratory investigations (routine liver & kidney function tests and hepatitis markers).

All candidates were subjected to:

1- Extraction of genomic DNA from peripheral blood leucocytes of EDTA anticoagulated blood which was done using the Thermo Scientific Gene JET Whole Blood Genomic DNA Purification Mini Kit (Cat. no. # K0781).

2- Determination of XPD Lys751Gln (rs13181) gene polymorphism using Real Time PCR.

3- Determination of Exo- 1 (K589E) Glu/Lys by Restriction Fragment Length Polymorphism (RFLP).

Identification of genetic polymorphisms of XPD Lys751Gln (A/C) (rs13181)

Amplification of the extracted DNA and Genotypic analysis was done by Real time PCR [25]. Real-Time PCR with sequence-specific primers was used to define the XPD Lys751Gln (A/C) gene SNP (rs13181) in exon 23. Real-time PCR allelic discrimination assay was designed using Taq-Man SNP Genotyping Assays (Applied Biosystems). Applied Biosystem step one Real-Time PCR System was used. The thermal cycling conditions were programmed as follows: 95°C for 10 min, 50 cycles of 92°C for 10 s and 60°C for 1 min. Controls were included in each run. Data analysis for allele discrimination was performed with the Applied Biosystems Real-Time PCR System software (Applied Biosystems, Foster City, CA. 94404 USA).

Identification of genetic polymorphisms of Exo- 1 (K589E) Glu/ Lys

A 306 pb PCR amplification fragment was generated using the primers 5'-GAC ACA GAT GTA GCA CGT AA-3' (Forward) and 5'-CTG CGA CAC ATC AGA CAT AT-3' (Reverse). After an initial denaturation at 94°C for 5 min, 35 PCR cycles were performed (94°C for 30 s, 55°C for 30 s, and 72°C for 30 s), followed by an elongation of 72°C for 10 min. The Exo 1 E589K polymorphism was analyzed by digestion of The PCR product with MseI (New England Biolabs Inc., MA) which resulted in two fragments of 196 and 110 bp in the

Table 1: Frequencies and Odds ratio of XPD Lys751Gln genotypes and alleles in the studied groups.

XPD Lys751Gln	Cases (n=50)		Controls (n=50)		*p* value
	Frequency	%	Frequency	%	
AA (Lys/Lys)	17	34	28	56	
AC (Lys/Gln)	26	52	18	36	0.084[1]
CC (Gln/Gln)	7	14	4	8	
C allele (Mutant type)	40	40	26	26	0.035[2]
A allele (Wild type)	60	60	74	74	

[1]OR oflys/glnplus gln/glnagainstlys/lys = 2.47 (CI = 1.10–5.55, p = 0.027)
[2]OR= 1.90 (CI = 1.04–3.46)

Table 2: Comparison between XPD Lys751Gln (AC + CC) genotypes versus AA genotype in HCC patients as regards different laboratory parameters.

	AA (n=17)	AC + CC (n=33)	*p* value
Mean ± SD			
H Hb (g/dl)	11.98 ± 2.31	11.84 ± 1.78	0.819
PC (%)	72.62 ± 18.48	73.47 ± 16.56	0.87
Al Albumin (g/dl)	3.3 ± 0.68	2.87 ± 0.6	**0.027***
Age (years)	50.18 ± 7.39	47.88 ± 6.20	0.251
Median (25^{th} - 75^{th} percentiles)			
MELD score	11 (9.15-18.75)	10.8 (8.1-13.4)	0.357
P PLT (x 10^3/ µl)	133 (80-222)	157 (128.5-174.5)	0.806
T TLC (x 10^3/ µl)	6.2 (5.05-7.2)	5.5 (4.5-6.95)	0.335
INR	1.3 (1.1-1.4)	1.2 (1.14-1.3)	0.622
T. Bil (mg/dl)	1.4 (0.9-2.95)	1.5 (0.95-2.35)	0.798
D. Bil (mg/dl)	0.4 (0.25-1.8)	0.5 (0.3-0.96)	0.734
ALT (U/L)	49 (23.5-56.5)	20 (16-40)	**0.011***
AST (U/L)	70 (53-100)	71 (46.5-90)	0.48
T. Total Ptn (g/dl)	7 (6.5-7.95)	6.8 (6.0-7.9)	0.418
ALP (U/L)	133 (85.5-179)	102 (74.5-151)	0.43
AFP (ng/ml)	405 (82.65-2858.5)	40 (7.65-204.5)	**0.007***
Urea (mg/dl)	32.1 (23.75-52.5)	31 (22.45-37.25)	0.301
Creatinine (mg/dl)	0.98 (8-1.3)	0.8 (0.6-1.2)	**0.045***

p value* < 0.05 was considered significant.

presence of the lys allele [26]. All PCR reactions were carried out in a Hybaid thermal cycler (Promega Corporation, USA) and PCR products were sized by electrophoresis on a 2% agarose gel stained with ethidium bromide.

HCV genotyping was done using Reverse Transcription Polymerase Chain Reaction (RT-PCR) on applied biosystems TR Step One Real time PCR system.

Statistical methods

Data obtained from the study were coded and entered using the software SPSS (Statistical Package for Social Science) Version17. Parametric data were summarized using mean ± SD, whereas non parametric data were summarized as median and percentiles for quantitative variables. Frequency and percentages were obtained for qualitative variables. Comparison among groups was done using the Chi-square test and the Fischer exact test for qualitative variable, t test and non-parametric Mann-Whitney U test were used to compare two groups, whereas analysis of variance and nonparametric test (Kruskal-Wallis test) were used to compare multiple groups. The Odds Ratio (OR) and their 95% Confidence Intervals (CIs) were calculated to estimate the strength of the association between the studied gene polymorphisms and the study population. A p value < 0.05 was considered significant [27].

Table 3: Frequencies and Odds ratio of EXO-1(K589E) Glu/ Lys Genotypes and alleles in the studied groups.

Exo- 1(K589E) Glu/ Lys	Controls (n=50)		Cases (n=50)		*p* value
	Frequency	%	Frequency	%	
GG (Glu/Glu)	31	62	27	54	
GA (Glu/Lys)	15	30	10	20	0.049[1]
AA (Lys/Lys)	4	8	13	26	
A allele (Mutant type)	23	23	36	36	0.044[2]
G allele (Wild type)	77	77	64	64	

[1]OR of lys/lys against glu/glu plus glu/lys = 4.04 (CI = 1.22–13.43, p = 0.031)
[2] OR= 1.88 (CI = 1.01–3.5)

Results

HCC cases were 38 males and 12 females with mean age of 48.66 ± 6.64 years. Our controls were chosen to be sex and age matched, with a mean age of 48.62 ± 7.28 years. There was no significant difference for age or gender (p >0.05). All of HCC patients were positive for HCV Ab and were genotype 4.

The XPD Lys751Gln polymorphism

We found that XPD (AC + CC) combined genotypes were significantly higher in HCC cases (66%) than in control subjects (44%) (OR = 2.47, 95% CI = 1.10 – 5.55, p value = 0.027), furthermore, the mutant C allele was significantly higher in HCC cases (40%) than in control subjects (26%) (OR = 1.90, 95% CI = 1.04 - 3.46, p value = 0.035) (Table 1). Regarding the laboratory findings of HCC patients, the mean serum albumin level was significantly lower in patients carrying the mutant allele (AC + CC) genotypes [2.87 ± 0.6 (g/dl)] than those with the wild; AA genotype (3.3 ± 0.68) (p = 0.027). We also found a significant increase in median levels of ALT [49 (23.5-56.5) U/L], AFP [405 (82.65-2858.5) ng/ml] and creatinine [0.98 (8-1.3) mg/dl] in patients with AA genotype (wild) when compared to those with combined (AC + CC) genotypes [20 (16-40) U/L] (p = 0.011), [40 (7.65-204.5) ng/ml] (p = 0.007) and [0.8 (0.6-1.2) mg/dl] (p = 0.045) respectively (Table 2). No statistical significant difference was found between the XPD (A/C) genotypes as regards clinical characteristics of HCC patients (p > 0.05).

The Exo- 1 (K589E) Glu/Lys polymorphism

We found that the AA genotype (mutant) was significantly higher than that in the combined genotypes (GG and GA) in HCC patients (26%) when compared with the control group (8%) (OR = 4.04, 95% CI = 1.22 – 13.43, p value = 0.031), furthermore, the mutant allele A was significantly higher in HCC cases (36%) than in control subjects (23%) (OR = 1.88, 95% CI = 1.01 - 3.50, p value = 0.044) (Table 3). Of the laboratory parameters, only serum creatinine was significantly higher in HCC patients with GG genotype (wild) comparing it to those carrying the other genotypes (p= 0.034), (Table 4).

Table 4: Association between Exo-1 K589E genotypes in HCC patients as regards different clinical and laboratory parameters.

Column1	Column2	Column3	Column4	Column5	Column6
		AA (n=13)	**GA (n=10)**		***p* value**
				GG (n=27)	
		Frequency (%)			
	Male	8 (61.5%)	5 (50%)	25 (92.6)	**0.010**[1]
Sex	Female	5 (38.5%)	5 (50%)	2 (7.4)	
DM	Yes	4 (30.8%)	6 (60%)	8 (29.6)	0.209
	No	9 (69.2%)	4 (40%)	19 (70.4)	
Smoking	Yes	8 (61.5%)	6 (60%)	18 (66.7)	0.911
	No	5 (38.5%)	4 (40%)	9 (33.3)	
Child Pugh	A	7 (53.8%)	3 (30%)	8 (29.6)	0.357
Score	B	2 (15.4%)	6 (60)	9 (33.4)	
	C	5 (29.4%)	1 (10%)	10 (37)	
Bilhrziasis	Yes	11 (84.6%)	3 (30%)	17 (63)	**0.028**[2]
	No	2 (15.4%)	7 (70%)	10 (37)	
Family	Yes	7 (53.8%)	5 (50%)	10 (37)	
History	No	6 (46.2%)	5 (50%)	17 (63)	0.552
Number of	< 2	9 (69.2%)	6 (60%)	19 (70.4)	0.83
Tumors	≥ 2	4 (30.8%)	4 (40%)	8 (29.6)	
Size of main	≤ 2cm	4 (30.8%)	3 (30%)	6 (22.2)	0.804
Tumor	>2cm	9 (69.2%)	7 (70%)	21 (92.6)	
Portal Vein thrombosis	Yes	1 (7.7%)	2 (20%)	2 (7.4)	0.499
	No	12 (92.3%)	8 (80%)	25 (92.6)	
Age, Mean ± SD		47.8 ± 7.0	49.7 ± 6.5	48.7 ± 6.8	0.794
(years)					
MELD score,		10.2 (8.0-12.8)	10.0 (9.1-11.3)	11.7 (8.9-18.5)	0.187
Median (25th - 75th percentiles)					
		Mean ± SD			
H Hb(g/dl)		12.1 ± 1.9	12.2 ± 1.4	11.7 ± 2.2	0.705
Total proteins (g/dl)		6.3 ± 1.4	7.4 ± 0.8	6.7 ± 1.5	0.181
		Median (25th - 75th percentiles)			
PLT (x 10^3/ µl)		165 (143-196)	145 (129.5-199.3)	137 (84-173)	0.344
T.L.C (x 10^3/ µl)		6.1 (5.1-7.3)	5.3 (4.3-6.6)	5.8 (5.0-6.8)	0.516
INR		1.2 (1.1-1.3)	1.2 (1.1-1.3)	1.2 (1.2-1.5)	0.532
T. Bil (mg/dl)		1.3 (0.8-2.2)	1.4 (1.2-2.4)	1.6 (0.9-4.0)	0.581
ALT (U/L)		24 (14.5-48.0)	19 (16.5-45.0)	34 (19-51)	0.173
AST (U/L)		57 (45.0-71.5)	88.5 (71.5-102.3)	70 (41-98)	0.06
ALB (g/dl)		2.7 (2.6-7.95)	3.0 (2.6-3.4)	3.1 (2.7-3.6)	0.562
ALP (U/L)		95 (72.5-140)	110.5 (88.5-175.5)	104 (81-157)	0.584
AFP (ng/ml)		106 (9.2-279.5)	165 (7.9-357.5)	85 (10.3-550.0)	0.981
Creatinine (mg/dl)		0.8 (0.7-1.2)	0.6 (0.5-0.9)	0.9 (0.8-1.3)	**0.034***

p value* < 0.05 was considered significant.

[1]OR of GG versus AA = 7.81 (CI = 1.26–48.36, *p* = 0.027),OR of GG versus GA = 12.5 (CI = 1.87–83.60, *p* = 0.009)

[2]OR of AA versus GA = 12.83 (CI = 1.69–97.19, *p* = 0.013)

We also found that in HCC patients, the frequency of males carrying the GG genotype was significantly higher than those with the GA or AA genotypes (For GG *vs.* GA: OR =12.5, CI = 1.87 – 83.60, *p* = 0.009, for GG *vs.* AA: OR = 7.81, CI = 1.26 – 48.36, *p* = 0.027) (Table

Table 5: Comparison of frequency distribution of Exo- 1 (K589E)polymorphism genotypes among male and female subjects.

Gender	Genotype	HCC	Controls	*P* value
Males		38	38	
	Lys/Lys	8	3	
	Glu/Lys	5	13	**0.049**[1]
	Glu/Glu	13	22	
Females		12	12	
	Lys/Lys	5	1	
	Glu/Lys	5	2	**0.015**[2]
	Glu/Glu	2	9	

[1]OR of (lys/lysvsglu/lys = 6.93, 95% CI = 1.29–37.22), $p = 0.027$
[2] OR of (lys/lys plus glu/lysvsglu/glu = 15.0, 95% CI = 2.02–111.17), $p = 0.012$

4 and 5). Moreover, we found higher association of Bilharziasis in HCC patients carrying the AA genotype (84.6%) compared to those having GA genotype (30%), OR = 12.83 (95% CI = 1.69 – 97.19), $p = 0.013$) (Table 4).

Discussion

Hepatocellular carcinoma has a high worldwide incidence, having a poor prognosis and survival rate that makes early diagnosis mandatory [28]. HCC management requires multidisciplinary approach because of the wide heterogeneity in clinical manifestations, different in biologic behavior, different causes of chronic liver disease and different therapeutic protocols [29]. Hepatitis viruses were reported to be the principal risk factors of DNA damage with chromosomal instability and insertion mutations leading to HCC development [30]. In the current study, we selected two single nucleotide polymorphisms, the XPD Lys751Gln and Exo- 1 (K589E) Glu/Lys to study their association with HCC in Egyptian patients.

DNA repair system maintains the stability of cellular functions and genomic integrity by the reversal of the damaged DNA caused by either endogenous metabolic products or by environmental carcinogens. Therefore, the DNA repair capacity may contribute to cancer patient outcomes [31,32]. Nuclear Emission Repair (NER) is the principal DNA repair pathway for the small base from oxidation and alkylation deleterious effects. XPD leads to opening of the DNA helix facilitating excision of damaged fragments. The two main polymorphisms involved in amino acid changes are 312 (Asp to Asn) and 751 Lys/Gln (AC).XPD 751Gln/Glnpolymorphism promotes carcinogenesis through reducing the enzyme activity and decreasing DNA repair abilities [30]. We demonstrated a statistically significant increase in the frequencies of combined (AC + CC) genotypes as well as the mutant C allele among HCC patients when compared to control group.

A previous study that was conducted in China supported our results. They reported that the frequency of the Lys/Lys (AA), Lys/Gln (AC) and Gln/Gln (CC) genotypes were (46.3%), (44.6%) and (9.1%) in HCC patients respectively while control subjects reported (56.9%), (38.7%) and (4.4%) respectively. They found a significant increase in the CC genotype frequency among HCC patients compared to the controls, (OR = 3.51, 95% CI = 1.50 - 6.31 and p value < 0.05). They also found that the frequency of the C allele was significantly higher in cases (31.4%) than in controls (23.8%), (OR = 1.42, 95% CI = 1.05 - 3.45) [10].

Moreover, another study which comes in concordance with ours found a significant association between XPD Lys751Gln (A/C) genotypes and HCC in patients with aflatoxin exposure. They demonstrated a significant increase in the frequency of AC genotype among cases (35.9%) when compared to the control group (26.3%), (OR = 1.75, 95% CI = 1.30 - 2.37 and p value < 0.001). They also found a significant increase in the frequency of CC genotype among cases (20.1%) comparing them to the control group (8.6%), (OR = 2.47, 95% CI = 1.62 - 3.76 and p value < 0.001), concluding that individuals carrying XPD codon 751 C allele were at a higher risk of HCC compared to those with the homozygote of XPD codon 751 A allele [33].

On the other hand, another study failed to find significant association between XPD Lys751Gln polymorphism and HCC susceptibility, where the frequency of AA genotype was (50%), AC genotype was (38%) and CC genotype was (12%) in HCC patients and (41%), (39%) and (20%) in the control group respectively ($p = 0.61$) [34]. A meta-analysis study that included seven studies for XPD Lys751Gln polymorphism reported an absence of association with HCC risk for all genetic models and suggested further large-scale studies considering gene-gene/gene-environment interactions which can explain the difference between our results from other studies [35].

On comparing the different clinical and laboratory parameters among HCC patients with different genotypes, we found that the mean albumin level was significantly lower in patients having the combined (AC + CC) genotypes than those with AA genotype which might suggest the role of the C allele in the development of chronicity in post-hepatitis liver disease. Serum creatinine, ALT and AFP levels were significantly higher in patients with XPD Lys751Gln (AA) genotype than those with combined (AC + CC) genotypes suggesting that, HCC patients with wild; AA genotype might a have more severe HCC outcome than those with (AC + CC) combined genotypes and that XPD Lys751Gln gene polymorphism might have a potential prognostic value in HCC.

In a study performed by Yue et al., 2013, they found a significant increase in the mean survival time among patients carrying the CC genotype comparing them to those carrying the AA genotype which suggested that XPD 751 Gln/Gln polymorphism had significant prognostic role on the survival of HCC patients. They also concluded that carriers of the CC genotype had more favourable outcome and reduced death rate in comparison to carriers of the AA genotype, which comes in harmony with our results.

Regarding the Exo- 1 (K589E) gene polymorphism, we found that both AA genotype and A (mutant) allele were significantly higher in HCC group comparing them to the control group. Our results were supported by a previous study which studied the association of Exo- 1 (K589E) gene polymorphism with HCC among Turkish population, they demonstrated a higher risk of HCC among subjects carrying homozygote AA (mutant), compared to those carrying the GG and heterozygote GA carriers (OR = 2.26 95% CI = 1.22–4.16, $p = 0.009$) [36]. They also found a higher risk of HCC in male subjects carrying the Exo 1 K589 AA genotype compared to those with the GG genotype (OR = 2.67, 95% CI = 1.27–5.61, $p = 0.009$). However, no significant association was reported between Exo- 1 (K589E) gene

polymorphism and HCC risk for females. In our study, the risk of HCC was significantly associated with Exo 1 K589 AA genotype in both male and female subjects. However, among HCC patients the frequency of males carrying the GG genotype was significantly higher than those with the GA or AA genotypes.

Conclusion

Our findings, XPD Lys751Gln (A/C) and Exo- 1 (K589E) G/A gene polymorphism exhibit a significant influence on HCC susceptibility in Egyptian patients and their detection could be used as a useful noninvasive molecular biomarker for the assessment of risk of HCC. Further studies are needed to validate the findings of our study, with larger sample volumes and comparing HCV G4 with different ethnic groups and other genotypes.

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