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ORIGINAL ARTICLE

Study of XPD Gene Polymorphism in HCV Positive HCC Egyptian Patients

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ABSTRACT

Background: Hepatocellular carcinoma (HCC) is one of the most frequently occurring tumorous cells globally, but its pathogenesis is unknown. Xeroderma pigmentosum gene D (XPD) is a critical Repair of DNA gene that has been implicated in protein mutation.

Aim: The objectives of this research were to assess the relationship between the XPD gene polymorphism (rs13181) (codon 751, Lys to Gln) and susceptibility to hepatocellular neoplasms, as well as to investigate the relationship between HCV and XPD polymorphisms.

Methods: Seventy-nine HCC patients and 63 patients with active HCV infection, and 52 healthy controls were involved. Real-time PCR carried out for genotyping of XPD Lys751Gln (rs 13181).

Results: Patients with XPD Lys751Gln AC genotype have a great risk of HCC development (p = 0.01, OR =1.505).

Conclusion: Our finding was that XPD Lys751Gln (A/C) polymorphism exhibit a major function in HCC early detection susceptibility in Egyptian patients. It has the potential to be a valuable noninvasive molecular indicator for evaluating the HCC threat.

Keywords: HCC; RT-PCR; XPD gene polymorphism

INTRODUCTION

Hepatocellular carcinoma (HCC), the much more commonly reported liver tumor, substantially leads to global tumor-related mortality and morbidity. The annual occurrence of new HCC patients is reported to be greater than 0.5 million[1].

It is well-founded that HCC production is a complicated biological function, and the exact mechanisms by which HCC occurs are still unknown. Numerous risk factors, including aflatoxin B1 (AFB1) consumption, smoking, chronic hepatitis B virus (HBV) infection, and more alcohol use, all lead to HCC susceptibility. Along with these causes, genetic polymorphisms contribute significantly to HCC susceptibility[2].

Numerous reports conducted over the last few decades have assessed and illustrated the relationship between repair of DNA genes and susceptibility to hepatocellular neoplasms. The most effective repair of the DNA pathway has been suggested to be nucleotide excision repair (NER). Xeroderma pigmentosum complementary class D (XPD) is a gene positioned on chromosome 19q13.3 that encodes a NER enzyme[3].

XDP affects the NER pathway since it is required to open the DNA duplex required to excise the DNA fragment comprising the deterioration base[4].

The XPD Lys751Gln (rs13181) polymorphism was demonstrated to influence repair of DNA ability, likely by modifying the amino acid sequence of the XPD protein, resulting in decreased repair ability and a raised tumor threat[5].

PATIENTS AND METHODS

Study population: This research was performed in the Tropical department of Theodor Bilharz Research Institute (TBRI), with 194 participants classified into three groups: group A included 79 cases with HCC, while group B involved 63 patients with Active HCV infection. Eventually, group C included 52 age and sex-matched volunteers as a control group. Participants with co-infections with HBV, alcohol use, or antiviral treatment were excluded from the sample. Patients that were involved in the research had informed consent. Additionally, the TBRI ethics committee approved these measures in compliance with the Declaration of Helsinki.

Genomic DNA extraction: The QIAamp DNA Mini Kit was utilized to collect genomic DNA (Qiagen; catalog No.: 51104). Proteinase K is utilized to remove DNA from whole blood. Cells are lysed after a brief incubation with proteinase K and a unique lysis buffer containing guanidine HCL that inactivates all nucleases immediately. Cellular nucleic acids adhere selectively to pre-packaged specific glass fibers in a highly pure filter tube. To remove PCR impurities, a sequence of rapid "wash - and - spin" steps is performed using 500 ul of Buffer AW1 and AW2. Finally, elution buffer (200 ul Buffer AE) was applied, and the mixture was incubated at 15-25 C for 1 minute to liberate the nucleic acid from the glass fiber, which is critical for removing PCR impurities.

Multiplication of the extracted DNA and detection of polymorphism: Allelic Discrimination (AD) is a multiplexed and end-point (data are obtained after the PCR process) test for detecting variants in a single nucleic acid sequence. The presence of two primer/probe pairs in every interaction enables genotyping of the two potential variations at a desired template sequence's single-nucleotide polymorphism (SNP) site. Allelic discrimination tests employ a PCR test that involves a fluorescent dye-labeled probe specific for each allele. The probes include two distinct fluorescent reporter dyes (FAM and VIC®) that differentiate allele multiplication. Every probe anneals to the complementary sequences between the reverse and forward primer locations throughout PCR. Only probes that hybridize to the allele can be cleaved by AmpliTaq Gold® DNA polymerase. Cleavage distinguishes the reporter dye from the quencher dye, resulting in an improvement in the reporter dye's fluorescence. Therefore, the fluorescence signal(s) produced by PCR multiplication identifies the existence of alleles in the specimen.

Mismatches between a probe and an allele minimize probe hybridization performance. Additionally, rather than cleaving the mismatched probe to release reporter dye, AmpliTaq Gold DNA polymerase is more likely to displace it.

Each PCR test included 2.5 ul diluted DNA, 12.5 ul of TaqMan Universal PCR Master Mix, 1.25 ul 20 TaqMan Genotyping Test Mix, and 8.75 ul distilled water. This PCR test was performed in a thermal cycler equipped with an ABI 7500, and the following software can be seen in Table 1.

Table 1: PCR amplification run

Pre-Read Run		Multiplication (RQ-PCR program)			Post-Read Run	
1 Cycle		Temperature	1 Cycle	Cycles	1 Cycle	
60 °C	1 min.	95°C	10 min	X1	60 °C	1 min
		95°C	15 sec	X40		
		60°C	60°C			

Statistical analysis: Microsoft Excel 2010 and the statistical package for social science (SPSS version 26.0) for Windows will be utilized to analyze the results (SPSS IBM., Chicago, IL). Continuous normally dispersed variations were demonstrated as mean ± SD with a 95 % confidence level, non-normally dispersed variations as median with a 25 and 75 percentile, and categorical variations as frequencies and percentages; a p-value <0.05 was considered statistically significant. The Student's t-test was utilized to compare the means of normally dispersed differences between classes; the Mann-Whitney assay was utilized to compare non-normal aspects, and the 2 test or Fisher's exact assay was utilized to compare the distributions of categorical differences between classes.

RESULTS

We found that there was a significant rise in AC hetero genotype in HCC group (45.6%) than HCV group (31.7%) (OR =1.505, 95% CI = 0.743 - 3.048, p= 0.01). Also, there was a significant rise in mutant genotypes (AC+CC) in HCC group (54.4%) in comparison to HCV group (41.3%) (OR = 1.419, 95% CI = 0.730 - 2.759, p= 0.01).Though, there was insignificant difference in allele frequencies between the control group and HCV group or HCC group regarding the genotypes frequency of the XDP gene. Moreover, the mutant C allele was greater in HCC patients (50%), although this rise is statistically insignificant. Wild genotype (AA) was significantly greater in the HCV group than the HCC group (p=0.01) (Table 2).

Table (2): Genotypes frequency of XDP gene among studied groups.

		CONTROL n=52	HCV n=63	HCC n=79	P. value		
					HCV Vs Control	HCC Vs Control	HCC Vs HCV
AA wild		27 (51.9%)	37 (58.7 %)	36 (45.6 %)	0.2	0.2	0.01*
AC heter		19 (36.5 %)	20 (31.7 %)	36 (45.6 %)	0.3	0.1	0.01*
CC homo		6 (11.5 %)	6 (9.5 %)	7 (8.9 %)	0.5	0.3	0.8
AC+CC		25(48.1 %)	26(41.3 %)	43(54.4 %)	0.2	0.2	0.01*
Allele	A (wild)	73(0.702)	94(0.746)	108(0.684)	0.3	0.7	0.2
	C(mutant)	31(0.298)	32(0.254)	50(0.316)			

*P value ≤ 0.05 significant; **P value ≤ 0.01 greatly significant.

Hardy-Weinberg Equilibrium: Regarding HCV patients, statistically non-significant differences were presented between the frequencies of observed and expected genotypes.

This proved that the XPD genotypes of the randomly collected samples were in H-W equilibrium data. (Table 3).

Table 3: Comparison between Observed and Expected SNPs Genotypes for HCV patients.

SNPs Genotype		n=63	Observed results (Current Study)	Expected results (H-W equilibrium)	p. value
XPD	AA	37	0.587	0.410	0.7
	AG	20	0.317	0.083	0.4
	GG	6	0.096	0.507	0.4
Total		63	1.000	1.000	

H-W equilibrium = Hardy-Weinberg equilibrium; n=Number,
P. Value is based on the X2 test, *p. value <0.05 is significant, ** p. value <0.01 is greatly significant.

In HCC patients, statistically significant differences were presented in AG genotype between the frequencies of observed and expected genotypes. This proved that the XPD genotypes of the randomly collected samples were not in H-W equilibrium data (Table 4).

Table 4: Comparison between Observed and Expected SNPs Genotypes for HCC patients.

SNPs Genotype		n=79	Observed results (Current Study)	Expected results (H-W equilibrium)	p. value
XPD	AA	36	0.456	0.325	0.8
	AG	36	0.456	0.042	0.04*
	GG	7	0.088	0.633	0.3
Total		79	1.000	1.000	

H-W equilibrium = Hardy-Weinberg equilibrium; n=Number,
P. Value is based on the X2 test, *p. value <0.05 is significant, ** p. value <0.01 is greatly significant.

DISCUSSION

HCC is prevalent in the world. It has a poor prognosis and a low survival rate that necessitates early detection[6].

Due to the broad heterogeneity of clinical symptoms, variations in biological activity, multiple factors of liver disease, and multiple therapeutic techniques, HCC care requires a multifaceted approach[7].

Hepatitis viruses have been identified as the primary cause of genetic deterioration, with chromosomal instability and mutations contributing to the production of HCC[8].

Genetic polymorphisms are typically classified as fragment length polymorphisms (FLPs), repetitive sequence polymorphisms (RSPs), or single nucleotide polymorphisms (SNPs)[9].

Genetic polymorphisms can alter the expression of proteins or the structure of cytokine particles, resulting in a variety of clinical symptoms and outcomes for HCC[10].

Repair of DNA systems is critical for normal cell cycle control and cell integrity preservation and the body's protective mechanisms against tumors. Alterations to the human genome's Repair of DNA mechanisms cause the aggregation of gene mutations and tumor growth[11].

Repairing genetic deterioration is critical for humans to avoid developing a lot of symptoms, such as tumors. Numerous studies have consistently demonstrated inter-individual variance in the Repair of DNA ability and that populations with impaired Repair of DNA ability are more susceptible to improving tumors. As a result, genetic variations in the repair of DNA genes that impair repair of DNA ability are thought to have a major effect on an individual's proclivity for tumors[12].

The complementation class D gene of Xeroderma pigmentosum encodes an adenosine triphosphate-dependent DNA helicase that mediates DNA unwinding in the 5'-3' direction. The enzyme is needed for the nucleotide excision repair (NER) mechanism, which would be the primary repair of the DNA route to eliminate large DNA damages due to compounds, oxidative stress, and environmental carcinogens[13].

SNPs account for approximately 90% of mutations in the human genome, and research has demonstrated that gene mutations can result in altered repair abilities, which can result in tumors[14].

Ultraviolet light, ionizing radiation, and various chemical variations all have the potential to cause double-strand breaks, DNA single, DNA protein cross-linking, base mismatches, and other genetic deterioration. The gene repair mechanism can correct these DNA errors, thus preserving the genetic information's stability. hMSH2, XPD, XRCC1, hOGG1, and XRCC3 are well-known fix genes[15].

The XPD gene is composed of 23 exons and 6 SNPs. XPD's primary feature is nucleotide excision[16]. Nevertheless, as a transcription factor II D (TFIID) component, XPD can contribute to p53-mediated apoptosis and transcription[17]. A single base change in XPD will alter the protein's function and result in tumorigenesis[18].

Numerous XPD polymorphisms have been found in the coding regions, such as a modification in the amino acid lysine to glutamine in codon 751 (Lys751Gln, rs13181) and a modification in the amino acid aspartic acid to asparagine in codon 312 (Asp312Asn, rs1799793)[19].

The Gln allele of XPD rs13181 and the Asn allele of XPD rs1799793 have been linked to decreased NER ability. Given the critical role of XPD in repairing DNA, the correlation between XPD polymorphisms and tumor threat is especially interesting[20].

We investigate the XPD Lys751Gln gene polymorphism and its interaction with HCC in Egyptian patients in recent research. We discovered a statistically significant rise in the AC genotype among HCC cases as compared to HCV cases. Additionally, when HCC cases were compared to HCV cases, a statistically significant rise in the frequency of combined (AC + CC) genotypes was reported, as was a significant rise in the frequency of the mutant C allele.

EL-Abd et al. found a statistically significant rise in the frequency of combined (AC + CC) genotypes and also the mutant C allele in HCC Egyptian cases when compared to the control group [21].

Prior research done confirmed our findings. They discovered a major rise in the frequency of the CC genotype in HCC cases relative to controls and a major rise in the frequency of the C allele in patients compared to controls[22].

Additionally, another study concurs with our established major connection between XPD Lys751Gln (A/C) genotypes and HCC in aflatoxin-exposed cases. They showed a statistically significant rise in the prevalence of the AC genotype among patients compared to the control group. Additionally, they discovered a substantial rise in the population of participants with the CC genotype as compared to the control group, indicating that populations bearing the XPD codon 751 C allele was at a raised threat of HCC as in comparison to those homozygous for the XPD codon 751 A allele[23].

Some studies found that the XPD rs13181 G allele was linked with a raised incidence of gastric tumor in a southern Chinese individual, using a case-control analysis of 361 patients and 616 controls [24]. Additionally, Jiang et al. discovered a connection between the XPD rs13181 polymorphism and gastric tumor[25].

In comparison, an alternate analysis dismissed to detect a meaningful relation between the HCC susceptibility and XPD Lys751Gln polymorphism, with AA genotypes occurring in 50% of HCC patients, AC genotypes occurring in 38% of HCC patients, and CC genotypes occurring in 12% of HCC patients ($p = 0.61$)[26].

In contrast to our findings, a meta-analysis of seven reports on the XPD Lys751Gln polymorphism found no correlation to HCC threat for all genetic designs. It recommended additional large-scale tests examining gene-gene/gene-environment connections to clarify the discrepancy between our findings and those of other reports[27].

Some reports discovered a major improvement in the survival time for individuals with the CC genotype as compared to those with the AA genotype. Additionally, they found that carriers of the CC genotype had a more positive result and a lower mortality rate than carriers of the AA genotype[8].

Other studies found no connection among the XPD rs13181 and rs1799793 polymorphisms and gastric tumor[28,29, 30].

Additionally, the majority of Caucasian research found no association between these gastric tumors and XPD polymorphisms. As a result, current data produced conflicting findings. Numerous meta-analyses were conducted in an early period to analyze the reported data. A meta-analysis study demonstrates that the XPD rs13181 and rs1799793 polymorphisms are related to a gastric tumor in Chinese but not in Caucasians[31].

Another study proposed that XPD rs1799793 can affect gastric tumor threat but found no relevant associations for XPD rs13181[32]. Another study hypothesized that XPD rs13181 had no effect on the pathogenesis of gastric tumor[33].

Nevertheless, some limitations applied to the recent analysis, including the fact that only Egyptians were involved. Leading to a shortage of study of gene-environment and gene-to-gene interactions because of insufficient original evidence, we cannot determine the genetic polymorphisms of other repairs of DNA genes involved in the NER, like XPC and XPA.

CONCLUSION

We found that XPD Lys751Gln (A/C) polymorphism had a major diagnostic function in the early detection of HCC cases. Additional research is required to confirm our results, using wider sample size and comparing HCV genotype 4 to other genotypes and other ethnic population.

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