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Association of UDP-Glucoronyl Transferase 1-A7 Polymorphism with Hepatocellular Carcinoma and Liver Cirrhosis

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Abstract:

Introduction: One of the most prevalent cancers worldwide is hepatocellular carcinoma (HCC). Enzymes called UDP-glucuronosyltransferases, or UGTs, are present in several living organisms, inclusive of bacteria and humans. Membrane-bound conjugating enzymes, or UGTs, catalyze the relocation of the glucuronic acid group of uridinediphosphoglucuronic acid (UDPGlcA) to the functional group of a specific substrate. This gene codes for many UDP-glucuronosyltransferases and is part of a multigene complex.

Aim of the study: Determine whether hepatocellular cancer and liver cirrhosis are associated with the UDP-Glucoronyl Transferase1-A7 polymorphism at codons 129 and 131.

Subjects and Methods: Seventy patients were enlisted from Cairo University's Kasr El Ainy tropical unit. To extract genomic DNA, whole blood was used as a sample. The real-time PCR method of melting curve analysis was used to identify the genetic variations in UGT1A7. examining codons 129 and 131 for mutations. Primer, surrounding the polymorphisms intrigued in exon 1 of UGT1A7 and fluorescence resonance energy transfer (FRET) probes, were planned based on the detailed nucleotide arrangement (GenBank U39570).

Results: Compared to wild type, there was a high prevalence of heterozygous (1,2) and homozygous (2,2) codon 129,131 in HCC cases (1,1). Liver cirrhosis cases had higher frequencies of heterozygous and homozygous genotypes compared to the wild type, while controls had higher frequencies of the wild type genotype.

Conclusion: The polymorphism at codons 129 and 131 of UGT1A7 has been linked to hepatocellular carcinoma (HCC) and is therefore regarded as a risk factor for both liver cirrhosis and HCC.

Keywords: Hepatocellular carcinoma; liver cirrhosis; Codon 129; codon 130; UGT1A7.

1. Introduction

Globally, hepatocellular carcinoma (HCC) is one of the foremost predominant malignancies. HCC is the fifth most prevalent cancer in the world for men and the ninth most frequent cancer in women, with over 500,000 new cases identified each year. With an extremely bad prognosis, it has become the third most frequent malignancy globally and ranks fourth in terms of cancer-related mortality. Liver cancer is a significant worry for men in the Middle East, particularly in some nations like Saudi Arabia and Egypt. An enzyme superfamily known as the human UDP-glucuronosyltransferases (UGTs) is responsible for the glucuronidation reaction-mediated metabolism of endogenous substances [1, 2].

On chromosome 2q37 lies the locus for the UGT1A gene [2]. Exon 1 of the UGT1A gene is alternatively spliced to produce the four usual exons (exons 2–5) that make up the UGT1 family's thirteen members [4]. Human UGT1A locus gene products express differently depending on the tissue. It has been discovered that

extrahepatic organs such as the human esophagus, stomach, and colon express some genes like the UGT1A7, UGT1A8, and UGT1A10 [5, 6].

Numerous substances that are significant to toxicology and medicine are metabolized by UGT1A7. The variant UGT1A73 allele (Lys129Lys131Arg208) is produced by the three missense variants at codons 129/131 and 208. The additional genetic changes at codons 115 and 139 show up to be less common than the UGT1A73 allele [7]. UGT1A7 has been implicated in the glucuronidation of carcinogens, including heterocyclic amines produced from food and polycyclic aromatic hydrocarbons [6, 8].

The UGT1A73 allele is a gene that increases the likelihood of developing orolaryngeal cancer in studies of disease susceptibility [9] HCC [10], colorectal cancer [11, 12] and pancreatic cancer [13]. The objective of this study was to find the association between the UDP-Glucuronyl Transferase1-A7 polymorphism with hepatocellular carcinoma and liver cirrhosis.

2. Subjects and Methods

2.1. Subjects

Seventy patients recruited from the tropical unit of Kasr El Ainy-Cairo University were incorporated into this study and classified as follows:

- **Group 1:** Thirty HCC patients (mean age: 57 ± 9.0 years) underwent the following procedures: Taking the patient's history, examining them to rule out bilharziasis, having them smoke, and figuring out their gender, Serum samples were taken out and subjected to surface antigen testing for hepatitis B (HbsAg) (negative), Anti-HCV (positive), AFP level, AST, ALT, Albumin, Bilirubin Total, Bilirubin direct, U/S or C.T for diagnosis of primary HCC, based on combination of focal lesions detected by any imaging technique U/S or C.T and or alpha feto-protein (AFP) level > 250 ng/ml for HCC.
- **Group 2:** Twenty patients with cirrhosis, with a mean age of 51 ± 10.9 years, were diagnosed based on their clinical presentation (fatigue, oedema, ascites), laboratory results, or histology. Serum samples were taken and subjected to tests for AFP level with values < 250 ng/ml, hepatitis B surface antigen (HBsAg)

(negative), Anti-HCV (positive), AST, ALT, Albumin, bilirubin total, bilirubin direct, and lastly imaging utilizing U/S or C.T. and endoscopic signs.

- **Group 3:** Twenty healthy Controls (volunteers) mean age 51.8 ± 8.6 years without liver disease with no evidence of hepatocellular carcinoma or cirrhosis either clinically, laboratory (negative) for hepatitis B (HBsAg) and C virus (Anti-HCV) with normal liver functions and by imaging(free from focal lesions and cirrhosis.

2.2. Methods

Sample Collection

3 ml serum samples to measure AFP levels, liver functions, anti-HCV, and HBsAg (AST, ALT, Albumin, Bilirubin total and direct). 2 ml venous blood was withdrawn from subjects under aseptic conditions into a sterile EDTA vacutainer tube. Samples were stored till extraction at -20 °c.

DNA extraction and Real-Time PCR

A sample of whole blood was used to extract genomic DNA. Using real-time PCR (polymerase chain reaction), the

genetic polymorphisms in UGT1A7 were identified by the usage of the high pure PCR template preparation kit, and DNA was withdrawn from whole blood.

Genotyping

Analyzing codons 129 and 131 for transformations. Due to an error within the issued research, primers encompassing the polymorphisms intrigued in exon 1 of UGT1A7 and fluorescence resonance energy transfer (FRET) probes were delivered based on the manufacturer's instructions. At Genome Unit Kasr El Ainy, the reaction mix was to begin with denatured for 12 minutes at 95°C in a computerized thermocycler (Roche light cycler 2.0). It was at that point subjected to 48 cycles of denaturation for 20 seconds at 95°C, annealing for 40 seconds at 56°C, elongation for 90 seconds at 72°C, and a last extension step for two minutes at 72°C. Melting curve analysis using FRET probes in the Light Cycler (Roche Diagnostics, Mannheim, Germany) was used to genotype the UGT1A7 alleles. The sensor probe was utilized to identify the polymorphisms at codons 129 and 131 59-LC 640-TTAAGTATTCTACTAATTTTTTGTCCT T-ph. (LC, Light Cycler Red attached to 59 terminus; ph, phosphate) and 59-

GGATCGAGAAACACTGCATCAAAAC AACTCTCC-FL as the anchor probe (FL, 5,6-carboxyfluorescein attached to 39-O-ribose) [14]. Melting curve studies revealed that the mutant allele generated a more stable duplex than the wild-type allele, leading to the formation of an allele-specific melting curve (N129K/R131K:58°C v 47°C). For analytical melting, the schedule was as follows: 95°C for 60 seconds, 38°C for 40 seconds, and a ramp rate of 0.1°C/s to 75°C.

2.3. Statistical method

Data management and analysis were performed using Microsoft Windows version 15 of SPSS 15.0 (Statistical Package for the Social Science; SPSS Inc., Chicago, IL, USA). The mean + SD was used to present parametric quantitative data. A one-way analysis of variance (ANOVA) and a post-hoc test were used to collate the means of the three groups, while the Student's t-test was employed to collate the means of the two groups. The Kruskal-Wallis and Mann-Whitney tests were used to compare medians for non-parametric quantitative data, which were expressed as the median (25th–75th quartiles). Using the correlation coefficient r —Pearson's for parametric data and Spearman's for non-parametric data—it

was possible to correlate two quantitative variables. The frequency and proportion of the qualitative data were reported. Association between qualitative information

was done utilizing the Chi-square test. Risk estimate was done by odds ratio. The *P*-value was considered significant at 0.05.

3. Results

This study was conducted on 70 cases. 30 cases with HCC with a mean age of 57 ±9.2 years, 20 patients with liver cirrhosis with a mean age of 51 ±11 years and 20 cases as control with a mean age of 51.8 ±8.6 years. Results showed a significant association between codon 129,131 with liver cirrhosis and HCC (*p*

=0.021). Codon 129, 131 frequency of heterozygous (N R/K K) and homozygous (KK) in HCC and liver cirrhosis cases were high compared with the control group. The frequency of wild type in the control group is high compared with HCC and liver cirrhosis (**Table 1**).

Table 1. UGT1A7 Genotype and allele frequency in HCC, liver cirrhosis and control groups.

Variables	HCC (N=30)	Liver cirrhosis (N=20)	Control (N=20)	<i>P</i> -value
Codon 129,131 (NR/KK) Heterozygous mutant	18 (60%)	11 (55%)	8 (40%)	0.021*
(K K) Homozygous mutant	12 (40%)	5 (25%)	3 (15%)	
(N R) Wild	0 (0%)	4 (20%)	9 (45%)	

* significant.

Risk estimation for homozygous (K K) and heterozygous (NR /KK) versus wild type (NR) in HCC and liver cirrhosis cases

was significant (OR =9.667 95 % CI: 1.034-90.412, *p* =0.032) (**Table 2**). The risk estimation for homozygous (K K) and

heterozygous (NR /KK) versus wild type (NR) in HCC and control was significant (OR =9.409, 95% CI: 2.442-36.26, $p =0.001$) (**Table 3**).

Table 2: Risk estimation of codon 129 and 131 genotypes in HCC and liver cirrhosis groups.

Group	HCC	Lc	OR	95 % CI	P-VALUE
Homozygous (KK) and heterozygous (NR/KK)	29 (96.6%)	15 (75%)	9.667	(1.034-90.412)	0.032*
Wild (NR)	1 (3.4%)	5 (25%)			

* significant.

Table 3: Risk estimation of codon 129 and 131 genotypes in HCC and control groups.

Group	HCC	Control	OR	95 % CI	P-VALUE
Homozygous (KK) and heterozygous (NR/KK)	46 (92%)	11 (55%)	9.409	(2.442-36.26)	0.001*
Wild (NR)	4 (8%)	9 (45%)			

* significant.

4. Discussion

Universally, hepatocellular carcinoma is the third most predominant kind of cancer, influencing 12.9 cases per 100,000 males and 4.6 occasions per 10,000 women. Hepatitis B infection (HBV), hepatitis C infection (HCV), liver cirrhosis, and hepatocarcinogenesis are emphatically related epidemiologically. It has moreover been illustrated that chromosomal variations from the norm impact genetic variations within the development of HCC [10].

The human UDP-glucuronosyl-transferases (UGTs) enzyme superfamily employments the glucuronidation reaction to metabolize endogenous compounds such as bilirubin, steroid hormones, and natural carcinogens such as benzo(a)pyrene and nitrosamines special to tobacco [7].

The UGT1A7 gene has nine alleles (UGT1A7*1–UGT1A7*9) that vary at the following positions: Gly115Ser, Asn129Lys,

Arg131Lys, Glu139Asp, and Trp208Arg [15, 16]. The variant allele UGT1A7*3, which is habitually seen within the population (17%) of *3/*3, is made by the three non-synonymous SNPs at codons 129/131 and 208, two of which are closely associated (129 and 131) [1].

Vogel et al. (2010) looked at the relationship between the UGT1A7 polymorphism and HCC in a German population [10]. In that study, individuals with one allele (UGT1A7*3) that has a low detoxifying ability had an increased chance of developing HCC. This conclusion has been validated in two more investigations, one on Taiwanese populations and the other on Japanese populations [17, 18].

The current study aimed to find an association between UDP-glucuronosyltransferase UGT1A7 genetic polymorphisms at codons 129 and 131 with hepatocellular carcinoma and liver cirrhosis. Real-time PCR (polymerase chain reaction) was utilized to identify the genetic variations in UGT1A7. Looking for mutations in codons 131 and 129. Fluorescence resonance energy transfer (FRET) probes and primers surrounding the polymorphisms intrigued in exon 1 of UGT1A7 were employed [19].

The present study showed a significant association between liver cirrhosis, HCC and UGT1A7 polymorphism (codon 129,131). Vogel and colleagues (2010) reported that the control population showed the presence of wild-type alleles in 29 (41%) compared with wild alleles in the control group of our study 6/20 (30%) [10]. In patients with HCC: wild-type alleles were found in only (6.8%) 4/59 of patients compared with wild-type in HCC in our study 2/50 (4%) which is correspondent to our study. Also, in the control group UGT1A7 *1/*2 in 13 (19%) compared with (*1*2) genotype in our study 6/20 (30%), UGT1A7 *2/*2 in 9 (13%) compared with (*2*2) genotype in our study 3/20 (15%), UGT1A7 *3/*3 in 7 (10%) compared with (*3*3) which was only present in cases in our study 6/50 (12%) [10]. Tseng and colleagues (2005) found that UGT1A7*1/*1 for cases 64 (29.5%) and for control 134 (46.0%), (OR: 0.49, 95% CI (0.34-0.71) [18]. UGT1A7*1/*2 for cases 55 (25.3%) and for control 66 (22.7%), (OR: 1.16, 95% CI: 0.77-1.75). UGT1A7*1/*3 for cases 53 (24.4%) and for control 54 (18.6%) (OR: 1.42, 95% CI: 0.92-2.18). UGT1A7*2/*2 for cases 13 (6.0%) and for control 12 (4.1%) (OR: 1.48, 95% CI: 0.66-3.31). UGT1A7 *2/*3 for cases 25 (11.5%) and for

control 19 (6.6%) (OR: 1.86, 95% CI: 1.00-3.48). UGT1A7*3/*3 for cases 7 (3.2%) and for control 6 (2.1%) (OR: 1.58, CI: 0.52-4.78). UGT1A7*1 for cases 172 (79.3%) and for control 254 (87.3%) (OR: 0.56, 95% CI: 0.35-0.90). UGT1A7*2 for cases 93 (42.9%) and for control 97 (33.3%), (OR: 1.50, 95% CI: 1.04-2.16). UGT1A7*3 for cases 85 (39.2%) and for control 79 (27.1%) (OR: 1.73, CI: 1.19-2.52) UGT1A7*2 and *3 alleles were significantly related to HCC development (UGT1A7*2: OR =1.50, 95% CI: 1.04-2.16; UGT1A7*3: OR =1.73, 95% CI: 1.19-2.52), while the UGT1A7*1/*1 was associated with decreased risk of HCC occurrence (OR =0.49, 95% CI: 0.34-0.71), which is matching our study [18]. Wang and colleagues (2004), when compared to the

*1/*1 alleles (genotypes), the frequencies of the UGT1A7*2/*3 and *1/*3 alleles (genotypes) in patients with HCC were found to be higher than those in patients without HCC (4 and 20%, respectively), with ORs of 6.08 (95% CI: 2.22–16.64) and 2.35 (95% CI: 1.26–4.35), respectively. These findings were based on a study conducted on Japanese patients. It's intriguing that the homozygous *2 or *3 allele had a lower OR than the frequency of the *2/*3 allele [17].

Conclusion

The polymorphism at codon 129,131 in UGT1A7 is linked to hepatocellular carcinoma (HCC) and is therefore regarded as a risk factor for both liver cirrhosis and HCC.

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Conflicts of Interest: All authors declare they have no conflicts of interest.

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