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Association of Lymphotoxin-α +252A/G, Tumor Growth Factor-β −509C/T and Tumor Necrosis Factor-α -308A/G Polymorphisms with Hepatocellular Carcinoma.

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Abstract

Background: Hepatocellular carcinoma (HCC) risk factors could either be host-genetic or environmental relation. This study aimed to assess the correlation of the three biallelic polymorphisms of lymphotoxin-alpha (LT-α) +252A/G, tumor growth factor-beta 1 (TGF-β1) −509C/T and tumor necrosis factor-alpha (TNF-α) -308A/G with HCC development regardless chronic hepatitis C (CHC) infection. Methods: These polymorphisms were studied among 220 consecutive participants. They were patients with/without HCV-related HCC, and with HCC related to etiologies other than HCV, in addition to the controls. Results: No significant difference between CHC patients and healthy controls regarding the distribution of genotypes of LTα +252A/G, TGF-β1 −509C/T and TNF-α -308A/G was observed. Regardless CHC infection, LTα GG genotype [29.2%, OR =1.99, 95% CI (0.98-4.07), P=0.039], TGF-β CT/TT genotypes [38.3%, OR =2.08, 95% CI (1.0-4.3), P=0.029], and TNF-α AG genotype [(35%, OR=1.27, 95% CI (1.05-1.54), P=0.028)] were more frequent in HCC compared to Non-HCC patients. Moreover, these genotypes were significantly associated (P<0.05) with HCC severity including tumor late stages, high grades, large tumor size, lymph node invasion and distant metastasis. Conclusion: our results demonstrating that LT-α +252A/G, TGF-β1 −509C/T and TNF-α -308A/G might be risk factors for HCC incidence. LT-α GG genotype, TGF-β CT/TT genotypes and TNF-α AG genotype might be correlated with HCC severity and progression.

Keywords: HCC; SNP; LT-α; TGF-β1; TNF-α

1. Introduction

Based on the World Health Organization reports (WHO), >3% of the world's population is infected with the hepatitis C virus (HCV); among them, >170 million individuals are chronic HCV infection [1]. Particularly, chronic HCV infection represents the 2nd most common cause of cancer-related mortality worldwide [2]. In Egypt, hepatocellular carcinoma (HCC) is the most common tumor in both sexes, and its incidence is elevated to 19.7% of the total malignancy cases [3]. HCC incidence is 15-20 times higher in HCV patients than uninfected individuals and rarely develops in the absence of cirrhosis or significant fibrosis [4, 5].

HCV is a hepatotropic positive-stranded RNA virus in the Flaviviridae family. Uncommon for RNA viruses, it manages to persist in up to 80% of cases, leading to chronic hepatitis C (CHC) with a high risk of developing fibrosis, cirrhosis, and ultimately HCC Although highly effective direct-acting antivirals can eliminate the virus (achieve sustained virological response) and have revolutionized clinical care of patients with CHC in the past decade, still an estimated 57 million people were living with HCV infection in 2020 [6]. Given these numbers, the WHO goal of eliminating HCV as a public health threat by 2030 appears highly ambitious. Annually, 1–4% of cirrhotic CHC patients develop HCC. In 2021, nearly 150 000 persons died owing to HCV-associated HCC, which was the highest number ever recorded [7].

The development of HCV-associated HCC usually occurs over years or even decades of ongoing infection. Regenerative hepatocyte growth is constantly stimulated by immune cells' ongoing removal of infected hepatocytes and by the death of cells in response to viral stress. Oxidative damage exacerbates the innate risk of genetic mutations resulting from incorrect DNA replication [8].

Reactive oxygen species (ROS) are mostly responsible for this; they are either released by the mitochondria of infected hepatocytes or secreted by immune cells. Furthermore, it has been documented that HCV uses the creation of ROS as part of its replication strategy [9].

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Evaluating genetic biomarkers for HCC susceptibility and their application in combination with conventional prognosis, staging, and diagnosis might decrease HCC mortality through patient care and early diagnosis [4]. Single-nucleotide polymorphisms (SNPs) are a tool that is involved in multistage hepatocarcinogenesis and may determine a patient's susceptibility to HCC development. Previous research identified many SNPs that disturb gene expression or function and are involved in HCC susceptibility, which may help to clarify HCC pathophysiologic mechanisms and predict population and individual risk [10].

When T cells are stimulated, LT-α is generated and is involved in several immune-stimulatory, antiviral, and inflammatory responses [11]. It has been linked to CHC pathogenesis as a major modulator of liver fibrogenesis and has the ability to cause cytotoxicity in cancer cells [12]. (A/G) single nucleotide polymorphism (SNP) at position 252 (rs909253) in the LT-α gene's first intron region, where the mutant allele is linked to increased corresponding cytokine expression [13].

The cytokine TGF-β is most commonly upregulated in tumor cells [14]. The identified −509C/T [rs1800469] SNP has been mostly investigated, and although no consistent conclusion has been drawn, several studies have evaluated the correlation of HCC risk with TGF-β1 −509C/T SNP, particularly in patients with chronic hepatitis B [15].

During HCV and HBV infection, circulating TNF-α levels elevate in correlation with the severity of tissue injury, fibrosis, and hepatic inflammation [16]. A biallelic SNP at the 308 position is of particular interest among several biallelic SNPs that have been recognized within the TNF-α gene. TNF-α -308 A>G SNP has been related to several malignant, infectious, autoimmune, and inflammatory diseases [17].

Limited studies have focused on TNF-α -308 A>G SNP association with HCC risk. In the current work, we intended to investigate the association between three biallelic SNPs LT-α +252A/G, TGF-β1 −509C/T, and TNF-α -308A/G and CHC infection. Then, we assumed that these SNPs may raise HCC risk through stimulated hepatic fibrosis, and thus we assessed their association with HCC development regardless of HCV infection.

2. Experimental

2.1. Patients

Two hundred twenty consecutive participants were enrolled in this study and divided into three main groups. Group 1 was 110 CHC patients (60 with and 50 without HCV-related HCC); group 2 was 60 HCC patients related to HCC etiologies other than HCV; and group 3 of 50 unrelated healthy individuals served as a control group; they were negative for HCV antibodies. All patients were recruited from two hospitals, Mansoura Oncology Center and Gastrointestinal Surgery Center, Faculty of Medicine, Mansoura University Hospitals, Mansoura University, from January 2021 to September 2021. The study was approved by the ethics committee, Faculty of Medicine, Ain Shams University, after obtaining informed consent from all the participants (approval code FMASU 1559/2013).

The HCC diagnosis was based on histological and pathological screening together with medical imaging, including CT and/or MRI. Patients with a genetic predisposition and those with end-stage renal failure, autoimmune diseases, diabetes mellitus, HIV infection, or any other malignancy were excluded. HCCs were staged based on the tumor-node-metastasis system (TNM) [18]. With the data confidentiality declaration, informed permission was obtained from each participant.

2.2. Methods

Collection of samples and biochemical analysis

Peripheral blood samples (5.5 ml) were taken from all participants. Each blood sample was divided into two parts: 2.5 mL were placed in EDTA tubes for the CBC and molecular analyses and 3 mL without anticoagulant that was centrifuged (5000 rpm/15 minutes) for the biochemical assays.

The biochemical measurements included serum alanine (ALT) and aspartate (AST) aminotransferase (BIOMED DIAGNOSTICS, Germany), total bilirubin (BioVision, Inc., USA), albumin (BIOMED DIAGNOSTICS, Germany), and creatinine (Abcam, USA) were completed on an automated biochemistry analyzer (Hitachi, Tokyo, Japan). Moreover, serum alpha-fetoprotein (AFP) level (Cat. No. ab193765, Abcam, USA) and serum HCV antibodies (MyBioSource, Inc., CA, USA) were measured by the ELISA kits using an EZ Red 800 microplate reader (Biochrom, USA). Hematological measurement, including hemoglobin, thrombocytes, RBCs, and WBCs counts, was carried out by a fully hematological analyzer (Abbott Diagnostics, USA).

DNA extraction

From 200 µl EDTA preserved whole blood, genomic DNA was isolated by phenol-chloroform and standard protein K digestion methods using DNA mini DNA extraction kit (Cat. No. #51106, QIAGEN, Hilden, Germany). To measure DNA concentration and purity, a NanoDropTM 1000 spectrophotometer was used.

Polymorphism Genotyping

Genotyping of the SNPs of the included genes was carried out by a tetra-primer amplification refractory mutation system PCR (T-ARMS-PCR) according to the method reported by Alhelf et al. [19]. Primers sequences were presented in (Table S1). Each PCR reaction (25 µl) contains [0.5 units of AmpliTaq Gold (Perkin-Elmer, USA), PCR buffer with 15 mM MgCl2, 2.5 µl

of GeneAmp 10X, 12.5 pmol of each primer, and 0.18 mM dNTPs]. The thermal cycler involved initial denaturation (95°C, 10 minutes), followed by 30 cycles including denaturation step (95°C, 1 minute), annealing step (64°C, 1 minute), and extension step (72°C, 1 minute). The final extension step was completed for 5 minutes at 72°C. PCR products were analyzed by 2% agarose gel electrophoresis and, under ultraviolet illumination (UVP Visi-BlueTm Transilluminators, Analytik Jena). DNA appeared as yellow/orange fluorescent bands were visualized using ethidium bromide.

2.3. Statistical analysis

The SPSS (SPSS Inc., Chicago, IL) was used for all statistical analyses. Variables were expressed as median (IQR) or means ± SD, appropriately. ANOVA and Kruskal-Wallis tests were used to compare quantitative variables between groups that were normally and non-normally distributed, respectively. Allele and genotype frequencies of genes were compared between cases and controls using the chi-squared test. Logistic regression analysis was used to determine the odds ratio (OR) and 95% confidence intervals (CI) of HCC risk and tumor severity features. The significance level was at $P<0.05$.

3. Results and Discussion

Results

The **(Table 1.a)** displayed the clinical and laboratory characteristics of HCC patients versus Non-HCC patients**.** Patients with HCC were associated with significant had increased serum bilirubin, AFP, and WBCs count and decreased serum albumin, hemoglobin, and platelet count compared to non-HCC patients ($p<0.05$). Among HCC patients, HCV-infected patients were associated with only a significant decrease ($P=0.0001$) in serum albumin compared to non-infected patients **(Table 1.b).**

HCC patients were classified according to tumor features, including tumor stage, grade, size, lymph node invasion, and distant metastasis, as showed in **(Table 2).**

Table 1.a. Clinical and laboratory characteristics of HCC patients versus Non-HCC patients

Variable	Non-HCC (n=50)	HCC (n=120)	*P* value
Age (years)	56.2±9.2	58.4±10.1	0.225
Gender (male/female)	35/15	86/34	0.169
AST (U/L)	56.5 (46.8-84.3)	61.5 (37-131.3)	0.141
ALT (U/L)	43 (34-50.3)	38.5 (27.3-84)	0.113
Bilirubin (mg/dL)	1.02 (0.7-1.4)	2 (1.2-4.97)	0.0001
Albumin (g/dL)	3.9±0.6	2.9±0.8	0.0001
Creatinine (mg/dL)	1.18±0.39	1.27±0.49	0.487
AFP (IU/ml)	5.2 (2.4-13.3)	53.5 (10.2-297)	0.0001
INR	1.22±0.22	1.26±0.33	0.554
Hemoglobin (g/dL)	13.6±1.9	11.2±1.8	0.0001
RBCs (10^6/mm^3)	5.0 ± 0.4	3.9 ± 0.6	0.154
WBCs (10^3/mm^3)	5.2±1.1	8.2±1.2	0.006
Platelets (10^3/mm^3)	198±32.3	110.0±30.1	0.0001

Table 1.b. Clinical and laboratory characteristics of HCV-infected patients versus HCV-non infected patients

Variable	HCV-infected	HCV-non infected	*P* value
Age (years)	58±9.4	60±12.1	0.225
Gender (male/female)	41/19	40/20	0.269
AST (U/L)	61.4 (46.8-130.5)	61.2 (37-131.1)	0.159
ALT (U/L)	42 (34-51.1)	40.5 (28.3-85)	0.119
Bilirubin (mg/dL)	1.9 (1.1-5.0)	1.8 (1.2-4.97)	0.091
Albumin (g/dL)	2.7±0.7	3.1±0.9	0.0001
Creatinine (mg/dL)	1.29±0.49	1.21±0.39	0.487
AFP (IU/ml)	54.5 (11.1-251.3)	44.9 (6.7-338.8)	0.213
INR	1.27±0.4	1.26±0.3	0.554
Hemoglobin (g/dL)	11.2±1.8	12.0±2.1	0.123
RBCs (10^6/mm^3)	3.9 ± 0.6	4.0 ± 0.7	0.119
WBCs (10^3/mm^3)	8.1±1.7	7.5±1.3	0.092
Platelets (10^3/mm^3)	109.9±35.2	120±32.1	0.173

Table 2. Classification of included HCC patients according to tumor stage, grade, size, lymph node invasion and distant metastasis.

Variable	Number (%)
Tumor stage	
Early (T1-T2)	**76 (63.3%)**
Late (T3-T4)	**44 (36.7%)**
Histological grade	
Low (G1-2)	**73 (60.8%)**
High (G3)	**47 (39.2%)**
Tumor size	
Small (<2.5 cm)	**40 (33.3%)**
Large (>2.5 cm)	**80 (66.4%)**
Lymph nodes invasion	
Negative	**75 (62.5%)**
Positive	**45 (37.5%)**
Distant metastasis	
Negative	**94 (78.3%)**
Positive	**26 (21.7%)**

As shown in **(Table 3),** the LT-α +252 AG genotype was predominant in all groups. In CHC patients, there was no AA genotype, while AG genotype was more frequent (80%) than in the healthy group (50%) without significant difference (P=0.06). In HCC patients GG genotype was more frequent than in the non-HCC group (29% vs. 14%), [OR =1.99, 95% CI (0.98-4.07), P=0.039].

Table 3. Association between LT-α +252 A>G genotypes and CHC progression

Polymorphism	Healthy	CHC	Non-HCC	HCC	*P* value
LT-α +252 A>G					
AA	7 (14%)	0 (0%)	0 (0%)	0 (0%)	
AG	25 (50%)	88 (80%)	43 (86%)	85 (70.8%)	0.061* 0.039**
GG	18 (36%)	22 (20%)	7 (14%)	35 (29.2%)	
Allele frequency					
A	39 (0.39)	88 (0.40)	43 (0.43)	85 (0.35)	0.912*
G	61 (0.61)	132 (0.60)	57 (0.57)	155 (0.65)	0.045**

Lymphotoxin-alpha,; CHC: chronic hepatitis C; HCC: hepatocellular carcinoma. * *P* value between CHC and healthy controls; ** *P* value between HCC and Non-HCC patients. *P*<0.05 was significant.

Regarding TGF-β-509 C/T SNPs, CC genotype was predominant in all groups. In healthy individuals, there was no TT genotype, while there were 5 patients in CHC with TT genotype, and all of them were HCC patients. No significant difference between CHC patients and healthy controls was observed. In HCC patients, CT genotype was more frequent than in the non-HCC group (38.3% vs. 16%), [OR=2.08, 95% CI (1.0-4.3), P=0.029], **(Table 4).**

Similarly, the TNF-α-308 GG genotype was predominant in all groups. In CHC, there was no AA genotype, while there was only 1 healthy individual with the AA genotype. No significant difference was noticed between CHC patients and healthy controls (P=0.188). In HCC patients, AG genotype was more frequent than in non-HCC group (35% vs. 14%), [OR=1.27, 95% CI (1.05-1.540), P=0.028], **(Table 5).**

Table 4. Association between TGF-β -509 C/T genotypes and CHC progression

Polymorphism	Healthy	CHC	Non-HCC	HCC	*P* value
TGF-β -509 C/T					
CC	24 (48%)	68 (61.8)	37 (74%)	69 (57.5%)	
CT	26 (52%)	37 (33.6%)	13 (16%)	46 (38.3%)	0.091*
TT	0 (0%)	5 (4.6%)	0 (0%)	5 (4.2%)	0.029**
Allele frequency					
C	74 (0.74)	173 (0.79)	87 (0.87)	184 (0.77)	0.855*
T	26 (0.26)	47 (0.21)	13 (0.13)	56 (0.23)	0.052**

TGF: Transforming growth factor; CHC: chronic hepatitis C; HCC: hepatocellular carcinoma. * *P* value between CHC and healthy controls; ** *P* value between HCC and Non-HCC patients. *P*<0.05 was significant.

Table 5. Association between TNF-α -308 A>G genotypes and CHC progression

Polymorphism	Healthy	CHC	Non-HCC	HCC	*P* value
TNF-α -308 A>G					
AA	1 (2%)	0 (0%)	0 (0%)	0 (0%)	0.188*
AG	9 (18%)	32 (29.1%)	7 (14%)	42 (35%)	
GG	40 (80%)	78 (70.9%)	43 (86%)	78 (65%)	0.028**
Allele frequency					
A	11 (0.11)	32 (0.14)	7 (0.07)	42 (0.17)	0.812*
G	89 (0.89)	188 (0.86)	93 (0.93)	198 (0.83)	
					0.055**

TNF: Tumor necrosis factor; CHC: chronic hepatitis C; HCC: hepatocellular carcinoma. * *P* value between CHC and healthy controls; ** *P* value between HCC and Non-HCC patients. *P*<0.05 was significant.

Findings revealed that LT-α +252 GG genotype **(Table 6)** and TGF-β -509 CT/TT genotypes **(Table 7)** were associated with HCC severity including tumor late stage, high grade, large size, lymph node invasion and distant metastasis. Also, TNF-α -308 AG genotype was associated with tumor late stage and high grades **(Table 8).**

Table 6. Association between LT-α +252 A>G genotypes and HCC severity

Classification	Number	LT-α +252 A>G		*P* value	OR (95%CI)
		AG n (%)	GG n (%)		
Tumor stage					
Early (T1-T2)	76	67 (88.2)	9 (11.8)	0.0001	2.9 (1.7-5.2)
Late (T3-T4)	44	19 (43.2)	25 (56.8)		
Histological grade					
Low (G1-2)	73	63 (86.3)	10 (13.7)	0.0001	2.2 (1.3-3.6)
High (G3)	47	23 (48.9)	24 (51.1)		
Tumor size					
Small (<2.5 cm)	40	34 (85)	6 (15)	0.030	2.23 (1.03-4.8)
Large (>2.5 cm)	80	52 (35)	28 (65)		
Lymph nodes invasion					
Negative	75	64 (82.7)	11 (14.7)	0.0001	2.03 (1.3-3.2)
Positive	45	22 (48.9)	23 (51.1)		
Distant metastasis					
Negative	94	74 (78.7)	20 (21.3)	0.008	1.37 (1.04-1.8)
Positive	26	12 (44.0)	14 (56.0)		

Table 7. Association between TGF-β -509 C/T genotypes and HCC severity

Classification	Number	TNF-α -308 A>G		*P* value	OR (95%CI)
		AG n (%)	GG n (%)		
Tumor stage					
Early (T1-T2)	76	22 (28.9)	54 (71.1)	0.043	1.66 (1.03-2.7)
Late (T3-T4)	44	20 (45.5)	24 (54.5)		
Histological grade					
Low (G1)	73	20 (27.4)	53 (72.6)	0.029	1.7 (1.1-2.7)
High (G2-3)	47	22 (46.8)	25 (53.2)		
Tumor size					
Small (<2.5 cm)	40	12 (30)	28 (70)	0.309	1.2 (0.9-1.5)
Large (>2.5 cm)	80	30 (37.5)	50 (62.5)		
Lymph nodes invasion					
Negative	75	23 (30.7)	52 (69.3)	0.226	0.81 (0.59-1.1)
Positive	45	19 (42.2)	26 (57.8)		
Distant metastasis					
Negative	94	30 (31.9)	64 (68.1)	0.145	0.86 (0.69-1.1)
Positive	26	12 (46.2)	14 (53.8)		

Table 8. Association between TNF-α -308 A>G genotypes and HCC severity

Classification	Number	TGF-β -509 C/T		*P* value	OR (95%CI)
		CC n (%)	CT/TT n (%)		
Tumor stage					
Early (T1-T2)	76	54 (71.1)	22 (28.9)	0.0001	1.82 (1.29-2.57)
Late (T3-T4)	44	15 (34.1)	29 (65.9)		
Histological grade					
Low (G1-2)	73	50 (68.5)	23 (31.5)	0.002	1.7 (1.2-2.4)
High (G3)	47	19 (40.4)	28 (59.6)		
Tumor size					
Small (<2.5 cm)	40	29 (72.5)	11 (27.5)	0.041	1.8 (1.02-3.3)
Large (>2.5 cm)	80	40 (50)	40 (50)		
Lymph nodes invasion					
Negative	75	51 (68)	26 (32)	0.019	1.45 (1.05-1.99)
Positive	45	20 (44.4)	25 (55.6)		
Distant metastasis					
Negative	94	59 (62.8)	35 (37.2)	0.036	1.23 (1.0-1.5)
Positive	26	10 (38.5)	16 (61.5)		

Discussion

As a multifactorial and complex process, both environmental and genetic factors influence liver pathogenesis, associating carcinogenesis [20]. Identifying and evaluating these factors could help understand several pathways involved in liver carcinogenesis; this may improve screening strategies for high-risk individuals [21]. Liver illness severity, malignant transformation, tumor growth, and predisposition to risk factors have all been associated with constitutional SNPs [22].

This study aimed to evaluate the association between biallelic SNPs of three genes LT-α +252A/G and TGF-β1 −509C/T in TNF-α -308A/G in CHC infection patients. These genes are implicated in hepatic fibrogenesis and may enhance HCC risk; thus, we evaluated their association with HCC development regardless of HCV infection. The main findings of our study were no detectable differences between CHC patients and healthy controls regarding the distribution of genotypes of the studied SNPs. Regardless of CHC infection, LT-α GG genotype, TGF-β CT/TT genotype, and TNF-α AG genotype were more frequent ($P<0.05$) in HCC compared to non-HCC patients. Moreover, these genotypes were associated with HCC severity, including tumor late stages, high grades, large tumor size, lymph node invasion, and distant metastasis.

Circulating levels of different cytokines were elevated during chronic viral infection [23, 24]. There is increased expression of hepatic protein and mRNA of LT-α and TNF-α in HBV-related HCC [25]. However, HCC cells produce LT-α and TNF-α, which are correlated with tissue injury and liver inflammation severity [26, 27]. According to these findings, it is believable to conjecture that the variant genotypes could be linked to elevated TNF-α activity and/or blood cytokine levels. Therefore, these SNP variations may be a causative risk factor for HCC and/or progressive fibrosis [28].

Our results are consistent with previous studies that these genes, SNPs, and genotypes are involved in hepatic carcinogenesis. In a similar population of our study, the LT-α +252 AG genotype was predominant in all groups [23]. Tsai et al. found that there are additive and independent interactions between chronic HBV-related HCC risk and LT-α +252 GG genotype. This genotype was related to higher liver damage and advanced fibrosis [28]. Galal et al. discovered that the LT-α GG genotype in CHC patients may be useful in identifying people who are at a high risk of developing HCC [23]. They also found that GG genotype was more frequent in CHC and controls as well as in HCC than liver cirrhosis. There was a greater association between HCC child classes and models of end-stage liver disease (MELD) scores and LTα genotypes than other genotypes [24].

Growing evidence demonstrated that TNF-α SNPs play pivotal roles in viral hepatitis related hepatocarcinogenesis and immunopathogenesis [24, 29]. Similar to our results, Talaat et al. revealed that TNF-α-308 SNP might be an independent HCC risk factor rather than a host genetic component linked to CHC [30].

In about 23 articles involving 3237 HCC patients and 4843 controls, a meta-analysis examining the relationship between five distinct SNPs of TNF-α and HCC risk was conducted by Wungu et al. They discovered that SNP − 308 G/A (AA vs. GG, OR=3.14) was associated with a significantly enhanced risk of HCC [31]. Also, Feng et al. found that AA genotype was significantly related to elevated HCC risk (OR=5.12) and AG/AA genotypes revealed 5.59-fold elevated HCC risk [32].

Jeng et al. discovered that the G-allele TNF-α 308 was an independent risk factor for HCC. They discovered that TNF-α-308 G-allele carriage is correlated with higher tumor grade and advanced clinical stage of HCC, which is similar to our findings [33].

In experimental models, strategies aimed at disrupting signaling pathways and/or synthesis of TGF-β1 significantly reduced hepatic fibrosis and, therefore, may reduce subsequent HCC risk [34]. In CHC patients, it has been reported that viral core protein stimulates TGF-β1 transcription, thus enhancing liver damage progression, including the occurrence of HCC [35]. The TGF-β signaling pathway shift from tumor suppression to fibrogenesis in liver disease is influenced by changes in TGF-β synthesis and the maintenance of chronic inflammation, which accelerates hepatic damage and raises the risk of HCC [34]. Up to now, limited studies have evaluated the correlation of TGF-β1-509C/T SNPs with the risk of HCC [36]. Similar to our findings, other research revealed a link between the development of HCC and TGF-β1 −509 T allele carriage [37].

The correlation of TGF-β1 different SNPs and HCC risk was examined in 14 studies, which were included in a meta-analysis by Guo et al. They discovered a significant association (P=0.0007) between the −509C/T SNP and HCC risk (OR=1.4) [38].

4. Conclusion

Finally, this study shows that LT-α +252A/G, TGF-β1 −509C/T, and TNF-α -308A/G may be linked to an increased risk of getting HCC. LT-α GG genotype, TGF-β CT/TT genotypes and TNF-α AG genotype might be correlated with HCC severity and progression. The main limitation of our study is the relatively small sample size; therefore, further investigations recruiting larger samples and considering gene-gene and gene-environmental interactions are required to verify this association and explore the underlying mechanisms of the involvement of these SNPs in HCC progression and to make better correlations between the studied SNPs and the different stratified groups of the disease.

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