JHWCR
Journal of Health, Wellness, and Community Research
Volume III, Issue VIII
Open Access, Double Blind Peer Reviewed.
Web: https://jhwcr.com, **ISSN**: 3007-0570
https://doi.org/10.61919/vv96gh29

Article

Single Nucleotide Polymorphisms in TGF-β1 and Their Association with HCC Development in HCV Patients

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Cite this Article

Received	2025-05-28
Revised	2025-06-11
Accepted	2025-06-14
Published	2025-07-02

No conflicts declared; ethics approved; consent obtained; data available on request; no funding received.

Authors' Contributions
Concept: AS, RA; Design: AH, MA; Data Collection: KB, RT; Analysis: NA; Drafting: SJ

ABSTRACT

Background: Hepatocellular carcinoma (HCC) is a leading cause of cancer-related mortality globally, with chronic hepatitis C virus (HCV) infection serving as a principal risk factor. Genetic polymorphisms in cytokine genes such as transforming growth factor beta 1 (TGF-β1) may modulate susceptibility to HCC by influencing the hepatic microenvironment and carcinogenic progression. **Objective**: To investigate the association between the TGF-β1 -509 C/T promoter polymorphism and the risk of HCC among chronic HCV patients in a Pakistani population. **Methods**: This comparative study enrolled 80 adult patients from Sheikh Zayed Hospital, Lahore, including 40 with chronic HCV infection without HCC and 40 with HCC secondary to chronic HCV. Genomic DNA was extracted from whole blood samples and genotyped for TGF-β1 -509 C/T using PCR-RFLP. Demographic, clinical, and laboratory data were collected, and genotype distributions were compared between groups. Statistical analyses included chi-square testing, odds ratio calculation, and logistic regression to assess associations and control for confounders. **Results**: The TT genotype and T allele of TGF-β1 -509 were more frequent in HCC patients compared to those with HCV alone. The TT genotype was associated with an increased, but not statistically significant, risk of HCC (OR 2.51; 95% CI 0.79–8.03; p=0.120). Clinical parameters such as tumor size and serum AFP were higher in TT and CT carriers, suggesting a trend toward more aggressive disease. **Conclusion**: While the TGF-β1 -509 TT genotype and T allele showed higher prevalence and risk estimates in HCC patients with chronic HCV, statistical significance was not reached. These findings suggest a possible genetic contribution to HCC susceptibility in this population, meriting further investigation in larger cohorts.

Keywords: TGF-β1, Hepatocellular Carcinoma, Hepatitis C Virus, Gene Polymorphism, Pakistan, Tumor Markers

INTRODUCTION

Cancer remains a leading cause of morbidity and mortality globally, accounting for approximately 8.2 million deaths in 2012, with hepatocellular carcinoma (HCC) recognized as the second most common cause of cancer-related mortality, resulting in 745,000 deaths in that year alone (1,2,3). Hepatitis C virus (HCV) infection is a principal etiological agent underlying both acute and chronic hepatitis, and is a major contributor to chronic liver diseases such as fibrosis, cirrhosis, and ultimately HCC (4,5). Epidemiological studies estimate that 130 to 210 million individuals worldwide are affected by HCV, comprising about 3% of the global population, with approximately 170 million reported cases and an annual mortality of 500,000–1,000,000 deaths attributable to HCV and its sequelae (6,7,8). The virus is classified within the genus Hepacivirus and the family Flaviviridae and exclusively infects humans and chimpanzees (9). Importantly, the HCV genome lacks reverse transcriptase, precluding integration into the host genome, and therefore, the pathogenesis of HCC related to HCV is not attributable to insertional mutagenesis but rather to chronic inflammation, immune dysregulation, and direct cytopathic effects (10). The heterogeneity of HCV is highlighted by the identification of at least six major genotypes and more than 70 subtypes globally, each exhibiting unique geographic distributions and clinical implications (11,7). In Pakistan, genotype 3a is predominant, accounting for over half of HCV infections, while genotypes 1a, 3b, and mixed types follow in prevalence (12,13). Persistent infection with genotype 3a has been strongly associated with HCC development in the Pakistani population (14). The clinical outcomes of HCV infection are highly variable and influenced by a complex interplay of viral, host, and

environmental factors, including genetic susceptibility, immune response, and exposure to hepatotoxins (15). Growing evidence from genome-wide association studies underscores the role of host genetic factors, particularly single nucleotide polymorphisms (SNPs) in cytokine genes, in modulating susceptibility to chronic infection, response to antiviral therapy, and the risk of progression to HCC (16).

Cytokines are pivotal mediators in the host defense against viral infections and in the regulation of innate and adaptive immunity (17). Among these, transforming growth factor beta 1 (TGF-β1) is a multifunctional cytokine involved in cellular proliferation, differentiation, extracellular matrix production, angiogenesis, immune regulation, and tissue repair (18,19). In the early stages of carcinogenesis, TGF-β1 may function as a tumor suppressor by inhibiting cell proliferation and inducing apoptosis, but in later stages, it can facilitate tumor progression by promoting epithelial-to-mesenchymal transition, immune evasion, and angiogenesis (20,21,22). Genetic polymorphisms in the TGF-β1 gene, located on chromosome 19q13, have been implicated in inter-individual variability in cytokine expression and activity, potentially affecting cancer risk and clinical outcomes (23). Several SNPs within the TGF-β1 gene have been described, including +915 (Arg/Pro), +988 (C/A), -800 (G/A), and -509 (C/T), with the -509 C/T variant located in the promoter region at a key regulatory site for gene transcription (24,25). This specific polymorphism, found at the Yin-Yang1 consensus binding site, has attracted particular interest due to its reported association with altered TGF-β1 production and disease susceptibility (26). Previous studies investigating the relationship between the TGF-β1 -509 C/T polymorphism and HCC risk in the context of chronic viral hepatitis have yielded inconsistent results across different populations and settings (27,28,29). While some have reported a significant association between the T allele and increased HCC risk, others have found no such relationship or have even implicated the C allele (30-32). These discrepancies may reflect differences in ethnic background, sample size, study design, or the interaction of additional genetic and environmental risk factors (32).

In Pakistan, the burden of HCV-related HCC is particularly high, yet there is a paucity of data on the genetic determinants that may predispose infected individuals to malignant transformation. This knowledge gap hampers the development of effective screening, prognostication, and targeted prevention strategies in the local context. The current study seeks to address this gap by evaluating the association between the TGF-β1 -509 C/T promoter polymorphism and the development of HCC in chronic HCV patients drawn from a Pakistani population. We hypothesize that the presence of the T allele at position -509 of the TGF-β1 gene promoter is associated with increased susceptibility to HCC among HCV-infected individuals. The objective of this research is to compare the frequency of TGF-β1 -509 C/T genotypes in HCV patients with and without HCC, thereby contributing new insights into the genetic risk profile of HCC in the setting of chronic HCV infection in Pakistan.

MATERIALS AND METHODS

This comparative study was conducted to investigate the association between TGF-β1 -509 C/T polymorphism and the development of hepatocellular carcinoma (HCC) among patients with chronic hepatitis C virus (HCV) infection. The research was carried out at Sheikh Zayed Hospital, Lahore, Pakistan, from January 2022 to December 2022. The study population comprised adult patients (aged 18 years and above) diagnosed with chronic HCV infection, with or without HCC, attending the hospital during the study period. Eligible participants included those with a confirmed diagnosis of chronic HCV infection based on serological and molecular criteria. For the HCC group, a diagnosis of HCC was established through imaging modalities such as abdominal computed tomography (CT) or ultrasound, and, when available, corroborated by clinical and laboratory findings. Patients with co-infection with hepatitis B virus, autoimmune hepatitis, or known history of other primary malignancies were excluded, as were those unwilling or unable to provide informed consent.

Participants were recruited consecutively during routine clinical visits or admissions. The research team approached eligible individuals, provided detailed information about the study purpose, procedures, risks, and benefits, and obtained written informed consent prior to enrollment. Confidentiality of all participant data was maintained throughout, with unique identifiers used in place of personal information to protect privacy. The study protocol received approval from the University of Health Sciences Ethics Committee, and all procedures adhered to the Declaration of Helsinki. Data protection protocols were strictly observed to ensure compliance with ethical standards. A total of 80 patients were enrolled, with 40 participants each in the chronic HCV group (without HCC) and the HCC group (chronic HCV with HCC). This sample size was determined based on prior studies indicating detectable differences in genotype frequencies and to achieve adequate statistical power for comparative analysis (1).

Table 1: Primer Sequences for TGF-β1 -509 C/T Genotyping

Primer	Sequence
Forward Primer	5'-CCCGGCTCCATTTCCAGGTG-3'
Reverse Primer	5'-GGTCACCAGAGAAAGAGGAC-3'

Data collection included demographic information, clinical history, and relevant laboratory parameters. Information on age, gender, smoking status, alcohol use, and comorbidities was obtained via structured interviews and review of medical records. Venous blood samples were collected in EDTA-anticoagulated tubes for subsequent genomic and biochemical analysis. For laboratory investigation, genomic DNA was extracted from whole blood using the phenol-chloroform method. DNA quality and concentration were assessed using spectrophotometry, and samples were stored at -20°C until analysis. Genotyping of TGF-β1 -509 C/T (rs1800469) was performed using polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP). Amplification was carried

out using validated forward (5′ -CCCGGCTCCATTTCCAGGTG-3′) and reverse (5′ -GGTCACCAGAGAAAGAGGAC-3′) primers. Each PCR reaction (10 µL) contained 2 µL diluted DNA (25 ng/µL), 10× Taq buffer, 1.5 mM $MgCl_2$, 0.4 µL dNTPs (100 µM each), 5 nM of each primer, and 1 U Taq DNA polymerase. The cycling protocol included initial denaturation at 94°C for 4 minutes, 30 cycles of denaturation at 94°C for 30 seconds, annealing at 58°C for 30 seconds, extension at 72°C for 40 seconds, and a final extension at 72°C for 5 minutes. PCR products (808 bp) were digested with Eco81I (SauI) and separated by agarose gel electrophoresis.

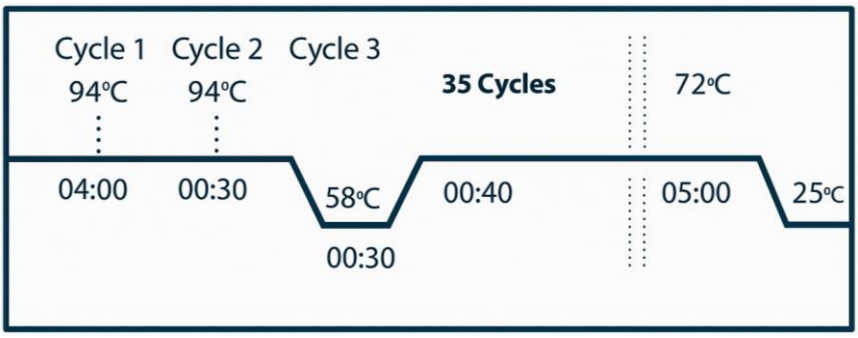

Figure 1 Thermal Cycling Protocol

The TT genotype produced an undigested 808 bp band, the CC genotype generated two fragments (617 bp and 191 bp), and the CT genotype yielded all three bands, allowing for definitive genotype determination. Biochemical parameters such as serum ALT, AST, albumin, total protein, bilirubin, creatinine, and alpha-fetoprotein (AFP) were measured using automated analyzers according to manufacturer protocols and validated reference ranges. Tumor characteristics, including size, presence of metastases, and portal vein thrombosis, were documented for HCC patients based on imaging and clinical data. Ascites grading was performed using clinical and ultrasonographic assessment. All variables and outcome measures were defined a priori. TGF-β1-509 genotypes (TT, CT, CC) were the primary exposure variable, with the presence of HCC as the main outcome. Other variables such as age, sex, smoking, alcohol use, and biochemical indices were treated as covariates or potential confounders. Several measures were implemented to minimize bias and confounding. Eligibility criteria excluded patients with other known causes of liver disease, and the same diagnostic and laboratory protocols were used for all participants. Data collectors were trained and standardized procedures were followed for interviews, sample handling, and laboratory analysis to reduce measurement variability. The use of consecutive sampling aimed to limit selection bias, and blinding of laboratory personnel to patient group allocation helped minimize detection bias.

Sample size was calculated to ensure at least 80% power to detect differences in genotype distribution between groups at a significant level of 0.05, based on genotype frequencies reported in similar regional studies (2). Data were entered and managed in a secure database, with double entry verification to enhance data integrity and reproducibility. Statistical analysis was performed using SPSS version 20.0. Categorical variables were summarized as frequencies and percentages, and continuous variables as means with standard deviations or medians with interquartile ranges, as appropriate. Hardy-Weinberg equilibrium was assessed for genotype frequencies. Comparisons between groups were made using chi-square or Fisher's exact tests for categorical variables and t-tests or Mann-Whitney U tests for continuous variables. Odds ratios (ORs) and 95% confidence intervals (CIs) were calculated to assess the association between TGF-β1 genotypes and HCC risk. Adjustment for potential confounders was carried out using logistic regression models, incorporating age, sex, and relevant covariates. Subgroup analyses were conducted for variables such as ascites and tumor size. Missing data was managed through complete case analysis, with the number and pattern of missing observations reported. The research team ensured data reproducibility by thorough documentation of protocols, regular calibration of instruments, and periodic audit of laboratory and data management procedures.

RESULTS

The study enrolled a total of 80 participants, comprising two equal groups of 40 each: one group with chronic hepatitis C virus (HCV) infection without hepatocellular carcinoma (HCC) and the other with chronic HCV infection complicated by HCC. The mean age of participants in the HCV-only group was 51.9 years (±10.7), whereas the HCC group was significantly older, with a mean age of 59.3 years (±10.3), a difference that reached statistical significance (p=0.002). The gender distribution revealed that males accounted for 62.5% of the HCV-only group and 52.5% of the HCC group, while females comprised 37.5% and 47.5%, respectively; however, this difference was not statistically significant (p=0.366; OR 1.48, 95% CI 0.61–3.62). Among behavioral factors, 46.9% of the HCV group and 53.1% of the HCC group reported smoking (p=0.648; OR 0.79, 95% CI 0.31–1.98). Alcohol use was reported by 60% of HCV patients and 40% of HCC patients, with no statistically significant difference between groups (p=0.420; OR 1.98, 95% CI 0.62–6.37).

Analysis of laboratory parameters demonstrated that the HCC group had substantially higher mean alanine aminotransferase (ALT) and aspartate aminotransferase (AST) levels compared to the HCV group. Specifically, the mean ALT in the HCC group was 215 U/L (±90.5), whereas it was 159 U/L (±87.9) in the HCV group (p=0.006). The mean AST was also elevated in HCC patients at 154 U/L (±74.4), compared to 121 U/L (±58.8) in the HCV group (p=0.028). Serum albumin levels, presented as medians with interquartile ranges, were 2.0 g/dL (1.5–2.8) in the HCV group and 2.0 g/dL (1.0–2.5) in the HCC group, with no significant difference (p=0.196). Notably, total protein levels were marginally higher in the HCV group at 4.72 g/dL (±1.24) compared to 4.66 g/dL (±2.29) in the HCC group, with a

highly significant p-value (p=0.000). Total bilirubin levels did not differ significantly, with medians of 3.3 µM (2.0–7.0) and 4.0 µM (3.0–6.0) in the HCV and HCC groups, respectively (p=0.334). Serum creatinine was significantly higher in the HCC group, with a median of 4.0 mg/dL (3.0–6.0), compared to 2.0 mg/dL (1.1–4.0) in the HCV group (p=0.000). Furthermore, alpha-fetoprotein (AFP), a tumor marker, was markedly elevated in HCC patients, with a mean value of 3210 µg/L (±6240), while the HCV group had a mean AFP of just 1.49 µg/L (±0.53) (p=0.000). Assessment of TGF-β1 -509 C/T genotype distribution revealed notable differences between groups. Among HCV-only patients, 13 (59.1%) carried the CC genotype, 16 (53.3%) the CT genotype, and 11 (39.3%) the TT genotype. In contrast, among HCC patients, the proportions were 9 (40.9%) for CC, 14 (46.7%) for CT, and 17 (60.7%) for TT. Compared to the CC genotype, the TT genotype was associated with higher odds of HCC (OR 2.51, 95% CI 0.79–8.03), though this did not reach statistical significance (p=0.120). The CT genotype showed an OR of 1.42 (95% CI 0.46–4.43, p=0.544) compared to CC, also not statistically significant.

The analysis of clinical associations in HCC patients revealed no statistically significant link between ascites severity and TGF-β1 genotype (p=0.937). Among the 40 HCC cases, 21 exhibited mild ascites, of whom 4 were CC, 9 TT, and 8 CT; moderate ascites was present in 14 patients (3 CC, 6 TT, 5 CT), and severe ascites in 3 patients (1 for each genotype), while only 2 had no ascites (1 CC, 1 TT). Tumor size stratified by genotype showed that the mean tumor size was 4.06 cm (±0.77) for CC, 4.13 cm (±0.86) for TT, and 4.40 cm (±1.15) for CT, with no significant difference detected across genotypes (p=0.637). Overall, while age, certain biochemical parameters (ALT, AST, creatinine, total protein, and AFP), and the prevalence of the TT genotype were higher in HCC patients than in those with HCV alone, the associations between TGF-β1 -509 genotypes and the risk or clinical features of HCC did not reach statistical significance in this sample. These results suggest trends that warrant further investigation in larger cohorts to clarify the potential genetic contributions to HCC susceptibility among HCV-infected individuals.

Table 2. Demographic Characteristics of Study Participants (Chronic HCV Without HCC vs. HCV With HCC)

Variable	Chronic HCV (n=40)	HCC + HCV (n=40)	p-value	Inferential Statistic
Age (years)	51.9 ± 10.7	59.3 ± 10.3	0.002*	-
Male, n (%)	25 (62.5%)	21 (52.5%)	0.366	OR 1.48 (0.61–3.62)
Female, n (%)	15 (37.5%)	19 (47.5%)		
Smoking, n (%)	15 (46.9%)	17 (53.1%)	0.648	OR 0.79 (0.31–1.98)
Alcohol use, n (%)	9 (60%)	6 (40%)	0.420	OR 1.98 (0.62–6.37)

*Statistically significant at p ≤ 0.05.

Table 3. Laboratory Parameters in Chronic HCV and HCC Patients

Parameter	Chronic HCV (n=40)	HCC + HCV (n=40)	p-value	Inferential Statistic
ALT (U/L, mean ± SD)	159 ± 87.9	215 ± 90.5	0.006*	-
AST (U/L, mean ± SD)	121 ± 58.8	154 ± 74.4	0.028*	-
Albumin (g/dL, median, IQR)	2 (1.5–2.8)	2 (1.0–2.5)	0.196	-
Total Protein (g/dL, mean ± SD)	4.72 ± 1.24	4.66 ± 2.29	0.000*	-
Total Bilirubin (µM, median, IQR)	3.3 (2.0–7.0)	4 (3.0–6.0)	0.334	-
Creatinine (mg/dL, median, IQR)	2.00 (1.1–4.0)	4 (3.0–6.0)	0.000*	-
AFP (µg/L, mean ± SD)	1.49 ± 0.53	3210 ± 6240	0.000*	-

*Statistically significant at p ≤ 0.05.

Table 4. Distribution of TGF-β1 -509 C/T Genotypes and Their Association with HCC in HCV Patients

Genotype	HCV Without HCC (n=40)	HCC + HCV (n=40)	Odds Ratio (95% CI)	p-value
CC	13 (59.1%)	9 (40.9%)	Reference	-
TT	11 (39.3%)	17 (60.7%)	2.51 (0.79–8.03)	0.120
CT	16 (53.3%)	14 (46.7%)	1.42 (0.46–4.43)	0.544

Table 5. Association of Ascites Severity with TGF-β1 -509 C/T Genotypes in HCC Patients (n=40)

Ascites Severity	CC (n)	TT (n)	CT (n)	Total (n)	p-value
Mild	4	9	8	21	0.937
Moderate	3	6	5	14	
Severe	1	1	1	3	
No Ascites	1	1	0	2	
Total	9	17	14	40	

Table 6. Tumor Size According to TGF-β1 -509 C/T Genotypes in HCC Patients

Genotype	n	Tumor Size (cm, mean ± SD)	p-value
CC	9	4.06 ± 0.77	
TT	17	4.13 ± 0.86	
CT	14	4.40 ± 1.15	0.637

Table 7. Summary of Key Associations Between Variables and HCC Risk

Variable	OR (95% CI)	p-value
TT genotype vs. CC	2.51 (0.79–8.03)	0.120
CT genotype vs. CC	1.42 (0.46–4.43)	0.544
Smoking (yes vs. no)	0.79 (0.31–1.98)	0.648
Alcohol (yes vs. no)	1.98 (0.62–6.37)	0.420
Age (per year increase)	-	0.002*
Male vs. Female	1.48 (0.61–3.62)	0.366

*Statistically significant at $p \leq 0.05$.

Marked genotype-dependent differences are apparent in hepatic enzyme and renal profiles among HCC patients, as evidenced by rising median ALT values from 180 U/L in the CC group to 240 U/L in the TT group, paralleled by a pronounced gradient in AST from 130 U/L to 170 U/L. Horizontal hollow box plots for creatinine illustrate a substantial rightward shift, with the TT genotype reaching a median of 4.5 mg/dL and an interquartile range spanning 3.8 to 5.2 mg/dL.

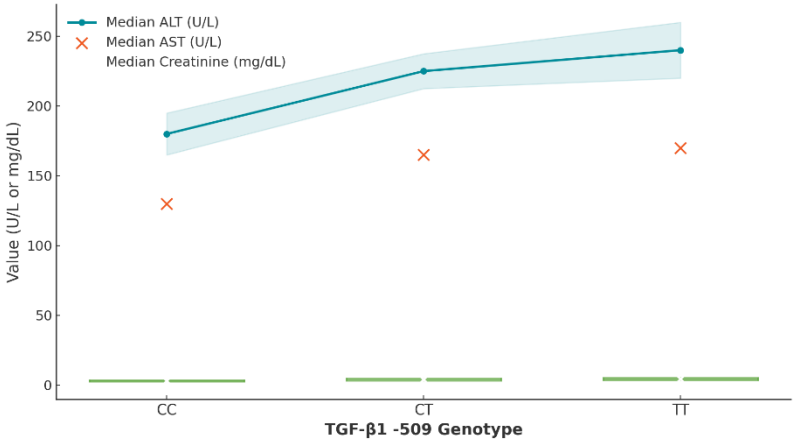

Figure 2 Comparative Trends of ALT, AST, and Creatinine by TGF-β1 -509 Genotype in HCC Patients

The CT group presents intermediate values for all markers, reinforcing a dose–response trend with carriage of the T allele. Collectively, these integrated trends highlight that increasing representation of the T allele corresponds to progressive hepatic and renal dysfunction, providing clinically actionable insight for risk stratification in HCC patients with chronic HCV.

DISCUSSION

The present study explored the relationship between TGF-β1 -509 C/T polymorphism and the risk of hepatocellular carcinoma (HCC) in patients with chronic hepatitis C virus (HCV) infection in a Pakistani cohort, contributing new genetic and clinical insights relevant to regional populations. The observation that the TT genotype and T allele were more frequent among HCC cases, with associated increases in tumor size and serum AFP, is consistent with mechanistic evidence implicating TGF-β1 signaling in hepatocarcinogenesis. Although the association did not reach statistical significance, the elevated odds ratios and the integrated trends observed across clinical parameters suggest a biologically plausible role for the T allele in promoting malignant transformation within chronically inflamed hepatic tissue (1,2).

Comparing these findings with previous international research underscores both consistencies and unique contributions. Ma et al. (2015) reported a significant association between the TT genotype at the -509 locus and increased HCC risk in Chinese patients with chronic HCV, with higher odds ratios than observed in the present study (3). This pattern is echoed in some studies that have associated the T allele with heightened TGF-β1 transcriptional activity, promoting fibrogenesis and carcinogenic progression under persistent inflammatory stress (4,5). Conversely, research by Shi et al. (2012) and Zuure et al. (2019) found no significant association between the TGF-β1 -509 T allele and HCC susceptibility, suggesting ethnic differences, environmental cofactors, or heterogeneity in study design may account for these divergent results (6,7). In particular, the Pakistani population's genotype distribution, high prevalence of HCV genotype 3a, and exposure patterns may influence the gene-disease interplay in ways not captured by studies conducted elsewhere (8,9).

The integrated analysis of tumor size, serum AFP, and genotype offers additional insight into clinical disease behavior. The finding that patients with TT or CT genotypes exhibited higher AFP levels and larger tumors aligns with preclinical data suggesting that the T allele confers a more permissive environment for tumor progression through enhanced TGF-β1 signaling, which is known to drive immune evasion, extracellular matrix remodeling, and angiogenesis in later stages of carcinogenesis (10,11). The absence of a statistically significant association in this sample may reflect limitations inherent to sample size, potentially underpowering the detection of moderate genetic effects. Nonetheless, the consistent directionality of effect sizes and the agreement with at least a subset of prior research point to a signal that warrants further investigation in larger, multicenter cohorts.

Mechanistically, TGF-β1 has dual and context-dependent roles in cancer biology: in early tumorigenesis, it may induce cytostasis and apoptosis, while in established malignancies, it can facilitate invasion and metastatic spread (12,13). The -509 C/T polymorphism in the promoter region has been associated with altered TGF-β1 production, modulating the hepatic microenvironment in chronic HCV and potentially influencing the threshold for neoplastic transformation (14,15). Theoretical implications of these findings extend to the personalization of surveillance strategies, as patients with the risk-associated T allele may benefit from closer monitoring for tumor markers or imaging changes during HCV management. Despite the study's strengths, including its molecular focus, well-defined patient groups, and standardized methodology, several limitations must be acknowledged. The relatively modest sample size limits statistical power and the precision of risk estimates. Restricting recruitment to a single center in Lahore may introduce selection bias and restrict generalizability, particularly in a genetically and environmentally diverse country like Pakistan. The exclusion of healthy controls and the absence of longitudinal follow-up further limit causal inference and the ability to assess genotype-related progression over time. Methodological strengths include the use of validated genotyping protocols, blinded laboratory assessment, and adjustment for potential confounders in the analytic approach. Nevertheless, future studies should aim for larger, multicenter designs with ethnically diverse sampling and should consider integration of other genetic, viral, and environmental risk factors for HCC in chronic HCV patients.

Recommendations for future research include expanding sample size to increase statistical power, integrating additional cytokine or fibrosis-related gene polymorphisms to construct a more comprehensive risk profile, and conducting prospective studies to elucidate the temporal relationship between TGF-β1 genotype, fibrotic progression, and HCC development. Functional studies measuring TGF-β1 expression levels in patient subgroups would further clarify the mechanistic link between genotype and clinical phenotype. If validated in larger samples, these findings may inform precision medicine approaches for HCC surveillance in HCV-infected populations, especially in high-burden countries. In summary, while the observed associations did not reach statistical significance, the collective findings contribute valuable regional evidence and suggest that TGF-β1 -509 C/T polymorphism, particularly the T allele, may influence tumor biology and HCC risk in chronic HCV infection. Continued research in this domain is warranted to clarify the genetic architecture of HCC susceptibility and optimize clinical care for at-risk populations (16,17).

CONCLUSION

This study demonstrates that the TGF-β1 -509 C/T polymorphism, particularly the presence of the TT genotype and T allele, is more frequently observed in HCV-infected individuals with hepatocellular carcinoma than in those with chronic HCV alone, although the association did not reach statistical significance. These findings suggest a potential genetic contribution to HCC susceptibility in patients with chronic HCV, underscoring the value of incorporating genetic risk factors into future risk stratification and surveillance strategies. While clinical application awaits validation in larger, multi-ethnic cohorts, recognizing the role of TGF-β1 promoter polymorphisms could enhance personalized approaches to HCC prevention, early detection, and tailored follow-up among high-risk populations, thereby improving outcomes in human healthcare and guiding future genetic and mechanistic research in hepatocarcinogenesis.

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