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SINGLE NUCLEOTIDE POLYMORPHISMS IN TGF-B1 AND THEIR ASSOCIATION WITH HCC DEVELOPMENT IN HCV PATIENTS

Original Research

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ABSTRACT

Background: Hepatitis C virus (HCV) infection remains a global public health concern, affecting over 170 million individuals and contributing substantially to the burden of liver-related morbidity and mortality. Chronic HCV infection can progress to fibrosis, cirrhosis, and hepatocellular carcinoma (HCC). Cytokine gene polymorphisms, including transforming growth factor-beta 1 (TGF-β1), may influence individual susceptibility to HCC. Variations in the TGF-β1 promoter region, such as the −509 C/T polymorphism, can alter cytokine expression and potentially affect disease progression.

Objective: To investigate the association between TGF-β1 −509 C/T gene polymorphism and the risk of developing HCC in patients with chronic HCV infection in a local Pakistani population.

Methods: A comparative study was conducted involving 80 patients recruited from Sheikh Zayed Hospital, Lahore. Group I comprised 40 patients with chronic HCV infection without HCC, and Group II included 40 chronic HCV patients with HCC. Diagnosis of HCC was confirmed by abdominal ultrasound and computed tomography. Genotyping of TGF-β1 −509 C/T polymorphism was performed using polymerase chain reaction–restriction fragment length polymorphism (PCR-RFLP) analysis. Statistical analysis was conducted using SPSS version 20.0, with chi-square tests to assess genotype and allele distribution. Odds ratios (OR) with 95% confidence intervals (CI) were calculated, and $p < 0.05$ was considered significant.

Results: The TT genotype was present in 11 (39.3%) HCV patients and 17 (60.7%) HCC patients [OR = 2.511, 95% CI: 0.786–8.029, p = 0.120], while the CT genotype was detected in 16 (53.3%) HCV patients and 14 (46.7%) HCC patients [OR = 1.422, 95% CI: 0.457–4.427, p = 0.544]. No statistically significant association was observed between genotype distribution and HCC; however, individuals carrying the TT genotype or T allele exhibited a higher OR compared to those with the CC genotype.

Conclusion: Although no significant association was found, the TGF-β1 −509 T allele may contribute to increased susceptibility to HCC in chronic HCV patients in the local population, highlighting the need for larger-scale studies.

Keywords: Alleles, Hepatitis C, Hepatocellular Carcinoma, Odds Ratio, Polymorphism, Polymerase Chain Reaction, Transforming Growth Factor beta1.

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INTRODUCTION

Cancer remains one of the foremost causes of mortality worldwide, accounting for approximately 8.2 million deaths in 2012 (1). Hepatocellular carcinoma (HCC) is the second leading cause of cancer-related deaths, responsible for an estimated 745,000 fatalities globally in the same year (2). Chronic hepatitis C virus (HCV) infection is a major etiological factor for HCC, particularly in countries such as Pakistan, where the prevalence of HCV-associated liver disease is high (3). HCV is a small, enveloped, positive-sense single-stranded RNA virus belonging to the genus *Hepacivirus* in the family *Flaviviridae*. It infects only humans and chimpanzees and, lacking reverse transcriptase, does not integrate into the host genome, thereby excluding insertional mutagenesis as a direct oncogenic mechanism (4,5). Globally, HCV affects 130–210 million individuals—about 3% of the world's population—and contributes to 500,000–1,000,000 deaths annually (6). The infection often progresses from acute to chronic hepatitis and may ultimately lead to cirrhosis and HCC (7). HCV exhibits marked genetic heterogeneity, with six major genotypes and over 70 subtypes identified worldwide (8). Treatment response varies between genotypes, and while differences in virulence and pathogenicity exist, the predominant genotype in Pakistan is genotype 3a, accounting for over half of all cases, followed by genotypes 1a, 3b, and mixed infections (9). Persistent genotype 3a infection has been strongly associated with HCC development in the Pakistani population (10). The progression from chronic HCV infection to HCC is influenced by a combination of viral kinetics, immune responses, host genetic makeup, and environmental factors (11). Among these, genetic determinants are increasingly recognized as critical modulators of disease susceptibility and progression. Cytokines, as key regulators of both innate and adaptive immunity, play a central role in viral control and in the pathogenesis of HCV-related liver injury (12). Genome-wide association studies have identified several cytokine gene single nucleotide polymorphisms (SNPs) that correlate with treatment outcomes and spontaneous viral clearance (13).

One cytokine of particular interest is transforming growth factor-beta 1 (TGF-β1), a member of the TGF-β family that also includes TGF-β2 and TGF-β3. TGF-β1 is released by various cell types during inflammation, tissue repair, and fibrogenesis, and is regarded as a key profibrogenic mediator in chronic liver disease (8-10). It has diverse functions, including regulation of cell proliferation, differentiation, extracellular matrix production, angiogenesis, immune modulation, and tumor progression (11). In chronic HCV infection, dysregulation of TGF-β1 secretion and signaling may contribute to impaired immune surveillance and enhanced fibrogenesis, thereby promoting hepatocarcinogenesis (12). Interestingly, TGF-β1 exhibits dual behavior—acting as a tumor suppressor in early stages and as a tumor promoter in advanced disease—although the mechanisms underlying this switch remain incompletely understood (13). The human TGF-β1 gene, located on chromosome 19q13, contains several polymorphic sites that influence its expression levels, including +915 (Arg/Pro), +988 (C/A), -509 (C/T), and -800 (G/A), as well as polymorphisms within exon 1 (14). Among these, the -509 C>T variant, located within a Yin-Yang 1 transcription factor binding site, has been most extensively studied for its association with altered TGF-β1 production (15). Given that cirrhosis and chronic inflammation are primary precursors to HCC, and that TGF-β1 is a pivotal mediator linking inflammation to fibrogenesis and oncogenesis, genetic variations in the TGF-β1 gene may have profound implications for HCV-related liver cancer risk (6,7). However, existing studies investigating the relationship between TGF-β1 -509 C>T polymorphism and HCC susceptibility have reported inconsistent findings, possibly due to differences in ethnic backgrounds, sample sizes, and study designs (10-14). In Pakistan, where HCV genotype 3a predominates and HCC incidence is rising, there is limited data exploring this genetic association. Therefore, this study aims to investigate the relationship between the TGF-β1 -509 C>T polymorphism and the risk of developing HCC among patients with chronic HCV infection in the Pakistani population, with the objective of identifying potential genetic markers that could inform early detection and targeted prevention strategies.

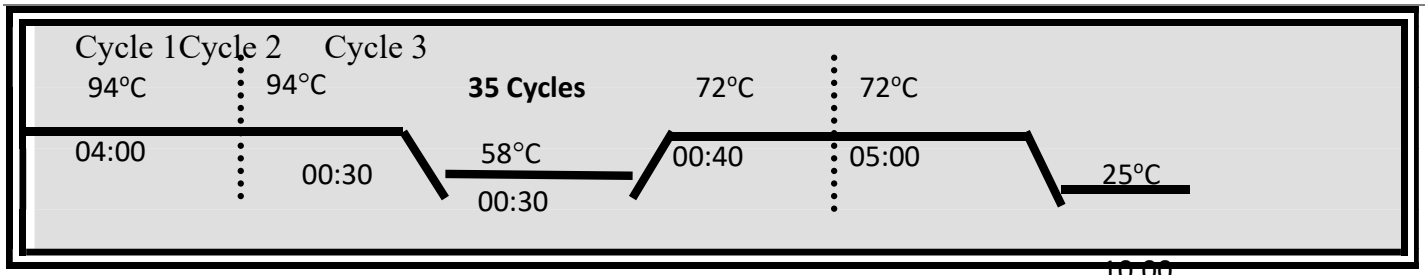
METHODS

This comparative study employed a purposive sampling technique and was conducted at Sheikh Zayed Hospital, Lahore. A total of 80 patients with chronic hepatitis C virus (HCV) infection were recruited and divided into two equal groups. Group I comprised 40 patients with chronic HCV infection but without hepatocellular carcinoma (HCC), whereas Group II included 40 chronic HCV patients diagnosed with HCC. The diagnosis of HCC was confirmed using abdominal ultrasound and computed tomography scans, ensuring accurate detection of tumor size, presence of metastases, and portal vein thrombosis. Inclusion criteria comprised adult patients with confirmed chronic HCV infection, with or without HCC, as per clinical and imaging findings. Exclusion criteria included patients with hepatitis B

virus (HBV) co-infection, other chronic liver diseases, previous liver transplantation, or any malignancy other than HCC. All participants provided written informed consent prior to enrollment. The study protocol was reviewed and approved by the Ethics Committee of the University of Health Sciences, Lahore, and was conducted in accordance with the Declaration of Helsinki. Venous blood samples were collected from all participants into EDTA-containing tubes for genomic DNA extraction. The phenol-chloroform method was employed to isolate genomic DNA, which was stored at −20°C until further analysis. Genotyping of the TGF-β1 gene polymorphism at position −509 (C/T) (rs1800469) in the promoter region was performed using the polymerase chain reaction–restriction fragment length polymorphism (PCR-RFLP) method. The primer sequences used for amplification were as follows: forward primer 5′-CCCGGCTCCATTTCCAGGTG-3′ and reverse primer 5′-GGTCACCAGAGAAAGAGGAC-3′. PCR amplification was carried out in a 10 µL reaction volume containing 2 µL of diluted DNA (25 ng/µL), 10× Taq buffer [10 mM Tris-HCl (pH 8.8 at 25°C), 50 mM KCl, 0.08% (v/v) Nonidet P-40], 1.5 mM $MgCl_2$, 0.4 µL of a mixture containing 100 µM of each dNTP, 5 nM of each primer, and 1 U of Taq DNA polymerase. Thermal cycling was performed on an iCycler PCR thermocycler (Bio-Rad, USA) under the following conditions: an initial denaturation at 94°C for 4 minutes, followed by 30 cycles of denaturation at 94°C, annealing at 58°C, and extension at 72°C, each for 1 minute, and a final extension step at 72°C for 5 minutes.

The PCR products, measuring 808 bp, were digested with Eco81I (SauI) restriction enzyme (Fermentas-Euromedex) and subjected to agarose gel electrophoresis for genotype determination. The TT genotype produced an undigested fragment of 808 bp, the CC genotype yielded two fragments of 617 bp and 191 bp, and the CT genotype displayed all three fragments (808 bp, 617 bp, and 191 bp). Statistical analyses were conducted using SPSS version 12.0 (SPSS Inc., Chicago, IL, USA). Continuous variables were expressed as means ± standard deviations (SD). Genotype frequencies were tested for Hardy–Weinberg equilibrium. Categorical variables, including demographic data, smoking and alcohol consumption status, and genotype distributions between groups, were compared using the χ^2 test or Fisher's exact test as appropriate. Odds ratios (ORs) with 95% confidence intervals (CIs) were calculated to assess the strength of association between genotypes and disease status. A two-tailed *P* value < 0.05 was considered statistically significant.

TGF-β1 polymorphism	**Sequence**
Forward Primer	**5′-CCCGGCTCCATTTCCAGGTG-3'**
Reverse Primer	**5′-GGTCACCAGAGAAAGAGGAC-3'**



RESULTS

The study included 80 participants, divided equally between patients with chronic HCV infection without HCC and those with HCV-associated HCC. The mean age was significantly higher in the HCC group compared to the non-HCC group (59.3 ± 10.33 years vs. 51.9 ± 10.72 years, $p = 0.002$). Gender distribution did not differ significantly, with males comprising 62.5% of the non-HCC group and 52.5% of the HCC group ($p = 0.366$). Smoking prevalence was slightly higher in the HCC group (53.1%) compared to the non-HCC group (46.9%), but this difference was not statistically significant ($p = 0.648$). Alcohol consumption was more frequent in the non-HCC group (60%) compared to the HCC group (40%), with no significant association observed ($p = 0.420$). Biochemical analysis revealed that mean serum ALT and AST levels were significantly elevated in the HCC group (215 ± 90.5 U/L and 154 ± 74.4 U/L, respectively) compared to the non-HCC group (159 ± 87.9 U/L and 121 ± 58.8 U/L, respectively), with *p* values of 0.006 and 0.028. Mean total protein concentration was slightly lower in the HCC group (4.66 ± 2.29 g/dL) than in the non-HCC group (4.72 ± 1.24 g/dL), and the difference was statistically significant ($p < 0.001$). Median total bilirubin was higher in the HCC group [4 (Q1–Q3: 3.0–6.0) µM] compared to the non-HCC group [3.3 (Q1–Q3: 2.0–7.0) µM], though not statistically significant ($p = 0.334$). Median creatinine levels were significantly elevated in the HCC group [4.0 (Q1–Q3: 3.0–6.0) mg/dL] compared to the non-HCC group [2.0 (Q1–Q3: 1.1–4.0)

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mg/dL] ($p < 0.001$). Alpha-fetoprotein (AFP) levels were markedly higher in the HCC group (3210 ± 6240 µg/L) than in the non-HCC group (1.49 ± 0.53 µg/L), with a highly significant difference ($p < 0.001$).

Genotypic distribution analysis of the TGF-β1 −509 C>T polymorphism showed that the CC genotype was most prevalent in the non-HCC group (59.1%) compared to 40.9% in the HCC group. The TT genotype was more frequent in the HCC group (60.7%) compared to the non-HCC group (39.3%), yielding an odds ratio (OR) of 2.511 [95% CI: 0.786–8.029], which was not statistically significant ($p = 0.120$). The CT genotype showed an OR of 1.422 [95% CI: 0.457–4.427] with no significant association ($p = 0.544$). In HCC patients, the presence of ascites showed no significant association with any TGF-β1 genotype ($p = 0.937$). Similarly, tumor size did not differ significantly across genotypes, with mean sizes of 4.056 ± 0.768 cm for CC, 4.129 ± 0.858 cm for TT, and 4.40 ± 1.148 cm for CT ($p = 0.637$). The allele frequency analysis showed that in the non-HCC group, the C allele frequency was 0.562 and the T allele frequency was 0.438, while in the HCC group, the C allele frequency was 0.475 and the T allele frequency was 0.525. Hardy–Weinberg equilibrium testing indicated no significant deviation from equilibrium in either group (HCV without HCC: $p = 0.272$; HCV with HCC: $p = 0.898$), suggesting that genotype distributions were consistent with expected population genetic proportions.

Table 1: Demographic Data of The Study Subjects

Variables		Group I n (%)	Group II n (%)	p-value
Gender	Male	25 (62.5%)	21 (52.5%)	0.366
	Female	15 (37.5%)	19 (47.5%)	
Age		51.9 ± 10.72	59.3 ± 10.33	0.002*
Smoking status		15 (46.9%)	17 (53.1%)	0.648
Drinking status		9 (60%)	6 (40%)	0.420

n=number, %=percentage, *p≤0.05=statistically significant

Table 2: Comparison of Biochemical Parameters Between Chronic HCV Patients with and Without Hepatocellular Carcinoma

Variables	Group I	Group II	*p*-value
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 U/L [Median(Q1-Q3)]	2 (1.5-2.8)	2 (1.0-2.5)	0.196
Total proteins (g/dL) (mean ± SD)	4.72 ± 1.24	4.66 ± 2.29	0.000*
Total bilirubin(µM) [Median (Q1-Q3)]	3.3 (2.0-7.0)	4 (3.0-6.0)	0.334
Creatinine (mg/dL) [Median (Q1-Q3)]	2.00 (1.1-4.0)	4 (3.0-6.0)	0.000*
AFPµg/L (mean ± SD)	1.49 ± 0.53	3210 ± 6240	0.000*

*p≤0.05=statistically significant

Table 3: Distribution of TGF-β 1 polymorphisms in HCV patients with and without HCC

TGF-β1 polymorphisms	HCV patients n=40 (%)	HCC patients n=40 (%)	OR (CI)	*p*-value
CC	13 (59.1%)	9 (40.9%)	Reference	
TT	11 (39.3%)	17 (60.7%)	2.511 (0.786-8.029)	0.120
CT	16 (53.3%)	14 (46.7%)	1.422 (0.457 – 4.427)	0.544

*p≤0.05=statistically significant, n= number

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Table 4: Association of Ascites Severity With TGF-β1 −509 C/T Genotypes in HCV-Related HCC Patients

`		Polymorphisms			Total	*p*-value
		CC	TT	CT		
Ascites	Mild	4	9	8	21	0.937
	Moderate	3	6	5	14	
	severe	1	1	1	3	
	No	1	1	0	2	
Total		9	17	14	40	

*p≤0.05=statistically significant

Table 5: Association of Tumor Size in HCC Patients with Polymorphisms Of TGF-B 1

Polymorphisms	HCC patients n (40)	Tumor size (mean ± SD)	*p*-value
CC	9	4.056 ± 0.768	0.637
TT	17	4.129 ± 0.858	
CT	14	4.40 ± 1.148	

*p≤0.05=statistically significant

Table 6: Allele Frequency Distribution and Hardy–Weinberg Equilibrium Results

Group	C allele frequency	T allele frequency	HWE p-value
HCV without HCC	0.562	0.438	0.272
HCV with HCC	0.475	0.525	0.898

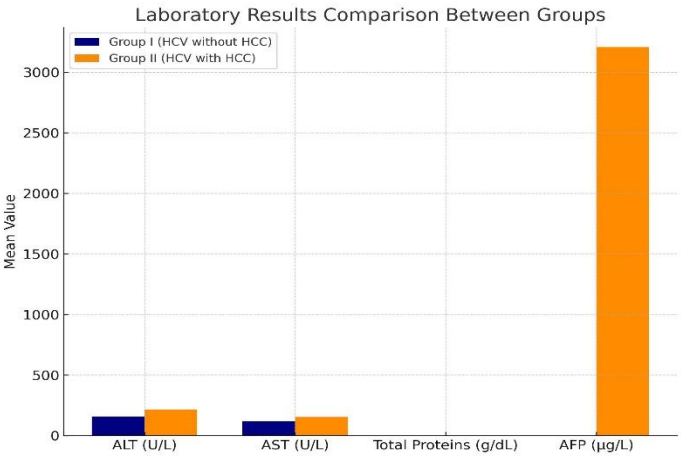

Figure 1 Laboratory Results Comparison Between Group

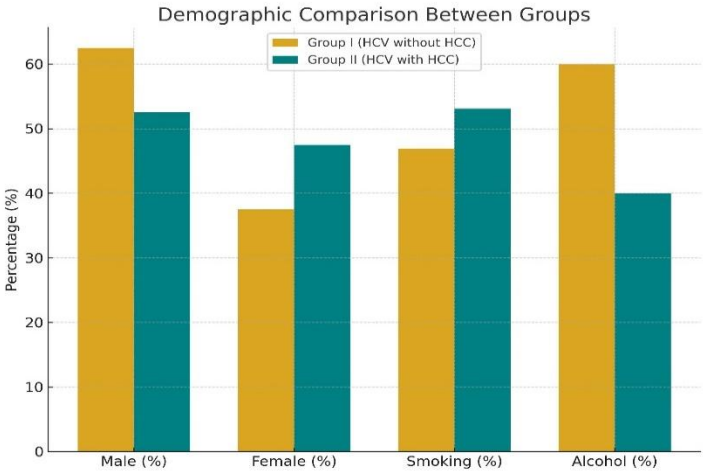

Figure 2 Demographic Comparison Between Groups

DISCUSSION

In this investigation, chronic HCV patients, with and without HCC, were evaluated for TGF-β1 polymorphism at the promoter region −509 C/T. The mean age was significantly higher in patients with HCC than in those without, reflecting a well-documented epidemiological trend in which advancing age is associated with a greater likelihood of malignant transformation in chronic liver disease (1-4). This observation aligns with multiple reports in which HCC patients demonstrated a higher age profile compared to non-HCC counterparts. Gender distribution in the present cohort indicated a relatively higher proportion of females, which may be influenced by the limited healthcare access among Pakistani women and the relatively small sample size. Conversely, larger epidemiological studies have typically reported a male predominance in both HCV and HCC cases, with some estimating male-to-female ratios exceeding 3:1

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in HCC incidence (5-7). The prevalence of smoking was greater in the HCC group compared to the non-HCC group, although the difference was not statistically significant. This pattern is consistent with prior studies in which smoking has been variably associated with HCC risk; while some investigations have reported a weak or non-significant association, others have demonstrated a modest dose-dependent increase in risk (8-11). Alcohol consumption was more frequent among HCV-only patients in the present study, yet literature generally supports a strong association between excessive alcohol intake and HCC development, particularly in synergy with viral hepatitis (12,13). Biochemical analysis demonstrated significantly elevated serum ALT and AST levels in the HCC group compared to the HCV group, a finding consistent with liver injury and disease progression. Higher levels of bilirubin, creatinine, and AFP in HCC patients further reflected advanced disease and impaired hepatic function. AFP, in particular, showed a highly significant difference between groups, reinforcing its value as a diagnostic biomarker in HCC, though with known limitations in sensitivity and specificity (14-16).

Genotypic distribution analysis of TGF-β1 −509 C/T polymorphism revealed a higher frequency of the TT genotype among HCC patients compared to those without HCC, with an elevated odds ratio suggesting a potential risk association. Although the differences did not reach statistical significance, the trend observed supports the possibility of the T allele being a contributory genetic factor in hepatocarcinogenesis among HCV-infected individuals. This aligns with prior research demonstrating increased HCC susceptibility among individuals carrying the TT genotype in certain populations (17-18). Contradictory evidence exists, with some studies associating the C allele with disease progression, while others found no significant genotype effect, highlighting the influence of ethnic, environmental, and clinical heterogeneity on genetic risk profiles (19-21). No significant association was found between TGF-β1 genotypes and the presence of ascites or tumor size in HCC patients, although ascites remains a common complication in advanced hepatic malignancy, often linked to cirrhosis and portal hypertension (22-23). The lack of association in the current cohort may be attributable to the sample size or the multifactorial nature of ascites development in HCC. The findings of this study suggest that the TGF-β1 −509 C/T polymorphism may contribute to an increased risk of HCC in patients with chronic HCV infection, although statistical significance was not achieved. The allele frequency analysis indicated a higher proportion of the T allele in the HCC group, and Hardy–Weinberg equilibrium was maintained in both groups, suggesting genetic stability in the studied population. These results warrant cautious interpretation but provide a basis for further investigation into the role of cytokine gene polymorphisms in hepatocarcinogenesis.

A key strength of this study lies in its focus on a specific high-prevalence genotype of HCV in a Pakistani cohort, contributing to localized genetic epidemiology data. However, the relatively small sample size limits the statistical power to detect modest associations, and the absence of multivariate adjustment for confounders such as liver fibrosis stage, viral load, and co-morbidities reduces the precision of the findings. Additionally, environmental exposures and lifestyle factors were not comprehensively quantified, which may confound genetic associations. Future research should employ larger, multi-center cohorts with stratification by HCV genotype, fibrosis stage, and treatment history. Incorporating functional assays to elucidate the biological impact of TGF-β1 −509 C/T variants on cytokine expression and immune regulation in hepatic tissue would strengthen mechanistic understanding. Longitudinal studies assessing genotype-specific disease progression from chronic HCV infection to HCC would provide valuable prognostic insights and may inform targeted screening strategies in high-risk subgroups.

CONCLUSION

This study demonstrated that the presence of the TT genotype or the T allele of the TGF-β1 −509 C/T polymorphism may indicate a greater tendency toward the development of hepatocellular carcinoma in individuals with chronic hepatitis C infection within the local population. While no statistically significant difference was observed in genotype frequencies between patients with and without HCC, the observed pattern suggests a potential genetic influence that could contribute to disease progression. These findings highlight the importance of further exploring cytokine gene polymorphisms as possible markers for identifying high-risk patients, which may ultimately support more targeted surveillance and early intervention strategies in HCV-related liver disease.

AUTHOR CONTRIBUTION

Author	Contribution
Almina Shafiq	Substantial Contribution to study design, analysis, acquisition of Data Manuscript Writing Has given Final Approval of the version to be published
Rabia Aslam	Substantial Contribution to study design, acquisition and interpretation of Data Critical Review and Manuscript Writing Has given Final Approval of the version to be published
Atika Hashmi	Substantial Contribution to acquisition and interpretation of Data Has given Final Approval of the version to be published
Madiha Asghar	Contributed to Data Collection and Analysis Has given Final Approval of the version to be published
Kanza Batool	Contributed to Data Collection and Analysis Has given Final Approval of the version to be published
Romeeza Tahi	Substantial Contribution to study design and Data Analysis Has given Final Approval of the version to be published
Nadeem Afzal	Contributed to study concept and Data collection Has given Final Approval of the version to be published
ShahJahan*	Writing - Review & Editing, Assistance with Data Curation

REFERENCES

1. Ghanem S, El Gedawy G, Yehia S, Bedair H, Awad S, Abdel-Razek W, et al. ADAM10 Gene Polymorphism and Its Relationship to Hepatocellular Carcinoma in Egyptian HCV Patients Receiving Direct-Acting Antiviral Therapies (DAAs). Asian Pac J Cancer Prev. 2023;24(1):149-55.
2. Ogawa E, Takayama K, Hiramine S, Hayashi T, Toyoda K. Association between steatohepatitis biomarkers and hepatocellular carcinoma after hepatitis C elimination. Aliment Pharmacol Ther. 2020;52(5):866-76.
3. Wei X, Yang C, Lin Q, Qiu M, Wen Q, Zhou Z, et al. Associations between modifiable risk factors and hepatocellular carcinoma: a trans-ancestry Mendelian randomization study. BMC Cancer. 2024;24(1):820.
4. AboShabaan HS, Alghannam O, Ismail F, El-Garawani IM, El-Shahat M, Talaat RM, et al. Bone morphogenic protein-7 (BMP-7) polymorphism: Susceptibility to cirrhosis and hepatocellular carcinoma after viral hepatitis in Egyptian patients. Clin Exp Hepatol. 2023;9(2):154-63.
5. Ghanm SE, Shebl NA, El Sayed IET, Abdel-Bary HM, Saad BF, Othman Saad W. Direct Relationship between Interleukin-10 Gene Polymorphism and Hepatocellular Carcinoma Complicated by Direct Acting Antiviral Treatment of Hepatitis C Virus. Asian Pac J Cancer Prev. 2021;22(10):3203-10.
6. Okumura T, Joshita S, Yamazaki T, Iwadare T, Wakabayashi SI, Kobayashi H, et al. HLA-G susceptibility to hepatitis B infection and related hepatocellular carcinoma in the Japanese population. Hum Immunol. 2023;84(8):401-7.
7. Aboushousha T, Emad M, Rizk G, Ragab K, Hammam O, Fouad R, et al. IL-4, IL-17 and CD163 Immunoexpression and IL-6 Gene Polymorphism in Chronic Hepatitis C Patients and Associated Hepatocellular Carcinoma. Asian Pac J Cancer Prev. 2021;22(4):1105-13.
8. Bondok RM, Barakat LA, Elsergany AR, Mahsoub N, Ghattas MH. Interferon-induced transmembrane protein 3 in hepatocellular carcinoma patients. BMC Cancer. 2023;23(1):584.
9. Abd El-Maksoud EM, Ibrahim NE, Farag MMS, Swellam M. Interleukin-17A and Interleukin-17F Gene Polymorphisms in Egyptian Patients with Chronic Hepatitis C and Hepatocellular Carcinoma. Asian Pac J Cancer Prev. 2025;26(1):153-9.
10. Khan N, Khan K, Badshah Y, Trembley JH, Ashraf NM, Shabbir M, et al. Investigating pathogenic SNP of PKCι in HCV-induced hepatocellular carcinoma. Sci Rep. 2023;13(1):12504.
11. Farid AA, Afify NA, Alsharnoby AA, Abdelsameea E, Bedair HM. Predictive Role of AURKA rs 1047972 Gene Polymorphism and the Risk of Development of Hepatocellular Carcinoma. Immunol Invest. 2022;51(5):1211-21.

12. Galal G, Tammam H, Abdel Aal A, Fahmy N, Sheneef A, Ahmed N, et al. Role of Lymphotoxin-α Gene Polymorphism in Hepatitis C Virus-Related Chronic Liver Disorders. Infect Drug Resist. 2021;14:1921-30.
13. Mohamed AA, Esmail OE, Ibrahim AMA, Makled S, Al-Hussain E, Elsaid A, et al. The role of PRDM1 gene polymorphism in the progression of hepatocellular carcinoma in Egyptian patients. J Med Virol. 2023;95(1):e28343.
14. Dawood RM, Abd El Meguid M, Elrobe W, Salum GM, Zayed N, Mousa S, et al. Significance of Hereditary Hemochromatosis Gene (HFE) Mutations in Chronic Hepatitis C and Hepatocellular Carcinoma Patients in Egypt: A Pilot Study. Asian Pac J Cancer Prev. 2021;22(9):2837-45.
15. El Sharnoby A, Bedair HM, Raia GYS, Hamed N, Sabry A, Khalaf FA, et al. Suppressor of Cytokine Signaling-3 Gene Polymorphisms and the Risk of Hepatocellular Carcinoma in Chronic Hepatitis C Patients in Egypt. Asian Pac J Cancer Prev. 2023;24(12):4253-61.
16. anzoor, S., et al. (2023). "Serum MicroRNAs as Predictors for HCV Progression and Response to Treatment in Pakistani Patients." 14(2): 441.
17. Marhemati, F., et al. (2020). "Transforming growth factor beta 1 (TGFβ1) polymorphisms and unexplained infertility: a genetic association study." 66(4): 267-280.
18. Minami, T., et al. (2021). "Impact of obesity and heavy alcohol consumption on hepatocellular carcinoma development after HCV eradication with antivirals." 10(4): 309-319.
19. Refolo, M. G., et al. (2020). "Inflammatory mechanisms of HCC development." 12(3): 641.
20. Shen, C., et al. (2023). "Hepatitis virus and hepatocellular carcinoma: Recent advances." 15(2): 533.
21. Takeda, H., et al. (2022). "Genetic landscape of multistep hepatocarcinogenesis." 14(3): 568.
22. Zahid, A., et al. (2023). "Prevalence of Hepatitis B&C in Pregnant females &Transmission through Sporadic Source and Dental Source among Pakistani Population: A Brief Review." 17(02): 8-8.
23. Zong, J., et al. (2020). "Serum tumor markers for early diagnosis of primary hepatocellular carcinoma." 413-422.