

Relationship of *CTLA-4* gene polymorphism to increased risk of Hepatocellular Carcinoma**Areeha Naveed¹, Sidra Zafar², Haris Khurram³, Saba Zafar^{1*}**¹*The Women University, Faculty of Life Sciences, Department of Biochemistry and Biotechnology, 60000, Multan, Pakistan.*²*Bahauddin Zakariya University, Faculty of Biological Sciences, Institute of Molecular Biology and Biotechnology, 60000, Multan, Pakistan.*³*National University of Computer and Emerging Sciences, Chiniot-Faisalabad Campus, Faculty of Sciences, ²Department of Sciences and Humanities, Chiniot, Pakistan***Abstract**

Cancer is a disease characterized by the uncontrolled growth and spread of abnormal cells through the circulatory and lymphatic systems. It is one of the leading causes of death worldwide, accounting for approximately 2–2.5 million deaths annually. Several factors contribute to cancer development, including genetic mutations, environmental exposure, tobacco use, obesity, alcohol consumption, hormonal imbalance, radiation, and carcinogens. Liver cancer is a major type of cancer associated with risk factors such as Hepatitis B virus (HBV), Hepatitis C virus (HCV), cirrhosis, alcohol intake, aflatoxin exposure, non-alcoholic steatohepatitis, and smoking. Liver cancer is classified into primary and secondary types, with hepatocellular carcinoma (HCC) being the most common primary liver cancer, accounting for nearly 90% of cases. Around 85% of cirrhotic patients eventually develop HCC. Current treatment options include liver transplantation, chemotherapy, immunotherapy, and local ablative therapies.

Cancer-related genes are broadly divided into tumor suppressor genes and oncogenes. Genes implicated in liver cancer include *TP53*, *CTNNB1*, *CCND1*, *VEGFA*, *HGF*, *NFE2L2*, *KEAP1*, and others. The present study focused on the *CTLA-4* gene as a candidate gene to investigate its association with hepatocellular carcinoma. Two SNPs, rs3087243 and rs5742909, were analysed along with demographic and clinical factors such as diabetes, smoking, BMI, exercise, HBV, and HCV infection.

A total of 100 blood samples were collected, including 50 liver cancer patients and 50 healthy controls. Genotyping was performed using TETRA-ARMS PCR followed by agarose gel electrophoresis. Statistical analysis using SPSS and logistic regression revealed that BMI, diabetes, smoking, and HBsAg positivity significantly increased liver cancer risk. In contrast, *CTLA-4* variants and several haplotypes appeared to have a protective role, with haplotype GACC showing a significant association with liver cancer susceptibility.

Keywords: Liver Cancer, Single Nucleotide Polymorphism, *CTLA-4*, Logistic regression.