Diagnostic Study of CD_{35} Protein-Gene As a Biochemical Variable To Prediction of Iraqi Hepatic Cancer Patients

Abstract

A number of cancers have been linked to the CD35 protein gene, which codes for a crucial molecule in inflammation and immunology. Hepatic carcinoma (HC) and CD35 polymorphisms have been linked in certain studies, however this is far from definitive. This research sought to examine the association between tag SNPs in CD35 and susceptibility to HC, as well as postoperative recurrence, to further knowledge of gene-environment interactions in HC.Of the 120 Iraqi participants surveyed for this research, 56 had HC and 64 were healthy. Several cancers have been linked to the CD35 protein gene, which codes for a crucial molecule in inflammation and immunology. Although some studies have indicated a correlation between hepatic carcinoma (HC) and CD35 polymorphisms, this is not yet definitive. This study examined CD35 tag SNPs and HC susceptibility and postoperative recurrence to better understand gene-environment interactions in HC. Our research included 120 Iraqis; 56 had HC and 64 were healthy. We used HaploView 4.2 software to pick three CD35 Tag SNPs (rs2296160, rs3849266 and rs7525160), and MALDI-TOF-MS was used for genotyping. Higher rates of HC were linked to CD35 rs7525160 mutant genotypes CC/CG. A stratification study showed that the CD35 rs7525160 CC/CG genotypes were significantly related with a pathological kind of poor HC prognosis and increased the incidence of HC in those under 60.The Cox proportional hazard ratio model shows that the rs7525160 CC/CG genotype increases post-surgery HC recurrence. Finally, the CD35 rs7525160 polymorphism may affect Iraqi HC incidence and prognosis.

Keywords: CD35-Protein-gene, genetic variation, metastasis, susceptibility, and hepatic cancer

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Introduction

Approximately 80% of initial liver malignancies are hepatic carcinomas (HCs), the fourth greatest cause of cancer deaths worldwide (1, 2). Liver cancer risk factors include alcohol, tobacco, aflatoxin exposure, obesity, diabetes, non-alcoholic fatty liver disease, and HBV and HCV. However, these risk indicators do not fully explain HC occurrence. Genetic variables linked to HC vulnerability have been found in many studies (7, 8).These discoveries underscore the significance of genetics in the development of HC. Consequently, a more comprehensive understanding of the molecular pathways that regulate the growth and development of HC may enable the development of effective treatment options and early diagnosis (9).

Tissue regeneration, lipid metabolism, and immune complex clearance are all performed by the complement system. It has traditionally been the initial line of defense against microbes (10). However, multiple studies have shown that the complement system may promote tumor development by prolonged cell proliferation, decreased apoptosis, and enhanced invasion and metastasis (11, 12, 13). The complement system relies on the liver more than any other organ (14). Liver damage, regeneration, and transplantation are among the several liver-related outcomes that follow (15). To activate the complement system, a huge family of proteins called complement regulatory proteins must be present (16). Complement receptor 1 (CR1/CD35; abbreviated CD35) is notable for its established role in immunological control by T-cells and B-cells, as well as its interaction with C3b and C4b to enhance neutrophil-mediated phagocytosis (17, 18). CD35's role in complement activation, innate immunity, and chronic inflammation makes it a key marker for gallbladder, nasopharyngeal, non-small cell lung, and gastric cancer risk. The link between CD35 polymorphisms and HC vulnerability is poorly understood. The current case-control research examined HC susceptibility and recurrence and identified CD35 SNPs to explore how gene-environment interaction influences HC risk.
(21).

Materials and Methods

Patients and controls

Between October 2025 and March 2026, doctors at Al-Hussein Teaching Hospital in Kerbala diagnosed 56 patients with liver cancer (Group 1), whereas 64 patients were classified as healthy (Group 2). During the recruitment process, each participant provided the case information. Each subject's age, sex, smoking status, and HC family history were collected from the individuals or their relatives as baseline information. HC patients' medical records, laboratory testing, and imaging revealed tumor size, amount, metastasis, Child-Pugh classification, and microvascular invasion.

Tag SNPs and Genotype Dignosis

The Human Genome Project and International HapMap Project provided CD35 SNPs. We used Iraqi HapMap data using HaploView 4.2 to search for CD35 tag SNPs with a MAF < 0.05 and r2 cut-off of 0.8. Three single nucleotide polymorphisms (rs2296160, rs3849266, and rs7525160) were also examined (Supplementary Table 1). Following the manufacturer's instructions, the TIANamp Genomic DNA Kit (Tiangen Biotech Co. Ltd., Beijing, China) extracted genomic DNA from whole blood samples (EDTA anticoagulant). A 20 ng/mL dilution of isolated DNA was kept at -20°C.

The MassARRAY system and the MALDI-TOF method were used for genotyping, following the manufacturer's instructions. Sequenom is located in San Diego, CA, USA. For certain tag SNPs, primer information is provided in Supplementary Table 2. Genotyping quality control confirmed genotypic consistency in 5% of randomly selected and duplicated samples. An extra 5% of samples were genotypically verified by direct sequencing.

Statistical Analysis

Analyses were done using SPSS 26. P-values under 0.05 indicated statistical significance for two-tailed observations. The chi-squared goodness-of-fit test evaluated the deviation of each SNP frequency from Hardy-Weinberg equilibrium (HWE) within the control group.We used a Mann-Whitney U test for continuous variables and a chi-square test with Fisher's exact test for categorical variables to compare the HC and control groups' demographics.

A logistic regression model linked HC risk to the CD35 tag SNP. Sex, HBV status, smoking, and alcohol intake were significant variables in OR and 95% CI. A Cox proportional hazards model and univariate survival analysis were performed to investigate RFS/OS and CD35 tag SNPs.

Results and Discussion

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After treatment at Kerbala's Al-Hussein Teaching Hospital's cancer division, 120 participants were 56 HC patients (41 males and 15 females) and 64 controls (34 males and 30 women). HC patients and controls were first included in Table 1. Nevertheless, age (categorized by 65 years) and familial history of liver cancer did not differ across the groups. Significant differences were seen across the groups categorized by gender, hepatitis B status, smoking, and alcohol use (P<0.05). Hepatectomy was performed on 56 hepatocellular carcinoma patients by March 2026.

Table 1: Levels of parameters under study to hepatic cancer patients and controls

Parameter	HC Patients n=56	Controls n=64	P-value
Age *< 60* *≥ 60*	43 13	52 12	0.090
Sex *Male* *Female*	41 15	34 30	0.000
Family history of HC *With HC* *Without HC*	3 53	3 61	0.400
Smoking *None S.* *With S.*	26 30	49 15	0.000
Hepatitis B *With H.* *Without H.*	43 13	13 51	0.000
Cirrhosis history *With C.* *Without* C.	35 21	no	no

P ≤ 0.05; statically significant. no; No significant.

Table P>0.05 shows that controls' CD35 genotype distribution matches Hardy-Weinberg equilibrium. Among the three CD35 single nucleotide polymorphisms, only the GG, CG, and CC genotypes of rs7525160 exhibited statistically significant frequencies (P<0.05). After adjusting for sex, smoking, and hepatitis B, individuals with at least one mutant allele C (CG+CC) exhibited a 1.58-fold increased likelihood of acquiring HC compared to those with the GG genotype (adjusted 95% CI [0.62-0.37]; P=0.01). The HC and control groups had comparable rs2296160 and rs3849266 genotypes (P>0.05) as shown in Table 2 and Figure 2.

Table 2: Reference SNPs of CD_{35} protein-gene in hepatic cancer patients and controls.

Reference SNPs	Genotype	HC Patients n=56	Controls n=64	(95% CI)[a]	P-value
rs2296160	AG GG/AG	41 15	34 30	0.73 0.26	0.40 0.70
rs3849266	CT TT/CT	26 30	49 15	0.46 0.53	0.50 0.60
rs7525160	CG CC/CG	35 21	29 35	0.62 0.37	0.05* 0.01*

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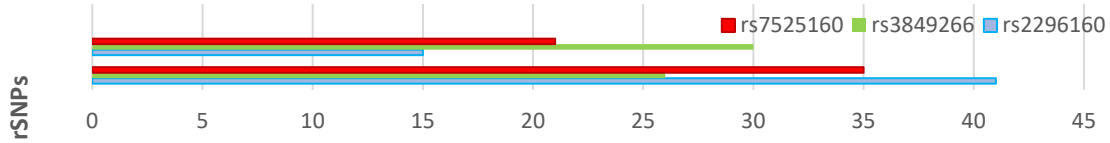
a:Adjusted for gender, smoking, and Hepatitis B ... *P ≤ 0.05 as a significant.



	HC Patients	Controls	
rs7525160	35	21	
rs3849266	26	30	
rs2296160	41	15	

Mutations

Figure 1: comparision of mutations among (rs7525160, rs3849266, and rs2296160) in hepatic cancer (HC) patients.

Chronic inflammation and the complement cascade, together with immunological and inflammatory responses, contribute to the development of cancer. Innate immunity depends on CD35, which suppresses C3 and C5 convertases in both classical and alternative routes to mitigate inflammation and limit the activation of the complement cascade (24). Additionally, it facilitates the degradation of these enzymes. Therefore, it is not implausible that the CD35 genetic polymorphism in the complement system could elevate the risk of cancer. Polymorphisms of the CD35 gene have been shown to be significantly related with a wide range of cancers including ovarian, small cell lung, nasopharyngeal, gallbladder, bladder, non-Hodgkin's lymphoma and gastric cancers in many studies (25, 26).

Not much is known about the link between CD35 polymorphisms and HC, however. We found the first evidence of an association between HC risk and one of the three CD35 tag SNPs (rs7525160) in an Iraqi sample. It should be noted that compared to the GG genotype, the CC/CG genotype of CD35 rs7525160 was linked to a higher risk of HC development.Genetic mutation-related diseases tend to manifest at younger ages. This is because, as demonstrated in multiple studies involving young patients (27), the interplay between genes and the environment may enhance sensitivity to cancerous tumors. Our results supported recent findings that the CD35 rs7525160 CC/CG genotype enhanced HC in individuals under 60. Better treatment and screening of high-risk groups have reduced recurrence and mortality (28), but HC is still the second leading cause of cancer deaths worldwide (29). There have been reports of AFP, tumor number, and tumor size as liver cancer prognostic variables; however, no practical indicators have been discovered as of yet (31).

Additionally, the subgroup analysis showed a significant difference in CD35 rs7525160 C allele genotype frequency between the control group and HC patients. Tumor sizes above 5 cm, TNM stages III/IV, cirrhosis, portal vein thrombosis, and AFP values over 400 ng/ml were notably indicative of this difference.

This data points to a possible association between the CC/CG genotype of CD rs7525160 and the kind of HC associated with a poor prognosis (32). We limited our analysis to 56 untreated patients who had hepatectomy in order to exclude the impact of different therapies on the HC prognosis. Our focus was on the connection of CD35 with RFS/OS. Through the use of Cox analysis, we demonstrated that rs7525160 stood out as a distinct factor predicting RFS in HC patients.

A significant association was identified between the CD35 rs7525160 CC/CG genotype and a reduced mean RFS in HC patients. The research revealed no association between CD35 polymorphism and overall survival (OS). This may be because several variables, including the patient's socioeconomic position and the therapy selected upon recurrence, impact both the OS and the postoperative recurrence of HC. It is necessary to rectify many issues with this research. Additionally, we have limited our results to the Iraqi population. Nonetheless, there is a substantial

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ethnic difference in the patterns of CD35 polymorphism allele frequency. Thus, our results are not applicable to other populations. Second, our work only examined CD35 polymorphism, HC susceptibility, and genetic prognosis; functional confirmation is required.Third, a bigger sample size is needed to study genetic polymorphism and HC metastasis.

Conclusions

Finally, among Iraqi participants younger than 60 years old, we observed that CD35 rs7525160 may be linked to heightened HC susceptibility. Moreover, the CD35 rs7525160 CC/CG genotype was associated with a pathological type and an independent risk factor for a shorter RFS time following hepatectomy, a bad prognosis for HC. Our findings also suggest that CD35 rs7525160 may screen and predict HC. If we want to know for certain how the CD35 polymorphism impacts HC risk and prognosis, we may need additional well-designed research with larger samples, longer follow-up periods, and individuals of different ethnicities.

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