Scientific Foundation SPIROSKI, Skopje, Republic of Macedonia
Open Access Macedonian Journal of Medical Sciences. 2022 Apr 14; 10(B):896-899.
https://doi.org/10.3889/oamjms.2022.9095
eISSN: 1857-9655
Category: *B - Clinical Sciences*
Section: *Gastroenterohepatology*

Association between CTLA-4 Gene Polymorphism and Risk of Hepatocellular Carcinoma in Chronic Hepatitis B Patients

Gontar Alamsyah Siregar[1]*, Zaimah Z. Tala[2]

[1]*Department of Internal Medicine, Faculty of Medicine, Universitas Sumatera Utara, Medan, Indonesia;* [2]*Department of Nutrition, Faculty of Medicine, Universitas Sumatera Utara, Medan, Indonesia*

Edited by: Ksenija Bogoeva-Kostovska
Citation: Siregar GA, Tala ZZ. Association between CTLA-4 Gene Polymorphism and Risk of Hepatocellular Carcinoma in Chronic Hepatitis B Patients. Open Access Maced J Med Sci. 2022 Apr 14; 10(B):896-899. https://doi.org/10.3889/oamjms.2022.9095
Keywords: Polymorphism; CTLA4-1661G>A; Hepatocellular carcinoma; Chronic Hepatitis B
***Correspondence:** Gontar Alamsyah Siregar, Department of Internal Medicine, Faculty of Medicine, Universitas Sumatera Utara, Medan, Indonesia.
E-mail: gontarsir@gmail.com
Received: 23-Feb-2022
Revised: 25-Mar-2022
Accepted: 04-Apr-2022

Funding: This research did not receive any financial support
Competing Interests: The authors have declared that no competing interests exist

Abstract

BACKGROUND: Hepatocellular carcinoma (HCC) is the most common form of liver cancer; most cases of HCC (approximately 80%) are associated with chronic hepatitis B virus (HBV) and prolonged latency to HCC development. Association CTLA-4-1661G > A had ever been reported in autoimmune cases. However, the association of CTLA-4-1661G > A polymorphism with HCC and chronic hepatitis B risk is hardly reported.

AIM: We aimed to investigate association between CTLA-4 gene polymorphism and risk of hepatocellular carcinoma in chronic hepatitis B patients.

METHODS: The study was conducted in May–November 2020 at Haji Adam Malik General Hospital, Medan, Indonesia. Eighty individuals were analyzed with a case–control study. The data analysis was performed with SPSS 18.0 software. Odds ratio (OR) with 95% confidence interval (CI) was used to evaluate the association of CTLA-4 polymorphism with the risk of HCC and chronic hepatitis B, which was calculated by Chi-squared independent test. Real-time PCR was used to examine CTLA-4 gene -1661G>A polymorphism. Genomic DNA was extracted from 5 ml frozen whole blood using the DNA Extraction Kit (Qiagen, Germany) according to the manufacturer's protocol.

RESULTS: There is an association between the CTLA-4-1661G>A polymorphism and the risk of HCC in chronic hepatitis B patients. Chronic hepatitis B patients with GG genotype had 2.55 times increased risk of developing HCC compared to GA + AA genotypes (p = 0.018). Hepatitis B patients with the G allele had 1.73 times increased risk of developing HCC compared to the A allele (p = 0.005).

CONCLUSION: CTLA4-1661G>A polymorphism is associated with the incidence of hepatocellular carcinoma in chronic hepatitis B patients.

Introduction

According to the International Agency for Research on Cancer, liver cancer is the fifth most common cancer in men worldwide (523,000 cases per year, 7.9% of all cancers) and the seventh in women (226,000 cases per year, 6.5% of all cancers). Hepatocellular carcinoma (HCC) is the most common form of liver cancer; most cases of HCC (approximately 80%) are associated with chronic hepatitis B virus (HBV) or hepatitis C virus (HCV) infections [1].

Hepatocellular carcinoma (HCC) is the most common form of liver cancer and accounts for ~90% of cases. Hepatitis B virus (HBV) infection is the most prominent risk factor for HCC development, accounting for ~50% of cases [2], [3], [4]. There is a high ecological correlation between areas of HBV prevalence and HCC incidence and mortality worldwide. Chronic HBV infection accounts for approximately 50% of the total cases and virtually all of childhood HCC; HB surface antigen (HBsAg) seroprevalence among persons with HCC varies widely: It is 3% in Sweden, 10% in the United States, 10%–15% in Japan, 19% in Italy, 55% in Greece, and 70% in South Korea. However, the HBV-related incidence of HCC is projected to increase for several decades, because of the high prevalence of chronic HBV infection and prolonged latency to HCC development [1].

Cytotoxic T-lymphocyte antigen-4 (CTLA-4) encoded by CTLA-4 belongs to the immunoglobulin supergene family. Its gene is located on chromosome 2 in q33, which is expressed on the surface of T cells in form of dimers. The capacity of CTLA-4 is for the most part to send inhibitory signals to adversely control the multiplication of lymphocytes and square the invulnerable reactions. Hereditary varieties or articulation problems of CTLA-4 might prompt a lessened inhibitory impact on the initiation of immune system microorganisms, in the meantime, immune system injury was enacted. As of late, studies concerning the connection between CTLA-4 polymorphisms and a few infections stand out. Association CTLA-4- 1661G> A had ever been reported in autoimmune cases [2]. However, the association of CTLA-4-1661G>A polymorphism with

HCC in chronic hepatitis B risk is hardly reported. We aimed to investigate association between CTLA-4 gene polymorphism and risk of hepatocellular carcinoma in chronic hepatitis B patients.

Methods

Population and research design

The study was conducted in May–November 2020 at Haji Adam Malik General Hospital, Medan, Indonesia. A total of 80 individuals were analyzed with a case–control study. The case group was HCC patients confirmed by hypervascular findings on arterial and portal vein phase imaging or delayed phase on three-phase CT scan. Patients with cholangiocarcinoma, hemangioma, liver metastases, or who underwent HCC treatment were excluded from the study. Meanwhile, the control group is healthy individuals who come for general examination or workers at Haji Adam Malik General Hospital.

Sample preparation

Real-time PCR was used to examine CTLA-4 gene -1661G>A polymorphism. Genomic DNA was extracted from 5 ml of frozen whole blood using the DNA Extraction Kit (Qiagen, Germany) according to the manufacturer's protocol.

DNA extraction

DNA extraction (spin column method) using high pure PCR template preparation reagent kit (Roche Applied Science), Primer and Probe: TaqMan SNP Genotyping Assay CTLA-4-1661 G>A, and Applied Biosystem.

Genotyping

Genotyping of the CTLA-4-1661G>A gene using forward primer: 5'-CTAAGAGCA TCCGCTTGCACCT-3' and reverse primer: 5' TTGGTGTGATGCACAGAAGCCTTTT-3'.

Statistical analysis

The data analysis was performed with SPSS 18.0 software. Odds ratio (OR) with 95% confidence interval (CI) was used to evaluate the association of *CTLA-4* polymorphism with the risk of HCC and chronic hepatitis B, which was calculated by Chi-squared independent test.

Table 1: Demographic characteristics of hepatitis B patients with and without HCC

Variables	HCC	Chronic hepatitis B	p-value
Gender, n (%)			
Male	31 (77.5%)	21 (52.5%)	0.019*
Female	9 (22.5%)	19 (47.5%)	
Age, years, mean (SD)	56.98 (12.05)	45.95 (11.02)	< 0.001*
BCLC stages, n (%)			
A	2 (5%)	NA	NA
B	7 (17.5%)		
C	13 (32.5%)		
D	18 (45%)		

*p < 0.05.

Results

Table 1 shows the demographic characteristics of hepatitis B patients with and without HCC. There was a relationship between male gender and the risk of HCC in chronic hepatitis B patients (p = 0.019). The mean age was significantly higher in chronic hepatitis B patients with HCC than without HCC (p < 0.001). The most common BCLC stage in HCC is Stage D (45%).

Association between CTLA-4 gene polymorphism and hepatocellular carcinoma in chronic hepatitis b patients

In the present study, the relationship between CTLA-4-1661G>A polymorphism and hepatocellular carcinoma in chronic hepatitis B was analyzed with linear regression analysis. p < 0.05 shows a significant difference. We made a comparison in genotypes and alleles frequencies of CTLA-4 Gene polymorphism between hepatocellular carcinoma and chronic hepatitis B patient. The result showed that frequencies of homozygous genotype G and G allele were remarkably higher in hepatocellular carcinoma than chronic hepatitis B. There is an association between GG genotype and G allele of CTLA-4-1661G > A polymorphism with the risk of HCC in chronic hepatitis B patients (Table 2).

Table 2: Association between CTLA-4 gene polymorphism in hepatocellular carcinoma and chronic hepatitis B patients

CTLA-4-1661G>A polymorphism	HCC (%)	CHB (%)	p value	OR (95% CI)
GG	12 (30)	1 (2.5)	0.001*	3.6 (1.61–7.90)
GA	20 (50)	25 (62.5)	0.234	1.6 (0.69–3.78)
AA	8 (20)	14 (35)		
GG+GA	32 (80)	26 (65)	0.055	2.1 (0.9–4.6)
AA	8 (20)	14 (35)		1 (ref.)
GG	12 (30)	1 (2.5)	0.018*	2.55 (1.73–3.8)
GA+AA	28 (70)	39 (97.5)		
G allele	44 (55)	27 (33.75)	0.005*	1.73 (1.15–2.7)
A allele	36 (45)	53 (66.25)		1 (ref.)

*p < 0.05.

Discussion

Our study showed that there was a relationship between male gender and the risk of HCC in chronic hepatitis B patients (p = 0.019). The mean age was

significantly higher in chronic hepatitis B patients with HCC than without HCC ($p < 0.001$). The most common BCLC stage in HCC is stage D (45%). Bouqis *et al*. also show that men are at increased risk for HCC partly because they have a greater incidence of viral hepatitis and alcoholic cirrhosis. The age distribution of HCC patients has shifted to younger ages, with the greatest proportional increases among individuals 45–60 years old [5].

The occurrence and development of HCC are a complex biological process influenced by many factors. The environmental factors are the external causes, whereas genetic susceptibility is an important internal cause and largely determines the risk degree of developing liver cancer among individuals. Human genes have abundant polymorphisms. Genetic mutations may lead to abnormal expressions of corresponding proteins. As a result, each individual may react differently to toxins or carcinogens, and thus may have different liver cancer susceptibility [6]. Cytotoxic T-lymphocytes (CTLs) play a major role in the fight against acute hepatitis B infection and the removal of viral infection from hepatocytes. In patients with chronic hepatitis B infection, viral T-specific cells did not have high activity, which would cause the production and secretion of adequate antiviral cytokines, inappropriate functioning of cytotoxic T lymphocytes, and thus the persistence of the virus in the body [7]. The determination of genetic polymorphisms is a new means to study the etiology of polygenetic disorders with complex inheritance patterns, such as cancer, diabetes, and hypertension [6], [7], [8].

T-cells play an important role in immune response and molecules that mediate regulation of T-cell activity could influence cancer susceptibility. CTLA-4 is one of the most studied negative regulators. The molecule is homologous to CD28, but with the opposite effect on T-cell activation [1]. Inhibition of CTLA-4 in T-cell activation is executed by two separate mechanisms. One mechanism involves competitive binding with CD28 for B7 on the antigen-presenting cell (APC). The other mechanism involves direct intracellular inhibitory signals mediated by the CTLA-4 cytoplasmic tail, which occurs in the early stages of an immune response [5].

One of the mechanisms adopted by tumor cells to evade the antitumor immune response is the upregulation of CTLA-4 expression. rs4553808 (CTLA-4-1661G > A) and rs733618 (CTLA-4-1722T > C) are located upstream of regulation and can affect binding to CCAAT C/EBPβ protein transcription. Studies have shown that the conversion of adenine to guanine at the -1661 site can lead to the formation of a new binding site for C/EBPβ which may lead to the CTLA-4c-1661A > G allele being considered a risk factor for cancer [9].

This study showed a statistically significant association between CTLA-4-1661G>A polymorphism and the incidence of HCC in chronic hepatitis B patients. Chronic hepatitis B patients with GG genotype had 2.55 times increased risk of developing HCC compared to GA + AA genotypes ($p = 0.018$). Hepatitis B patients with the G allele had 1.73 times increased risk of developing HCC compared to the A allele ($p = 0.005$). Samaneh *et al*. also analyzed that the AA genotype was more common in the control group than patients ($p = 0.0003$; OR = 0.15, 95% CI = 0.05–0.42) with the "A" allele significantly more common in controls than patients ($p = 0.0003$) [9]. On the other hand, GA+AA genotypes and A allele are significantly higher in the chronic hepatitis B group than in the HCC group.

Yang *et al*. found that the CTLA-4 rs3087243 G>A polymorphism was associated with an increased risk of HCC, with a potential association with the AA/GA versus GG genotype [10]. In this meta-analysis, a significant association was found between the CTLA-4-1661A/G polymorphism and an increased risk of cancer in the heterozygous, dominant, and allele models. The difference in the results of this study is due to differences in ethnicity and geography [11]. In a previous study, CTLA4 polymorphisms may be used as non-invasive biomarkers in identifying the right patients for immunotherapy, and in predicting and monitoring the treatment response of HBV-related liver disease including HCC is an interesting issue to be investigated [12]. We hope that advanced sequence analysis of the host genome will provide a better understanding of the host-virus and the genetic factors involved in the development of HCC. Further research is needed to evaluate and understand the role of host-HBV interactions in HBV-related HCCs to develop effective diagnoses and treatments.

This study is limited by the relatively small sample size of the patient population. The findings were based on patient data from a single center. The CTLA4 polymorphisms may vary ethnically and this study only examined the role in the Indonesian population.

Conclusions

The CTLA-4-1661G>A polymorphism is significantly associated with the risk of HCC in chronic hepatitis B patients

References

1. El-Serag HB. Epidemiology of viral hepatitis and hepatocellular carcinoma. Gastroenterology. 2012;142(6):1264-73. http://dx.doi.org/10.1053/j.gastro.2011.12.061
PMid:22537432
2. Llovet JM, Kelley RK, Villanueva A, Singal AG, Pikarsky E,

Roayaie S, *et al*. Hepatocellular carcinoma. Nat Rev Dis Primers. 2021;7(1):6. https://doi.org/10.1038/s41572-020-00240-3

3. Darmadi D, Ruslie RH. Association between prothrombin induced by Vitamin K absence-II (PIVKA-II) and Barcelona clinic liver cancer (BCLC) stage, tumor size, portal venous thrombosis in hepatocellular carcinoma patients. Sains Malays. 2021;50(2):475-80. http://doi.org/10.17576/jsm-2021-5002-18
4. Darmadi D, Ruslie RH, Pakpahan C. Vascular endothelial growth factor levels difference among hepatocellular cancer patients based on Barcelona clinic liver cancer staging. Open Access Maced J Med Sci. 2021;9:797-800. https://doi.org/10.3889/oamjms.2021.6598
5. Bouqbis L, Izaabel H, Akhayat O, Pérez-Lezaun A, Calafell F, Bertranpetit J, *et al*. Association of the CTLA4 promoter region (-1661G allele) with Type 1 diabetes in the South Moroccan population. Genes Immun. 2003;4(2):132-7. http://dx.doi.org/10.1038/sj.gene.6363933
6. Gu X, Qi P, Zhou F, Ji Q, Wang H, Dou T, *et al*. +49G > A polymorphism in the cytotoxic T-lymphocyte antigen-4 gene increases susceptibility to hepatitis B-related hepatocellular carcinoma in a male Chinese population. Hum Immunol. 2010;71(1):83-7. http://dx.doi.org/10.1016/j.humimm.2009.09.353

   PMid:19778566
7. Ghorbani P, Mollaei HR, Arabzadeh SA, Zahedi MJ. Upregulation of single nucleotide polymorphism of PD-1 gene (rs10204525) in chronic hepatitis B patients. Int Arch Med Microbiol. 2019;2(1):1-8. http://dx.doi.org/10.23937/2643-4008/171000
8. Liu Z, Song Z, Sun J, Sun F, Li C, Sun J, *et al*. Association between CTLA-4 rs231775 polymorphism and hepatocellular carcinoma susceptibility. Int J Clin Exp Pathol. 2015;8(11):15118-22.

   PMid:26823853
9. Wagner M, Jasek M, Karabon L. Immune checkpoint molecules-inherited variations as markers for cancer risk. Front Immunol. 2021;11:606721. https://doi.org/10.3389/fimmu.2020.606721

   PMid:33519815
10. Yang J, Liu J, Chen Y, Tang W, Liu C, Sun Y, *et al*. Association of CTLA-4 tagging polymorphisms and haplotypes with hepatocellular carcinoma risk: A case-control study. Medicine (Baltimore). 2019;98(29):e16266. http://dx.doi.org/10.1097/md.0000000000016266

    PMid:31335675
11. Yan Q, Chen P, Lu A, Zhao P, Gu A. Association between CTLA-4 60G/A and-1661A/G polymorphisms and the risk of cancers: A meta-analysis. PLoS One. 2013;8(12):e83710. http://dx.doi.org/10.1371/journal.pone.0083710
12. Wang L, Li N, Fan X, Wang X, Zhang X, Zhang K, *et al*. Circulating CTLA-4 levels and CTLA4 polymorphism associate with disease condition and progession and hepatocellular carcinoma patients' survival in chronic hepatitis B virus infection. Int Immunopharmacol. 2020;82:106377. http://doi.org/10.1016/j.intimp.2020.106377

    PMid:32163858