The relationship between hepatitis C - related hepatocellular carcinoma and *IL-23 receptor* gene polymorphism

**Said Abdel Baky** **Gad1\*, Ashraf Khalifa1, Samia Hussein2,3\*, Abeer A. Abdelrahman2, Ola M. Elfarargy4, 5, and Ahmad I. Elagrody1**

1 Department of Internal Medicine, Gastroenterology and Hepatology, Faculty of Human Medicine, Zagazig University, Egypt.

2 Medical Biochemistry& Molecular Biology Department, Faculty of Human Medicine, Zagazig University, Egypt.

3Department of Basic Medical Sciences, Ibn Sina University for Medical Sciences, Amman, Jordan.

4Medical Oncology Department, Faculty of Human Medicine, Zagazig University, Egypt.

5Armed Force College of Medicine, Egypt.

\* The corresponding author

Address of the corresponding author: Medical Biochemistry and Molecular Biology Department, Faculty of Medicine, Zagazig University, Zagazig, Egypt.

**E-mail of the corresponding author:** samiahussein82@hotmail.com.

**Running head:** *IL-23R* polymorphism andHCV- related HCC.

**DOI:** [**10.21608/AJGH.2022.151904.1009**](10.21608/AJGH.2022.151904.1009)**.**

**Type of manuscript:** original research.

**Conflict of interest:** N / A.

**Funding source:** This research received no specific grant from any funding agency in the public, commercial, or not-for-profit sectors.

**Date of submission:** 22- July-2022.

**Revised:** 02-October-2022.

**Accepted:** 05-October-2022.

**First online:** 08-October-2022.

**Abstract:**

**Aims:** This study evaluated the relationship between *developing interleukin-23 receptor (IL- 23R) gene polymorphism and hepatitis C-related hepatocellular carcinoma (HCC)* in Egyptian patients.

**Patients & Methods:** The current study included 250 subjects. They were divided into three groups: one hundred patients with hepatitis C virus (HCV) infection without HCC, one hundred patients with HCV and HCC, and fifty healthy non-hepatic volunteers as a control group. *IL- 23R* gene polymorphism (rs10889677) was genotyped by restriction fragment length polymorphism- polymerase chain reaction (RFLP- PCR).

**Results:** There was a significant difference between the studied groups regarding the frequency of genotypes and alleles (P =0.006 and < 0.001, respectively). Additionally, under the recessive inheritance model, IL-23R polymorphism is significantly associated with HCC development (P=0.001).  Moreover, a significant protective effect of the rs10889677 C allele in HCC susceptibility was detected (OR = 0.15, 95% CI = 0.05–0.39, P < 0.001). AC and CC genotypes also had a significant protective effect (OR = 0.16, 95% CI = 0.05–0.46, P < 0.001).

 **Conclusions:** there is a possible relationship between IL- 23R (rs10889677) polymorphism and HCC risk in Egyptian individuals where AC and CC genotypes and C alleles are protective.

**Keywords:** HCC; HCV; *IL-23R;* gene polymorphism.

**Introduction**

Egypt is considered to have the highest hepatitis C virus (HCV) infection prevalence worldwide. The Demographic Health Survey (DHS) of 2015 showed a seroprevalence of 10% among the age group between 15 and 59 years [1]. The most common HCV genotype among the Egyptian population is genotype 4, representing 2-3% of world genotypes [2]. Hepatocellular carcinoma (HCC) is a highly heterogeneous tumor complicating chronic HCV infection and is mainly initiated by many genetic changes [3]. HCC is the world's third and sixth most prevalent cause of cancer death in men and women, respectively [4].

HCC usually develops on top of liver cirrhosis which results from several factors, including alcohol abuse, infection, especially HCV and HBV, and nonalcoholic liver disease [5]. Besides, several genetic and environmental variables contribute to this condition. For example, several polymorphisms in cytokine genes were reported to associate with an increased vulnerability to infection with HCV or an increased tendency to HCC progression [6,7].

The interleukin-23 receptor (IL- 23R) shares the same site as the IL-12R. It triggers memory T helper (Th) 17 cell-mediated inflammatory activity [8], which is essential in the inflammatory and immunological surveillance of HCV and HBV carcinogenesis [9, 10]. Many studies have investigated the role of *IL-23R* gene polymorphism in different disorders, such as inflammatory bowel disease [11], rheumatoid arthritis [12], and stomach cancer [13]. However, there is currently no solid evidence relating the *IL-23R* gene polymorphism to HCV-related HCC development. As a result, our current research focused on this variation in the gene and how it leads to HCC in patients with HCV in Egypt.

**Materials and Methods**

This case-control study was carried out at the Department of Internal Medicine, Zagazig University, in collaboration with the Departments of Medical Biochemistry& Molecular Biology and Medical Oncology, Zagazig University, from January 2021 to January 2022. The study was performed after receiving approval from the Institutional Review Board of the Faculty of Medicine, Zagazig University. Patients involved in the study gave written consent.

This study included 250 subjects: one hundred having HCV and newly diagnosed with HCC and one hundred having HCV without HCC. Another 50 healthy subjects without a history of liver disease were included as a control group.

All patients with liver disease other than HCV were excluded from the study. Also, subjects with HIV or other malignancies were excluded from the study.

HCV infection was diagnosed by antibody positivity by enzyme-linked immunosorbent assay (ELISA) (Anaheim, CA 92801, USA). HCV RNA was detected by real-time polymerase chain reaction (COBAS amplifier, TaqMan 48, Roche Mannheim, Germany). History taking and general examination were performed for all participants. Then, the patients were subjected to the following investigations: total serum bilirubin, total protein, serum albumin, alanine transaminase (ALT), aspartate transaminase (AST), α-fetoprotein (AFP), prothrombin time (PT), and platelet count. HCC was diagnosed by ultrasonography and typical radiological criteria in triphasic magnetic resonance imaging (MRI) and triphasic computerized tomography (CT).

Ten millimeters of venous blood was withdrawn, and genomic DNA was isolated from ethylene diamine tetra-acetic acid (EDTA) collected blood using a genomic DNA Mini kit (Geneaid, Taiwan). Extracted DNA was checked for quality and quantity using Nanodrop spectrophotometry (ND 1000-NanoDrop®). The samples were stored at - 20°C till further analysis.

The restriction fragment length polymorphism-polymerase chain reaction (RFLP-PCR) method was used to investigate the polymorphism in the *IL-23R* gene (rs10889677). We used forward primer 5′-ATCGTGAATGAGGAGTTGCC -3′ and reverse primer 5′- TGTGCCTGTATGTGTGACCA -3′. The PCR cycling conditions were 5 minutes at 94°C followed by 35 cycles of 1 minute at 94°C, 1 minute at 65°C, and 2 minutes at 72°C, with a final step at 72°C for 20 minutes. A 10-μL PCR product was digested at 37°C for 4 hours in a 15-μL reaction containing 5 U of MnlI (New England Biolabs, Beverly, MA). Digested products were separated on a 2.5% agarose gel stained with ethidium bromide. The detected genotypes were AA (286, 185bp), AC (286, 225, 185, 61bp), and CC (225, 185, 61bp) [14].

**Statistical analysis:**

 The sample size was calculated using Epi Info program 6 (Atlanta, Georgia, USA). The statistical package SPSS Version 20 inc. (Chicago, USA) was used to analyze the collected data. Quantitative data were represented as mean± standard deviation. The Chi-square test (χ2) was used to compare proportions as appropriate. T-test was used to detect the difference between two groups and one-way ANOVA for multigroup comparisons. A *P* Value < 0.05 was considered statistically significant at a 95% confidence interval.

**Results**

There were no significant differences in age, sex, or mean age at 1st HCV diagnosis between the examined groups. Compared to the control group, the patient groups demonstrated substantial elevations in total bilirubin, ALT, AST, ALP, GGT, and AFP. In contrast, they showed significant declines in protein and serum albumin. Comparing HCV cases with and without HCC, there were substantial differences in protein, albumin, ALT, and AFP, as well as highly significant differences in AST and GGT. In contrast, no significant difference in total bilirubin or PT was detected (Table 1).

Table 1: **The demographic and laboratory characteristics of the diseased and control groups.**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Parameters** | **Control group (n=50)** | **HCV patients****without HCC (n=100)** | **HCV patients****with HCC (n=100)** | ***P***  |
|  **Age (years)**  | 56.65±5.25 | 55.62±7.31 | 57.68±7.42 | 0.130 |
| **Sex** *Male**Female* | 28 (56%)22 (44%) | 55 (55%)45 (45%) | 57 (57%)43 (43%) | 0.960 |
| **Age at first HCV diagnosis (years)** |  | 36.26±5.46 | 37.49±6.70 | 0.164 |
| **Total Bilirubin (mg/dL)** | 0.82±0.21 | 1.03±0.31a | 1.13±0.47a | **<0.001\*\*** |
| **Total Protein (g/dL)** | 7.21±0.82 | 6.80±1.34a | 6.49±1.44ab | **0.001\*** |
| **Albumin (g/dL)** | 4.02±0.41 | 2.68±0.93a | 2.27±0.82ab | **<0.001\*\*** |
| **ALT (U/L)** | 21.94±13.60 | 68.39±17.20a | 76.53±19.06ab | **<0.001\*\*** |
| **AST (U/L)** | 17.61±9.58 | 66.44±14.32a | 83.74±23.38ab | **<0.001\*\*** |
| **ALP (U/L)** | 91.88±28.63 | 193.85±56.86a | 203.12±69.32a | **<0.001\*\*** |
| **GGT (U/L)** | 22.87±7.00 | 46.66±16.79a | 56.86±17.72ab | **<0.001\*\*** |
| **AFP (ug/L)** | 4.74±0.41 | 8.65±2.47a | 146.45±130.80ab | **<0.001\*\*** |
| **PT (seconds)** | 12.60±1.61 | 12.58±2.54 | 13.20±1.80a | 0.04\* |

 a Against controls; b HCV without HCC against HCV with HCC; \*: significant P<0.05; \*\*: highly significant P<0.001

The frequency of genotypes of IL-23R (rs10889677) gene polymorphism in the studied groups is presented in figure 1. Our results revealed a significant difference in the frequency of genotypes among the three studied groups (P=0.006). However, using Chi for trend, there was no significant difference in genotypes between HCV without the HCC group and the control group (P=0.369). At the same time, there were substantial differences in genotypes between HCV with the HCC group and the control group and HCV without the HCC group (P=0.001 and 0.01, respectively) (Table 2).

Regarding allele frequency, our results revealed a significant difference between the studied groups (P<0.001). Using Chi for trend, there was no significant difference in allele frequency between HCV without the HCC group and the control group (P=0.145). However, there were substantial differences in allele frequency between HCV with the HCC group and both the control group and HCV without the HCC group (P<0.001 and 0.001, respectively) (Table 2).

Under the recessive inheritance model, IL-23R polymorphism was significantly associated with HCC development (P=0.001), with a considerable difference between HCV individuals with HCC and HCV patients without HCC (P = 0.009). Also, a highly significant difference was detected between HCV individuals with HCC and control subjects (P<0.001) (Table 2).

Table 2: **Different genotypes and allelic frequencies of the IL23R (****rs10889677 A>C) gene polymorphism.**

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| **Genotype** | **Control group (n=50)** | **HCV patients without HCC (n=100)** | **HCV patients with HCC (n=100)** | ***P*** | **Chi for trend** | **OR (95% CI)** | ***P*** |
| ***Allele frequency******A******C*** | 83 (83%)17 (17%) | 178 (89%)22 (11%) | 194 (97%)6 (3%) | <0.001\*\* | 0.145$<0.001@\*\*0.001#\* | 0.60 (0.30–1.19) @0.15 (0.05-0.39) # | 0.07<0.001\*\* |
| **Genotypes*****AA******AC******CC*** | 36 (72%)11 (22%)3 (6%) | 82 (82%)14 (14%)4 (4%) | 94(94%)6 (6%)0 (0%) | 0.006\* | 0.369$<0.001@\*\*0.01#\* |   Reference0.55 (0.23–1.34) @0.58 (0.12–2.75) @0.20 (0.07-0.60) #**------------------#** | 0.1030.2570.001\*----- |
| ***Recessive model******AA******AC + CC*** | 36 (36%)14 (28%) | 82 (82%)18 (18%) | 94 (94%)6 (6%) | 0.001 | 0.158$<0.001@\*\*0.009#\* | Reference 0.56 (0.25-1.25) @0.16 (0.05-0.46) # | 0.08<0.001\*\* |

$:HCV patients without HCC against control group; @ HCV with HCC group against control group; #: HCV with HCC group against HCV without HCC; OR: Odds Ratio; \*: significant; \*\*: highly significant

  A significant protective effect of the rs10889677 C allele in HCC susceptibility was detected (OR = 0.15, 95% CI = 0.05–0.39, P < 0.001). AC and CC genotypes also had a significant protective effect (OR = 0.16, 95% CI = 0.05–0.46, P < 0.001).

**Discussion**

HCC is the most conflicting complication of HCV [15,16]. Many treatment modalities are present at the time but with poor prognosis, especially with an aggressive form of HCC [17]. Therefore, there was an urgent need to study the association between genetic variations and the occurrence of HCC [18]. *IL-23R* polymorphism has been studied extensively with autoimmune and inflammatory diseases such as rheumatoid arthritis [12]. Besides, *IL-23R* gene polymorphisms were documented to be closely associated with the development of cancers such as stomach cancer [13]. However, other studies stated that IL-23 is a protective cytokine [19]. So, we studied the correlation between *IL-23R* single nucleotide polymorphism (SNP) rs10889677 and the occurrence of HCV-related HCC in Egyptian patients.

Our study revealed that *IL-23R* gene polymorphism (rs10889677) was significantly associated with HCC risk development. This finding is similar to that found by Amer et al. (2017) and Pan and Wang (2019) [21]. In our study, AC and CC genotypes and C alleles seem protective against HCC development. Similarly, a significant association between the A allele and ankylosing spondylitis susceptibility was detected [22].

The impact of IL-23 in carcinogenesis is not well understood, most probably tissue-specific. Wendling showed that IL-23 is a carcinogenic cytokine [23], contrary to other studies that stated that IL-23 is a protective cytokine [19]. IL-23 is an innate immune-suppressive cytokine in several carcinogenesis models in rats. It was demonstrated that IL-23 and IL-23R play a crucial role in T helper 17 (Th-17) cell-mediated immunity, tumor-promoting proinflammatory operation, failure of CD8+T immune surveillance, and pathogenesis of cancers [24, 25]. Hori et al. and Kim et al. stated that the IL-23R signaling pathway in T cell regulatory state (Tregs) also enhances Tregs' immunosuppressive effect, leading to impaired immune response and promoting the invasion of the immune system by cancer [26,27]. From the previous links between the two kinds of T cells, IL-23R most probably play an essential role in cancer development and progression. In some experimental studies, genetic deletion or antibody-mediated elimination of IL-23R leads to increased settling of cytotoxic T cells into the tissue rendering an increased tendency to protect against carcinogenesis development [17]. Because IL-23 promotes and activates inflammatory processes and tumorigenesis, blocking IL-23 may benefit cancer development prevention and targeted cancer therapy [14].

**Limitations:**

The risk of HCC is affected by several factors other than genetic factors. Furthermore, screening for associations between genetic variants and HCC risk requires a large sample size and long-term follow-up. Additional research is needed, including a large population of different races and ethnicities using more polymorphic sites to translate these findings into clinical application.

**In conclusion,**this study revealed that *IL-23R* *rs10889677* AC and CC genotypes and C allele possibly protect Egyptian HCV-infected individuals against developing HCC.

**Footnotes.**

**Funding**

This research received no specific grant from public, commercial, or not-for-profit funding agencies.

**Declaration of competing interest**

There are no conflicts of interest related to this study.

**Authors' contributions:** All authors contributed equally to this work.

The institutional review board of Zagazig University, Faculty of Medicine, approved this research.

**Funding:** None

**Data Availability Statement:** Available upon reasonable request from the corresponding author.

**Conflicts of Interest:** The authors declare no conflict of interest.

All authors had direct exposure to the study data and read and agreed with the final text.

Peer-Reviewers: Ayman Sadek (Assistant professor of medicine), Marwa Shabana (lecturer of clinical pathology), Amr Shaaban Hanafy (professor of internal medicine), Amany Mohammed Abdallah (Assistant professor of community medicine).

**E- Editor:** Osama Ahmed Khalil, Salem Youssef Mohamed.

**Copyright ©.** This open-access article is distributed under the [Creative Commons Attribution License (CC BY)](AbdAllah%2C). The use, distribution, or reproduction in other forums is permitted, provided the original author(s) and the copyright owner(s) are credited. The original publication in this journal is cited by accepted academic practice. No use, distribution, or reproduction is permitted, complying with these terms.

**Disclaimer:** All claims expressed in this article are solely those of the authors and do not necessarily represent their affiliated organizations or those of the publisher, the editors, and the reviewers. Any product evaluated in this article or its manufacturer's claim is not guaranteed or endorsed by the publisher.

**References**

1. Ministry of Health and Population [Egypt], El-Zanaty and Associates [Egypt], ICF International Egypt Health Issues Survey 2015. Cairo, Rockville, MD: Ministry of Health and Population, ICF International; 2015.

2. Nguyen MH, Keeffe EB. Prevalence and treatment of hepatitis C virus genotype 4, 5, and 6. Clin Gastroenterol Hepatol. 2005; 3(10 Suppl 2): S97-S101. doi:10.1016/s1542-3565(05)00711-1

3. European Association for The Study of The Liver; European Organisation for Research and Treatment of Cancer. EASLEORTC clinical practice guidelines: management of hepatocellular carcinoma [published correction appears in J Hepatol. 2012 Jun; 56 (6):1430]. J Hepatol. 2012; 56(4):908-943. doi: 10.1016/j.jhep.2011.12.001

4. Karimkhanloo H, Mohammadi-Yeganeh S, Ahsani Z, Paryan M. Bioinformatics prediction and experimental validation of microRNA-20a targeting Cyclin D1 in hepatocellular carcinoma. Tumour Biol. 2017;39(4):1010428317698361. doi:10.1177/1010428317698361

5. Al-Qahtani AA, Al-Anazi M, Abdo AA, et al. Genetic variation at -1878 (rs2596542) in MICA gene region is associated with chronic hepatitis B virus infection in Saudi Arabian patients. Exp Mol Pathol. 2013;95(3):255-258. doi: 10.1016/j.yexmp.2013.08.005.

6. Yang YM, Kim SY, Seki E. Inflammation and Liver Cancer: Molecular Mechanisms and Therapeutic Targets. Semin Liver Dis. 2019;39(1):26-42. doi:10.1055/s-0038-1676806

7. Ma X, McKeen T, Zhang J, Ding WX. Role and Mechanisms of Mitophagy in Liver Diseases. Cells. 2020;9(4):837. Published 2020 Mar 31. doi:10.3390/cells9040837

8. Eken A, Singh AK, Treuting PM, Oukka M. IL-23R+ innate lymphoid cells induce colitis via interleukin-22-dependent mechanism. Mucosal Immunol. 2014;7(1):143-154. doi:10.1038/mi.2013.33

9. Dubinsky MC, Wang D, Picornell Y, et al. IL-23 receptor (IL-23R) gene protects against pediatric Crohn's disease. Inflamm Bowel Dis. 2007;13(5):511-515. doi:10.1002/ibd.20126

10. Xu Y, Liu Y, Pan S, et al. IL-23R polymorphisms, HBV infection, and risk of hepatocellular carcinoma in a high-risk Chinese population. J Gastroenterol. 2013;48(1):125-131. doi:10.1007/s00535-012-0620-1

11. Weersma RK, Zhernakova A, Nolte IM, et al. ATG16L1 and IL23R are associated with inflammatory bowel diseases but not celiac disease in the Netherlands. Am J Gastroenterol. 2008;103(3):621-627. doi:10.1111/j.1572-0241.2007. 01660.x

12. Song GG, Bae SC, Choi SJ, Ji JD, Lee YH. Associations between interleukin-23 receptor polymorphisms and susceptibility to rheumatoid arthritis: a meta-analysis. Mol Biol Rep. 2012;39(12):10655-10663. doi:10.1007/s11033-012-1955-7

13. Chen B, Zeng Z, Xu L, et al. IL23R +2199A/C polymorphism is associated with decreased risk of certain subtypes of gastric cancer in Chinese: a case-control study. Cancer Epidemiol. 2011;35(2):165-169. doi: 10.1016/j.canep.2010.08.006

14. Chien MH, Hsin CH, Chou LS, et al. Interleukin-23 receptor polymorphism as a risk factor for oral cancer susceptibility. Head Neck. 2012;34(4):551-556. doi:10.1002/hed.21779

15. Tarao K, Rino Y, Ohkawa S, et al. Association between high serum alanine aminotransferase levels and more rapid development and higher rate of incidence of hepatocellular carcinoma in patients with hepatitis C virus-associated cirrhosis. Cancer. 1999;86(4):589-595.

16. Yoshida H, Shiratori Y, Moriyama M, et al. Interferon therapy reduces the risk for hepatocellular carcinoma: national surveillance program of cirrhotic and noncirrhotic patients with chronic hepatitis C in Japan. IHIT Study Group. Inhibition of Hepatocarcinogenesis by Interferon Therapy. Ann Intern Med. 1999;131(3):174-181. doi:10.7326/0003-4819-131-3-199908030-00003

17. Parmiani G, Anichini A. T cell infiltration and prognosis in HCC patients. J Hepatol. 2006;45(2):178-181. doi: 10.1016/j.jhep.2006.06.005

18. Langowski JL, Zhang X, Wu L, et al. IL-23 promotes tumour incidence and growth. Nature. 2006;442(7101):461-465. doi:10.1038/nature04808

19. Volpe E, Servant N, Zollinger R, et al. A critical function for transforming growth factor-beta, interleukin 23 and proinflammatory cytokines in driving and modulating human T(H)-17 responses. Nat Immunol. 2008;9(6):650-657. doi:10.1038/ni.1613

20. Amer T, El-Baz R, Mokhtar AR, El-Shaer S, Elshazli R, Settin A. Genetic polymorphisms of IL-23R (rs7517847) and LEP (rs7799039) among Egyptian patients with hepatocellular carcinoma. Arch Physiol Biochem. 2017;123(5):279-285. doi:10.1080/13813455.2017.1320680

21. Pan X, Wang G. Correlations of IL-23R gene polymorphism with clinicopathological characteristics and prognosis of hepatocellular carcinoma patients after interventional therapy. *Genomics*. 2019;111(4):930-935. doi: 10.1016/j.ygeno.2018.05.023

22. Han R, Xia Q, Xu S, Fan D, Pan F. Interleukin-23 receptor polymorphism (rs10889677 A/C) in ankylosing spondylitis: Meta-analysis in Caucasian and Asian populations. *Clin Chim Acta*. 2018; 477:53-59. doi: 10.1016/j.cca.2017.11.038

23. Wendling D. Interleukin 23: a key cytokine in chronic inflammatory disease. Joint Bone Spine. 2008;75(5):517-519. doi: 10.1016/j.jbspin.2008.03.004

24. Voo KS, Wang YH, Santori FR, et al. Identification of IL-17-producing FOXP3+ regulatory T cells in humans. Proc Natl Acad Sci U S A. 2009;106(12):4793-4798. doi:10.1073/pnas.0900408106

25. Libioulle C, Louis E, Hansoul S, et al. Novel Crohn disease locus identified by genome-wide association maps to a gene desert on 5p13.1 and modulates expression of PTGER4. PLoS Genet. 2007;3(4): e58. doi: 10.1371/journal.pgen.0030058

26. Hori S, Nomura T, Sakaguchi S. Control of regulatory T cell development by the transcription factor Foxp3. Science. 2003;299(5609):1057-1061. doi:10.1126/science.1079490

27. Kim JM, Rasmussen JP, Rudensky AY. Regulatory T cells prevent catastrophic autoimmunity throughout the lifespan of mice. Nat Immunol. 2007;8(2):191-197. doi:10.1038/ni1428.