Online ISSN: 2682-2628
Print ISSN: 2682-261X

# IJCBR

## International Journal of Cancer and Biomedical Research

https://jcbr.journals.ekb.eg

Editor-in-chief
Prof. Mohamed Labib Salem, PhD

# Association between the ACE (I/D) Gene Polymorphism and Hepatocellular Carcinoma Risk in Egyptian HCV Patients

Naglaa S. Elabd, Belal A. Montaser, Heba F. Khader , Suzy F. Gohar, Khalid S. Makboul, Moamena S. Elhamoly

EKB Egyptian Knowledge Bank بنك المعرفة المصري

PUBLISHED BY
EACR EGYPTIAN ASSOCIAN FOR CANCER RESEARCH
Since 2014

**International Journal of Cancer & Biomedical Research**
**(IJCBR)** https://jcbr.journals.ekb.eg

IJCBR is an Int. journal published by the Egyptian Society of Cancer Research (EACR, established in 2014, http://eacr.tanta.edu.eg) and sponsored by the Egyptian Knowledge Bank (EKB: www.ekb.eg).

IJCBR has been approved by the Supreme Council of Universities, Egypt with score 7 (http://egjournal.scu.eg). The journl is cited by google scholar and registered by Publons (https://publons.com). The journal has recently been evaluated in 2020 by Nature Springer with a good standing.

**Scope of IJCBR**

- Drug discovery from natural and synthetic resources
- BioMedical applications of nanotehnology
- Sem cell biology and its application
- Basic and applied biotechnology
- Inflammation and autoimmune diseases
- In slico models and bioinformatics
- In vitro and In vivo preclinical animal models
- Cellular and molecular cancer biology
- Cancer Immunology and Immunotherapy
- New methods for prediction, early detection, diagnosis prognosis and treatment of dieases.
- Immunology in health and dieases
- Anti-microbial defense mechanisms
- Cellular and molecular physhiology and pahthology of diseases

**IJCBR Editor,**
**Prof. Mohamed Labib Salem, PhD**
Professor of Immunology
Faculty of Science, Tanta Universiy, Egypt

INTERNATIONAL JOURNAL OF CANCER AND BIOMEDICAL RESEARCH (IJCBR)

RESEARCH ARTICLE

# Association between the ACE (I/D) Gene Polymorphism and Hepatocellular Carcinoma Risk in Egyptian HCV Patients

**Naglaa S. Elabd[1], Belal A. Montaser[2], Heba F. Khader[3] , Suzy F. Gohar[4], Khalid S. Makboul[5], Moamena S. Elhamoly[1]**

[1]*Tropical Medicine Department, Faculty of Medicine, Menoufia University, Egypt*
[2]*Clinical Pathology Department, Faculty of Medicine, Menoufia University, Egypt*
[3]*Medical Biochemistry and Molecular Biology Department, Faculty of Medicine, Menoufia University, Egypt*
[4]*Clinical Oncology Department, Faculty of Medicine, Menoufia University, Egypt*
[5]*Internal Medicine Department, Faculty of Medicine, Al-Azhar University, Cairo, Egypt*

## ABSTRACT

**Background**: Hepatocellular carcinoma is one of the most fatal malignancies worldwide and is related to many risk factors. Chronic HCV is associated with a 20–30-fold increased risk for HCC. Angiotensin-converting enzyme (ACE) is overexpressed in many cancers and plays a major role in angiogenesis and carcinogenesis. **Aim**: We aimed to elucidate the effect of the ACE I/D gene polymorphism in patients with HCV-related liver cirrhosis and HCC, and its relationship to clinical parameters. **Patients and Methods**: The study included 180 participants, cirrhotic (n=60), HCC (n=60) and control healthy subjects (n=60). Liver and renal function tests, alpha-fetoprotein, HCV antibodies and triphasic CT were assessed. ACE Gene polymorphism was assessed by Nested PCR. **Results**: We observed higher frequencies of DD (36.7%) and DI (51.7%) genotypes, along with the D allele (62.5%), in HCC patients compared to those of cirrhotic cases (10%, 40% and 30%, respectively) and control subjects (6.7%, 38.3%, and 25.8%, respectively). DD and DI genotypes increased the risk and predicted the occurrence of HCC by OR 25.932 and OR 6.354, respectively. The D allele conveys significant risk for HCC compared to control and cirrhotic groups with OR 4.785 and OR 3.889, respectively. Both DD genotype and D allele are significantly correlated with larger tumor size and metastasis. **Conclusion**: The ACE I/D polymorphism (DD genotype and D allele) is significantly associated with HCC risk in HCV patients and is correlated with increased tumor growth and advanced stage.

**Keywords:** HCV, hepatocellular carcinoma, ACE I/D, polymorphism, Nested PCR

Editor-in-Chief: Prof. M.L. Salem, PhD - Article DOI: 10.21608/JCBR.2020.29926.1037

## ARTICLE INFO

***Article history***

Received: May 10, 2020
Revised: June 11, 2020
Accepted: June 16, 2020

**Correspondence to:**
Naglaa S. Elabd, MD,
Tropical Medicine Department,
Faculty of Medicine,
Menoufia University, Egypt
Tel.: 00201092304322
E-mail: naglaa_elabd@yahoo.com

## INTRODUCTION

Hepatocellular carcinoma (HCC) is the sixth leading cause of cancer worldwide (Chow et al., 2016) and a noteworthy medical problem in Egypt, being the fourth cause of cancer and the second cause of cancer mortality in males and females, respectively (Global Burden of Disease Liver Cancer Collaboration et al., 2015). Hepatitis C virus (HCV) is one of the major hazard factors for HCC, causing 10%–25% of cases around the world (Huang, 2011), while in Egypt, 71% of HCC patients are positive for anti-HCV antibodies (El-Zayadi et al., 2001). Likewise, in the Nile Delta, HCV rather than HBV is linked to the development of HCC (Abd-Elsalam et al., 2018). The worldwide distribution of HCC mirrors the distributions of such viral infections (El-Serag, 2011).

Chronic HCV infection is associated with a 20–30-fold increased risk of developing HCC compared to uninfected people. Approximately 2.5% of patients with chronic HCV develop HCC (Bowen and Walker, 2005). HCV carcinogenesis is mediated by virally induced factors and host-induced immunologic responses (Farazi and De Pinho 2006). The outcome of HCV infection is influenced by both immunologic and genetic factors (Alric, 1997). Studies on monozygotic twins propose that having genetic factors could explain 50% or more of the variance in major

outcomes of infectious diseases. Various studies have illustrated a genetic predisposition for viral infections (Fierro et al., 2014).

The angiotensin-converting enzyme (ACE) gene is located on chromosome 17q23, traversing 21 kb and composed of 26 exons and 25 introns (Erdos, 1990). A polymorphism of ACE gene caused by the insertion (I) or deletion (D) of a 287 bp Alu repetitive sequence in intron 16 (rs1799725) creates three genotypes, DD, II, and ID (Wolfarth et al., 2005). There is a strong association between the ACE polymorphism and the plasma level of ACE. Mean ACE activity levels in II carriers were approximately half that found in DD genotype individuals (Hassanina et al., 2014). Numerous cell types in different tissues express angiotensin I-converting enzyme (ACE), a type I cell surface zinc metallopeptidase that produces angiotensin II, the primary player in the renin-angiotensin system (RAS) (Bauvois, 2004).

Some studies performed on brain, pancreatic, and gastric cancers have shown the expression of many RAS elements in both tissue and cell lines (Juillerat-Jeanneret et al., 2004). The RAS system is directly involved in cancer cell proliferation, differentiation, apoptosis, and angiogenesis (George et al., 2010). It also has a major function in the progression of liver fibrosis and the induction of HCC (Yoshiji et al., 2011).

To our knowledge, the relationship between the ACE gene polymorphism and the risk of developing HCC in cirrhotic patients has not been clarified yet. Hence, we aimed to elucidate the effect of the ACE I/D gene polymorphism in patients with HCV-related liver cirrhosis and HCC, in addition to its relationship to clinical parameters in these patients.

## PATIENTS AND METHODS

This cross-sectional study included one hundred and eighty participants. They were selected from the Tropical Medicine and Clinical Oncology Departments, Menoufia University Hospital, Menoufia University, Menoufia, Egypt between January 2017 and January 2018. Laboratory investigations and genetic analysis were completed in the Clinical Pathology and Medical Biochemistry and Molecular Biology Departments, Faculty of Medicine, Menoufia University, Menoufia, Egypt. Subjects were classified into three groups: Group I included 60 patients with liver cirrhosis on a background of chronic HCV with a mean age of 55.50±4.67 years; they were 39 males and 21 females. HCV was diagnosed by detecting HCV antibodies and furtherly confirmed by real-time PCR. Group II was composed of sixty HCV patients with a mean age of 60.20±5.38 years, including 50 males and 10 females, diagnosed with HCC on a background of cirrhosis by abdominal ultrasonography and triphasic CT. Group III included sixty HCV negative healthy subjects with matched age and gender as control group.

For all participants, history and clinical evaluation were collected. Patients with liver cirrhosis related to any etiology other than chronic HCV, as well as patients with cancer elsewhere in the body, were excluded. All participants provided informed consent affirmed by the Faculty of Medicine, Menoufia University ethical committee and as directed as per the Declaration of Helsinki.

Seven mL of venous blood was withdrawn from all participants as follows: 2ml of fresh blood was collected into EDTA tube for DNA extraction. The remaining 5ml were transferred to plain tube and serum was separated for assessment of laboratory investigation. Laboratory investigations included automated enzyme-linked fluorescent immunoassay (ELFA) used for detecting serum HBsAg, HCV Abs, and AFP using mini VIDAS systems (bioMérieux, Marcy l'Etoile, France) in a one-step immunoassay sandwich method with definite fluorescent recognition. Kinetic UV optimized method IFCC (LTEC Kit, England) was used to detect both ALT and AST enzymes. Serum albumin was estimated using a quantitative method of the enhanced specificity of bromocresol green colorimetric assay using the DIAMOND diagnostic kit (Germany). Prothrombin time was determined using a BIOMED-LlQUIPLASTIN diagnostic kit (Germany). The corresponding international normalized ratio (INR) was determined using appropriate tables provided by the manufacturer. The quantitation of HCV RNA was performed by real-time PCR Taqmanprobes.

### Genetic analysis

Three milliliters of venous blood was cleared into ethylenediaminetetraacetic acid (EDTA) tubes under standard safety measures and quickly stored at -20°C. Genomic DNA was isolated by the Gene JET Genomic DNA Purification Kit following the manufacture instructions (Thermo Fisher Scientific, MA,). Extracted DNA was dictated using a Thermo Scientific Nanodrop apparatus.

### Nested PCR for Genotyping of ACE I/D gene

The primer sequences and ACE genotyping for ACE gene, a 278 base pair AluYa5 sequence insertion/deletion (I/D) polymorphisms (rs1799725) in intron 16 was determined by as previously described by Rigat (1992). Nested PCR amplification was performed by preparing 50 µl composed of 10 µl of genomic DNA, 25 µl of PCR DreamTaq™ Green PCR Master Mix (Fermentas), which includes DNA polymerase, Green buffer, MgCl2, and dNTPs and is already enriched with two tracking dyes and a density reagent that allow for direct loading of the PCR product on a gel, along with 2 µl of both GIIS Primer and GAS Primer with 50 pmoles/µl and 11 µl of nuclease-free water.

### PCR primers

Forward primer (GIIS)
5'CTCAAGCACGCCCCTCACAGGACTG-3'.

Reverse primer (GAS) 5'-
GATGTGGCCATCACATTCGTCATCAGAT-3'.

Nested primer (FYM) 5'-
ATCACGAGGTCAGGAGATCGGGAGAC-3'.

### PCR conditions

The PCR mixture was exposed to 95°C for 5 min as initial denaturation following 20 cycles of amplification composed of 95°C for 60 s, 62°C for 45 s, and 72°C for 45 s. Finally, reactions were cooled to 4°C. Then, we added 1 µl of both GIIS and FYM primers to the mixture. PCR was then resumed for another 20 cycles. The nested primer is inward to the insertion point to only amplify the I allele. Reaction results were visualized under ultraviolet light after loading on a 3% agarose gel stained with ethidium bromide. Based on whether the insertion was present, a 490-bp-length fragment was produced, specifying the I allele, and a 190-bp length fragment, specifying the D allele. The nested primer was used to avoid the failure of the amplification of I and its possible rating as Rigat (1992) (Figure 1).

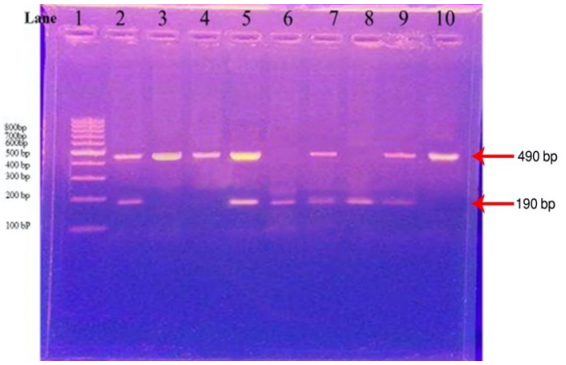


**Figure 1.** A representative agarose gel showing PCR lane 1: 100-bp DNA ladder; lanes 2, 5, 7, and 9: I/ D heterozygous (190 and 490 bp bands); lanes 3, 4, and 10: I/I homozygous (490 bp); and lanes 6 and 8: homozygous D/D (190 bp).

### Statistical analysis

Data were analyzed using IBM SPSS software program version 20.0. (Armonk, NY: IBM Corp). Tests used to verify the normality of the distribution of variables was the Shapiro-Wilk. Comparisons between different groups for categorical variables included Chi-square test [Fisher's or Monte Carlo]. One-way ANOVA was used to compare the studied groups and was followed by the post hoc test (Tukey) for pairwise comparisons. For comparing different groups with abnormally distributed quantitative variables, Kruskal-Wallis test was utilized and was followed by post hoc test (Mann Whitney). P-values less than 0.05 were considered statistically significant.

## RESULTS

The results of this study demonstrated matched age and gender distributions between the three studied groups, as there was no significant difference in these factors (P= 0.176, p= 0.070 respectively). ALT, AST, total bilirubin, and INR were markedly higher, and serum albumin was lower, in both HCV-related cirrhotic and HCC patients than in healthy controls (p<0.001), with no difference between the patient groups. The levels of alpha-fetoprotein exhibited highly significant variations (P<0.001) between all studied groups, with significantly elevated levels in HCC patients (Table 1).

No significant variation was detected between patient groups with respect to the clinical manifestations of cirrhosis (jaundice, ascites, upper GIT bleeding, CHILD Pough score, and splenomegaly) (P>0.05). However, hepatic encephalopathy and anorexia were more frequently noted in HCC patients than in cirrhotic patients (P=0.019 and <0.001, respectively) (Table 1).

As indicated by Hardy-Weinberg equilibrium (HWE), ACE I/D distribution among patients was $X^2$=0.732, P=0. 391 and in controls was $X^2$=7.882, P= 0.997. All findings concurred with HWE (Robert et al., 2007).

Our results on the genotypes and allele distributions of the ACE I/D polymorphism, HCC patients exhibited the highest prevalence of DD (36.7%) and DI (51.7%) genotypes compared to that of cirrhotic patients (10% and 40% respectively, P1< 0.001) and controls (6.7% and 38.3% respectively, P1<0.001), with no differences between cirrhotic patients and controls (P2=0.754). In addition, the excess levels of mutant D allele versus I allele were observed in HCC (62.5%) compared to both cirrhotic patients (30%) and healthy controls (25.8%) (P=0.001), with no difference between cirrhotic patients and controls (P2=0.472) (Table 2).

**Table 1.** Comparison of the three studied groups according to demographics and clinical parameters

| | Group I (Cirrhosis) (n = 60) | Group II (HCC) (n = 60) | Group III (Control) (n = 60) | Test of sig. | p | Significance between groups | | |
|---|---|---|---|---|---|---|---|---|
| | | | | | | I vs. II P1 | I vs. III P2 | II vs. III P3 |
| Gender | | | | | | | | |
| Male | 39(65%) | 50 (83.3) | 45 (75.0%) | $\chi^2$=5.315 | 0.070 | - | - | - |
| Female | 21(35%) | 10 (16.7) | 15 (25.0) | | | | | |
| Age | 55.1 ± 4.8 | 58.2 ± 10.2 | 57.5 ± 11.6 | F=1.755 | 0.176 | - | - | - |
| AFP | 22(4 – 122) | 396(33 – 5100) | 2.9(1.7 – 5.1) | H=154.060* | <0.001* | <0.001* | <0.001* | <0.001* |
| ALT | 55.0 (21 – 103) | 72.5 (21 – 344) | 26.0 (20 – 34) | H=84.962* | <0.001* | 0.064 | <0.001* | <0.001* |
| Bilirubin | 2(1 – 5.4) | 1.8(1 – 5.6) | 0.6(0.3 – 1) | H=117.851* | <0.001* | 0.714 | <0.001* | <0.001* |
| Albumin | 2.3(1.6 – 4.9) | 2.9(1.7 – 4.1) | 4.3(3.8 – 5) | F=143.234* | <0.001* | 0.139 | <0.001* | <0.001* |
| INR | 1.6(0.9 – 2.6) | 1.3(1 – 2.7) | 1(0.9 – 1) | H=111.616* | <0.001* | 0.965 | <0.001* | <0.001* |
| AST | 53 (21 – 166) | 55 (21 – 255) | 27 (22 – 42) | H=67.218* | <0.001* | 0.333 | <0.001* | <0.001* |
| Child Score | | | | | | | | |
| A | 25 (41.7%) | 20(33.3%) | - | $\chi^2$=5.370 | 0.068 | | | |
| B | 18 (30%) | 30(50%) | - | | | | | |
| C | 17 (28.3%) | 10(16.7%) | - | | | | | |
| Encephalopathy | | | | | | | | |
| No | 51 (85.0%) | 40 (66.7%) | - | $\chi^2$=5.502* | 0.019* | | | |
| Yes | 9 (15.0%) | 20 (33.3%) | - | | | | | |
| Ascites | | | | | | | | |
| No | 26(43.3%) | 34 (56.7%) | - | $\chi^2$=12.915* | 0.005* | | | |
| Mild | 12(20%) | 1 (1.7%) | - | | | | | |
| Moderate | 5(8.3%) | 11 (18.3%) | - | | | | | |
| Severe | 17(28.3%) | 14 (23.3%) | - | | | | | |
| Jaundice | | | | | | | | |
| No | 44 (73.3%) | 40 (66.7%) | | $\chi^2$=0.635 | 0.426 | | | |
| Yes | 16(26.7%) | 20 (33.3%) | | | | | | |
| Anorexia | | | | | | | | |
| No | 52(86.7%) | 31 (51.7%) | | $\chi^2$=17.232* | <0.001* | | | |
| Yes | 8(13.3%) | 29 (48.3%) | | | | | | |
| Tumor size | | 3.5(2 – 11 | | | | | | |
| <5 | - | 42(70%) | | - | - | | | |
| ≥5 | - | 18(30%) | | | | | | |

Data were described using numbers and percentages, the mean ± SD or median (min. – max.).
*: Statistically significant at p < 0.05, AFP: Alpha-fetoprotein, ALT: alanine aminotransferase,
INR: international normalized ratio, AST: aspartate aminotransferase, $\chi^2$: Chi-square test, H: H for Kruskal Wallis test, Pairwise comparison bet. Every 2 groups was done using Post Hoc Test (Dunn's for multiple comparisons test), F: F for ANOVA test, Pairwise comparison bet. every 2 groups was done using Post Hoc Test (Tukey),

**Table 2.** ACE I/D polymorphism in the three studied groups

| | Group I (Cirrhosis) (n = 60) | Group II (HCC) (n = 60) | Group III (Control) (n = 60) | $\chi^2$ | P | Significance between groups | | |
|---|---|---|---|---|---|---|---|---|
| | | | | | | I vs. II P1 | I vs. III P2 | II vs. III P3 |
| ACE I/D Polymorphism | | | | | | | | |
| II | 30(50%) | 7(11.7%) | 33(55%) | 37.054* | <0.001* | <0.001* | 0.754 | <0.001* |
| DI | 24(40%) | 31(51.7%) | 23(38.3%) | | | | | |
| DD | 6(10%) | 22(36.7%) | 4(6.7%) | | | | | |
| Allele | | | | | | | | |
| I | 84(70%) | 45(37.5%) | 89(74.2%) | 40.494* | <0.001* | <0.001* | 0.472 | <0.001* |
| D | 36(30%) | 75(62.5%) | 31(25.8%) | | | | | |

Data were described using numbers and percentages, *: Statistically significant at p <0.05

**Table 3.** Odds ratio

| | Group I (Cirrhosis) (n = 60) | Group II (HCC) (n = 60) | Group III (Control) (n = 60) | I vs II | | I vs III | | II vs III | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | OR | 95% C.I. | OR | 95% C.I. | OR | 95% C.I. |
| ACE I/D Polymorphism | | | | | | | | | |
| II® | 30(50%) | 7(11.7%) | 33(55%) | | | | | | |
| DI | 24(40%) | 31(51.7%) | 23(38.3%) | 5.536* | 2.08 – 14.75 | 1.148 | 0.54 – 2.44 | 6.354* | 2.39 – 16.89 |
| DD | 6(10%) | 22(36.7%) | 4(6.7%) | 15.714* | 4.63 – 53.30 | 1.650 | 0.42 – 6.41 | 25.932* | 6.78 – 99.19 |
| Allele | | | | | | | | | |
| I® | 84(70%) | 45(37.5%) | 89(74.2%) | | | | | | |
| D | 36(30%) | 75(62.5%) | 31(25.8%) | 3.889* | 2.27 – 6.66 | 1.230 | 0.70 – 2.17 | 4.785* | 2.76 – 8.30 |

OR: Odds ratio, CI: Confidence interval, ®: Reference, Group I: Liver cirrhosis, Group II: HCC, Group III: Control

**Table 4.** Relationship between the ACE I/D polymorphism and different parameters in patients with liver cirrhosis (group I)

| | ACE I/D Polymorphism | | | Test of sig. | p | Allele | | Test of sig. | P |
|---|---|---|---|---|---|---|---|---|---|
| | II (n= 30) | DI (n= 24) | DD (n= 6) | | | I (n= 84) | D (n= 36) | | |
| Gender | | | | | | | | | |
| Male | 17(56.7%) | 18(75%) | 4(66.7%) | $\chi^2$=2.003 | 0.410 | 52(61.9%) | 26(72.2%) | $\chi^2$=1.179 | 0.278 |
| Female | 13(43.3%) | 6(25%) | 2(33.3%) | | | 32(38.1%) | 10(27.8%) | | |
| Age | 55.7 ± 3.6 | 55.5 ± 5.2 | 50.8 ± 7.2 | F=2.798 | 0.069 | 55.62 ± 4.07 | 53.94 ± 6.10 | t=1.510 | 0.138 |
| AFP | 22(5 – 87) | 23(5 – 122) | 7(4 – 54) | H=2.591 | 0.274 | 22(5 – 122) | 18(4 – 122) | U=1352.0 | 0.359 |
| Child Score | | | | | | | | | |
| A | 14(46.7%) | 9(37.5%) | 2(33.3%) | $\chi^2$=2.316 | 0.700 | 37(44%) | 13(36.1%) | $\chi^2$=0.840 | 0.657 |
| B | 8(26.7%) | 9(37.5%) | 1(16.7%) | | | 25(28.9%) | 11(30.6%) | | |
| C | 8(26.7%) | 6(25%) | 3(50%) | | | 22(26.2%) | 12(33.3%) | | |

Data were described using numbers and percentages, the mean ± SD or median (min. – max.). *: Statistically significant at p <0.05, t: student's t-test, U: Mann Whitney test

**Table 5.** Relationship between the ACE I/D polymorphism and different parameters in the HCC group (group II)

| | ACE I/D Polymorphism | | | Test of sig. | P | Allele | | Test of sig. | P |
|---|---|---|---|---|---|---|---|---|---|
| | II (n= 7) | DI (n= 31) | DD (n= 22) | | | I (n= 45) | D (n= 75) | | |
| Gender | | | | | | | | | |
| Male | 7(100%) | 24(77.4%) | 19(86.4%) | $\chi^2$=1.744 | 0.377 | 38(84.4%) | 62(82.7%) | $\chi^2$=0.064 | 0.800 |
| Female | 0(0%) | 7(22.6%) | 3(13.6%) | | | 7(15.6%) | 13(17.3%) | | |
| Age | 64.3 ± 7.3 | 57.6 ± 10.2 | 57 ± 10.6 | F=1.488 | 0.234 | 59.7 ± 9.8 | 57.2 ± 10.3 | t=1.274 | 0.205 |
| AFP | 420(293 – 420) | 350(33 – 3500) | 417(84 – 5100) | H=0.097 | 0.953 | 392(33 – 3500) | 400(33 – 5100) | U=1644.50 | 0.816 |
| Child Score | | | | | | | | | |
| A | 3(42.9%) | 4(12.9%) | 13(59.1%) | | | 10(22.2%) | 30(40%) | | |
| B | 4(57.1%) | 20(64.5%) | 6(27.3%) | $\chi^2$=13.809* | 0.004* | 28(62.2%) | 32(42.7%) | $\chi^2$=4.871 | 0.088 |
| C | 0(0%) | 7(22.6%) | 3(13.6%) | | | 7(15.6%) | 13(17.3%) | | |
| | P1=0.148 | P2=0.361 | | | | | | | |
| Tumor size | | | | | | | | | |
| <5 | 6(85.7%) | 28(90.3%) | 8(36.4%) | $\chi^2$=17.927* | <0.001* | 40(88.9%) | 44(58.7%) | $\chi^2$=12.233* | <0.001* |
| ≥5 | 1(14.3%) | 3(9.7%) | 14(63.6%) | | | 5(11.1%) | 31(41.3%) | | |
| | P1=1.000 | P2=0.035* | | | | | | | |
| Metastasis | | | | | | | | | |
| No | 5(71.4%) | 26(83.9%) | 5(22.7%) | $\chi^2$=20.517* | <0.001* | 36(80%) | 36(48%) | $\chi^2$=12.000* | 0.001* |
| Yes | 2(28.6%) | 5(16.1%) | 17(77.3%) | | | 9(20%) | 39(52%) | | |
| | P1=0.592 | P2=0.030* | | | | | | | |

Data were described using numbers and percentages, the mean ± SD or median (min. –max.), *: Statistically significant at p <0.05, P1: II vs DI, P2: II vs DD

Analyzing the risk association of the studied gene, both the DD and DI genotypes of ACE I/D could be considered as risk factors that predict the occurrence of HCC, with OR 25.932 [95% CI: 6.78–99.19] and OR 6.354 [95% CI: 2.39–16.89], respectively, compared to controls with OR 15.714 [95% CI: 4.63 – 53.30] and cirrhotic group OR 5.536 [95% CI: 2.08–14.75]. Furthermore, D allele demonstrated significant risk for HCC patients compared to control and cirrhotic groups with OR 4.785 [95% CI: 2.76 – 8.30] and OR 3.889 [95% CI: 2.27–6.66], respectively (Table 3).

As shown in Table 4, ACE I/D polymorphism in HCV patients was not significantly associated with age, gender, AFP, or child score. However, in HCC patients, there was a significant correlation with child score (P=0.004), tumor size (P<0.001), and metastasis (P<0.001). The DD genotype and D allele exhibited significant predominance in tumor size greater than 5 cm, in contrast to the II genotype and I allele (P=0.035 and P<0.001, respectively). Additionally, the DD genotype and D allele exhibited significant association with distant metastasis (P=0.030 and P=0.001, respectively) (Table 5).

## DISCUSSION

HCC is a predominant tumor, having an incidence rate of sixth and third around the world regarding cancer-related deaths (Pu et al., 2018). The most significant occurrences of HCC emerge from tissues with long-standing inflammation and fibrosis, which initiates tumor progression and induces reluctance to treatment (Jeng et al., 2017). The pathogeneses of HCC is complicated and is related to many risk factors. In any case, HCC occurs in a low level of individuals who are vulnerable to such hazards, proposing genetic susceptibility and interplay between genetic factor and additional factors as a reason for HCC, including hepatitis B and C viral infection, which has important pathogenic consequences leading to HCC (Sümbül et al., 2012; Kuo et al 2017).

Like all tumors, HCC relies on the formation of a vascular network and has been reported that angiotensin-converting enzyme (ACE), a regulator of the renin-angiotensin system (RAS), is overexpressed in many cancers, such as HCC, and plays a major role in angiogenesis and carcinogenesis (Pajares et al., 2012; Yoshiji et al., 2011).

In this study, we made the distinction between Egyptian patients with the DD genotype or the D allele of ACE D/I polymorphism and associated HCC risk in a background of HCV infection, as well as associating these factors with advanced tumor staging, larger tumor size, and the presence of metastasis, in contrast to II and I allele. Consistent with our results, Zha et al., (2015) found, in a Chinese Dai population, that being a DD carrier is correlated with an elevated HCC risk compared to being a II carrier. In addition, they presume the potential ability for HCC prevention by the modification of ACE function after the confirmation of results by additional studies. Similarly, George et al., (2010) reported that the expression and actions of RAS affect malignancy, implying that RAS inhibi5tors, which are now used to manage hypertension, cardiac, and vascular diseases, could alter cancer management protocols.

In agreement with our findings for HCC, in a study on cancer breast patients, Singh et al., (2018) found that the DD genotype and D allele of ACE (I/D) polymorphisms are associated with more advanced breast cancer and increased tumor size in an Indian population.

The plasma levels of ACE are more increased in subjects with the ACE DD genotype than in in the II genotype (Rigat et al., 1990). Castellon et al., (2007) assumed that the *ACE* I/D polymorphism was related to tumor development with respect to ACE level and subsequent angiotensin-II. Angiotensin-II may enhance the cancer cell expression of vascular endothelial growth factor (VEGF) (Yoshiji et al., 2007).

Simões E Silva et al., (2017) suggested that angiotensin II may stimulate liver stellate cell propagation and induce transforming growth factor (TGF)-b1 expression, suggesting that Ang II participates in hepatic fibrosis. In another study, it was proposed that angiotensin II enhances angiogenesis, contributing to liver fibrogenesis, carcinogenesis, and HCC growth Yoshiji et al., (2002), in agreement with our results.

As broadly recommended antihypertensive medications, angiotensin-converting enzyme inhibitors (ACEi) and receptor blockers (ARB) may likewise have advantageous effects as anti-tumor therapies and may be related to improved survival in HCC and cirrhotic patients (Pinter et al., 2017). These inhibitors are also reportedly correlated with the decreased recurrence rate of breast cancer (Chae et al., 2011) and improved response of rectal cancer to radiation therapy (Morris et al., 2016). However, in a previous study, Yuan et al., (2013) determined that the D allele of ACE in a Chinese population conveyed a decreased risk for HCC.

Variable studies have been performed concerning this subject. However, the results are controversial. As per the study of Liu et al., (2012) on a Chinese Taiwan population, the ACE DD genotype exhibits an elevated risk of oral cancer versus the II genotype. A meta-analysis by Ruiter et al., (2011) noted the association of D allele with breast cancer risk postmenopausal women and in prostate cancer. Otherwise, previous reports did not observe a significant connection between the ACE I/D polymorphism and malignancy hazard (Gao et al., 2012 and Zhang et al., 2011).

The current study suggests that the ACE I/D polymorphism (DD genotype and D allele) is significantly associated with HCC risk in HCV patients in Egypt, correlating with increased tumor growth and advanced stage.

## Conflicts of interest

All authors have approved this article and declare no conflicts of interest.

## References


Abd-Elsalam S, Elwan N, Soliman H, Ziada D, Elkhalawany W, Salama M, Hawash N, Arafa M, Badawi R, Shehata WM, Khalil HS, and Elmashad N (2018). Epidemiology of liver cancer in Nile delta over a decade: A single-center study. South Asian J Cancer, 7:24–6.

Alric L, Fort M, Izopet J, Vinel JP, Charlet JP, Selves J, Puel J, Pascal JP, Duffaut M, and Abbal M (1997). Genes of the major histocompatibility complex class II influence the outcome of hepatitis C virus infection. Gastroenterology, 113:1675–81.

Bauvois B (2004). Transmembrane proteases in cell growth and invasion: new contributors to angiogenesis? Oncogene, 23:317.

Bowen DG, and Walker CM (2005). Adaptive immune responses in acute and chronic hepatitis C virus infection. Nature, 436:946.

Castellon R, and Hamdi HK (2007). Demystifying the ACE polymorphism: from genetics to biology. Curr Pharm Des, 13(12):1191-1198.

Chae YK, Valsecchi ME, Kim J, Bianchi AL, Khemasuwan D, Desai A, and Tester W (2011) Reduced Risk of Breast Cancer Recurrence in Patients Using ACE Inhibitors, ARBs, and/or Statins. Cancer Invest, 29:585–93.

Chow PKH, Choo SP, Ng DCE, Lo RHG, Wang MLC, Toh HC, Tai DW, Goh BK, Wong JS, Tay KH, Goh AS, Yan SX, Loke KS, Thang SP, Gogna A, Too CW, Irani FG, Leong S, Lim KH, and Thng CH (2016). National Cancer Centre Singapore Consensus Guidelines for Hepatocellular Carcinoma. Liver Cancer, 5:97–106.

El-Serag HB (2011). Hepatocellular Carcinoma. N Engl J Med, 365:1118–27.

El-Zayadi AR, Abaza H, Shawky S, Mohamed MK, Selim OE, and Badran HM (2001). Prevalence and epidemiological features of hepatocellular carcinoma in Egypt—a single center experience. Hepatol Res, 19:170–9.

Erdos EG (1990). Angiotensin I converting enzyme and the changes in our concepts through the years. Lewis K. Dahl memorial lecture. Hypertension, 16: 363-368.

Farazi PA, and DePinho RA (2006). Hepatocellular carcinoma pathogenesis: from genes to environment. Nat Rev Cancer, 6:674.

Fierro NA, Gonzalez-Aldaco K, Torres-Valadez R, Martinez-Lopez E, Roman S, and Panduro A (2014). Immunological, Metabolic and Genetic Factors in Hepatitis C Virus Infection. World J Gastroenterol, 20(13):3443-56. doi: 10.3748/wjg.v20.i13.3443

Gao M, Wang Y, Shi Y, Liu D, Liang Y, Yu Y, Zhaobin J, Zhu L, and Jin S (2012). The relationship between three well- characterized polymorphisms of the angiotensin converting enzyme gene and lung cancer risk : a case-control study and a meta-analysis. J Renin Angiotensin Aldosterone Syst, 13: 455–460.

George AJ, Thomas WG, and Hannan RD (2010). The renin–angiotensin system and cancer: old dog, new tricks. Nat Rev Cancer, 10:745.

Global Burden of Disease Liver Cancer Collaboration, Akinyemiju T, Abera S, Ahmed M, Alam N, Alemayohu MA, et al (2017). The

Burden of Primary Liver Cancer and Underlying Etiologies From 1990 to 2015 at the Global, Regional, and National Level: Results From the Global Burden of Disease Study 2015. JAMA Oncol, 3:1683–91.

Hassanina OM, Moustafab M, and El Masry TM (2014). Association of insertion–deletion polymorphismof ACE gene and Alzheimer's disease in Egyptian patients. The Egyptian Journal of Medical Human Genetics, 15: 355-360.

Huang Y-T, Jen C-L, Yang H-I, Lee M-H, Su J, Lu S-N, Iloeje UH, and Chen CJ (2011). Lifetime risk and sex difference of hepatocellular carcinoma among patients with chronic hepatitis B and C. J Clin Oncol, 29:3643–50.

Jeng KS, Jeng CJ, Jeng WJ, Chang CF, and Sheen IS (2017). Role of C-X-C chemokine ligand 12/C-X-C chemokine receptor 4 in the progression of hepatocellular carcinoma. Oncol Lett, 14:1905–10.

Juillerat-Jeanneret L, Celerier J, Chapuis Bernasconi C, Nguyen G, Wostl W, Maerki HP, Janzer RC, Corvol P, and Gasc JM (2004). Renin and angiotensinogen expression and functions in growth and apoptosis of human glioblastoma. Br J Cancer, 90:1059–68.

Kuo HH, Lin RJ, Hung JT, Hsieh CB, Hung TH, Lo FY, Ho MY, Yeh CT, Huang YL, Yu J, and Yu AL (2017). High expression FUT1 and B3GALT5 is an independent predictor of postoperative recurrence and survival in hepatocellular carcinoma. Sci Rep, 7:10750.

Liu YT, Lin LW, Chen CY, Wang CP, Liu HP, Houng JY, Chung FM, and Shieh TY (2012). Polymorphism of angiotensin I-converting enzyme gene is related to oral cancer and lymph node metastasis in male betel quid chewers. Oral Oncol, 48:1257–62.

Morris ZS, Saha S, Magnuson WJ, Morris BA, Borkenhagen JF, Ching A, Hirose G, McMurry V, Francis DM, Harari PM, Chappell R, Tsuji S, and Ritter MA (2016). Increased tumor response to neoadjuvant therapy among rectal cancer patients taking angiotensin-converting enzyme inhibitors or angiotensin receptor blockers. Cancer, 122:2487–95.

Pajares MJ, Agorreta J, Larrayoz M, Vesin A, Ezponda T, Zudaire I, Torre W, Lozano MD, Brambilla E, Brambilla C, Wistuba II, Behrens C, Timsit JF, Pio R, Field JK, and Montuenga LM (2012). Expression of tumor-derived vascular endothelial growth factor and its receptors is associated with outcome in early squamous cell carcinoma of the lung. J Clin Oncol, 30:1129–36.

Pinter M, Weinmann A, Wörns MA, Hucke F, Bota S, Marquardt JU, Duda DG, Jain RK, Galle PR, Trauner M, Peck-Radosavljevic M, and Sieghart W (2017). Use of inhibitors of the renin–angiotensin system is associated with longer survival in patients with hepatocellular carcinoma. United Eur Gastroenterol J. 5:987–96.

Pu W, Li J, Zheng Y, Shen X, Fan X, Zhou J-K, He J, Deng Y, Liu X, Wang C, Yang S, Chen Q, Liu L, Zhang G, Wei YQ, and Peng Y (2018). Targeting Pin1 by inhibitor API-1 regulates microRNA biogenesis and suppresses hepatocellular carcinoma development. Hepatology, 68:547–60.

Rigat B, Hubert C, Alhenc-Gelas F, Cambien F, Corvol P, and Soubrier F (1990). An insertion/deletion polymorphism in the angiotensin I-converting enzyme gene accounting for half the variance of serum enzyme levels. J Clin Invest, 86(4):1343-6.

Rigat B, Hubert C, Corvol P, and Soubrier F (1992). PCR detection of the polymorphism of the human angiotensin converting enzyme gene (DCP1 (dipeptidyl carboxypeptidase 1) Nucl Ac Res, 20 (6):1.433.

Robert L. Nussbaum, Roderick R. Mcinnes, Huntington F. Willard, Ada Hamosh, Thompson & Thompson GENETICS IN MEDICINE, 7th edition. CHAPTER 9. 2007; by Saunders, an imprint of Elsevier Inc.

Ruiter R, Visser LE, Van Duijn CM, and Stricker BH (2011). The ACE insertion/deletion polymorphism and risk of cancer, a review and meta-analysis of the literature. Curr Cancer Drug Targets, 11(4):421-430.

Simões E Silva AC, Miranda AS, Rocha NP, and Teixeira AL (2017). Renin angiotensin system in liver diseases: Friend or foe? World J Gastroenterol, 23:3396–406.

Singh A, Srivastava N, Amit S, Prasad SN, Misra MP, and Ateeq B (2018). Association of AGTR1 (A1166C) and ACE (I/D) Polymorphisms with Breast Cancer Risk in North Indian Population. Transl Oncol, 11(2):233-242.

Sümbül AT, Akkız H, Bayram S, Bekar A, Akgöllü E, and Sandıkçı M (2012). p53 codon 72 polymorphism is associated with susceptibility to hepatocellular carcinoma in the Turkish population: a case–control study Mol Biol Rep, 39:1639-1647.

Wolfarth B, Bray MS, Hagberg JM, Pérusse L, Rauramaa R, Rivera MA, Rankinen T, and Bouchard C (2005). The human gene map for performance and health-related fitness phenotypes: the 2004 update. Med Sci Sports Exerc, 37(6):881-903.

Yoshiji H, Kuriyama S, and Fukui H (2002). Angiotensin-I-converting enzyme inhibitors may be an alternative anti-angiogenic strategy in the treatment of liver fibrosis and hepatocellular carcinoma. Possible role of vascular endothelial growth factor. Tumour Biol, 23(6):348-356.

Yoshiji H, Noguchi R, Ikenaka Y, Kaji K, Aihara Y and Fukui H (2011). Impact of Renin-Angiotensin System in Hepatocellular Carcinoma. Curr Cancer Drug Targets, 11:431–41.

Yuan F, Zhang L-S, Li H-Y, Liao M, Lv M, and Zhang C (2013). Influence of Angiotensin I-converting enzyme gene polymorphism on hepatocellular carcinoma risk in china. DNA Cell Biol, 32:268–73.

Zha Y, Gan P, Liu Q, and Tan J (2015). Relationship between polymorphism of angiotensin-converting enzyme gene insertion/deletion and risk of hepatocellular carcinoma in a Chinese Dai population. JRAAS - J Renin-Angiotensin-Aldosterone Syst, 16:695–9.

Zhang Y, He J, Deng Y, Zhang J, Li X, Xiang Z, Huang H, Tian C, Huang J, and Fan H (2011). The insertion/deletion (I/D) polymorphism in the Angiotensin-converting enzyme gene and cancer risk: a meta-analysis. BMC Med Genet, 12:159.

**Egyptian Association for Cancer Research (EACR)**
http://eacr.tanta.edu.eg/

EACR is an NGO society that was declared by the Ministry of Social Solidarity (Egypt) No. 1938 in 19/11/2014 based on the initiative of Prof. Mohamed Labib Salem, the current Chairman of EACR. EACR aims primarily to assist researchers, in particular young researchers in the field of cancer research through workshops, seminars and conferences. Its first international annual conference entitled "Anti-Cancer Drug Discovery" was successfully organized in April 2019 (http://acdd.tanta.edu.eg). Additionally, EACR aims to raise the awareness of the society about the importance of scientific research in the field of cancer research in prediction, early diagnosis and treatment of cancer. EACR is also keen to outreach the scientific community with periodicals and news on cancer research including peer-reviewed scientific journals for the publication of cutting-edge research. The official scientific journal of EACR is "International Journal of Cancer and biomedical Research (IJCBR: https://jcbr.journals.ekb.eg) was successfully issued in 2017 and has been sponsored by the Egyptian Knowledge Bank (EKB: www.ekb.eg).

**EACR Chairman,**
**Prof. Mohamed Labib Salem, PhD**
Professor of Immunology
Faculty of Science, Tanta Universiy, Egypt

**International Journal of Cancer & Biomedical Research**
**(IJCBR)** Online ISSN 2682-2628



For more information, contact

Hamdi Kandil
Tanta University, Egypt
Email: Ijcbr100@gmail.com