Angiotensin-Converting Enzyme Gene Polymorphism (insertion/deletion) and The Risk of Hepatocellular Carcinoma in Egyptian HCV and HBV Patients

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Abstract: Background: Chronic infection with Hepatitis C virus (HCV) and Hepatitis B virus (HBV) are the major risk factor for the development hepatocellular carcinoma (HCC) worldwide. Hepatitis C infection represents a major health problem in Egypt with a reported prevalence of more than 20%. About 60 to 80% of patients develop chronic infection, which may progress to complications. Different studies have illustrated a genetic predisposition for viral infections and development of complications. The angiotensin-converting enzyme (ACE) gene is directly involved in the process of cancer cell proliferation, differentiation, apoptosis and angiogenesis. It also plays a vital role in inducing liver fibrosis and developing hepatocellular carcinoma. **Aim:** The aim of this study was to study whether ACE insertion/deletion (I/D) gene polymorphism associated with risk of HCC in Egyptian HCV and HBV patients. **Patients and Methods:** The study was conducted on one hundred and fifty subjects who were divided into three groups: 60 patients with chronic HCV & HBV, 60 patients with HCV & HBV related HCC and 30 healthy gender and age matched subjects. Full clinical examination and history were taken, Liver function tests, Alpha fetoprotein by ELISA and polymerase chain reaction was used to determine the distribution of allele and genotype frequency of ACE I/D polymorphism. **Results:** The results showed that there were a significant statistical difference between HCC patients and healthy controls regarding the genotype and allele frequencies of the ACE I/D polymorphism[DD (OR = 26, 95% CI, 2.61- 259.32, *P* = 0.002) and DI (OR = 6.0; 95% CI, 1.26 – 28.55; *P* = 0.02)]. The D allele was correlated with a significant increased HCC risk when compared with the I allele (OR = 6.91, 95% CI, 2.45– 19.5 *P*> 0.001). The DD genotype were more frequently increased in HCC group than in chronic HCV & HBV group with significant statistical difference (OR = 5.2, 95% CI, 1.25 - 21.57, *P* = 0.02) between both groups, and the D allele was correlated with a significant increased HCC risk when compared with the I allele (OR = 2.59, 95% CI, 1.24 – 5.41 *P*> 0.01).There were significant statistical differences between different genotypes as regard tumor size in HCC group*(P* = 0.01).There were no significant statistical differences between different genotypes as regards liver functions and alpha fetoprotein in HCC cases*(P* >0.05). **Conclusion:** The results suggested The DD and DI genotypes were correlated with a significant increased HCC risk as compared with the II genotype and the D allele was correlated with a significant increased HCC risk.

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1. Introduction:

Liver cancer rapidly reduces quality of life and typically causes death 6 months–1 year from diagnosis. (1) Globally, it is the fifth leading cause of cancer and the third leading cause of cancer death. (1,2). This cancer varies widely in incidence throughout the world, with rising incidence in Egypt. The primary risk factors for hepatocellular carcinoma (HCC) are hepatitis B virus (HBV), hepatitis C virus (HCV), dietary aflatoxin exposure, and chronic alcohol consumption. (1,2)

In Egypt, Hepatocellular carcinoma was reported to account for about 4.7% of of chronic liver disease (CLD) patients. (3) In 2005, El-Zayadi *et al.* (4) reported a remarkable increase in the proportion of HCC among chronic liver disease patients from 4.0% to 7.2% over the last decade. This rising proportion may be explained by the increasing risk factors such as the emergence of HCV over the same period of time, the contribution of HBV infection, improvement of the screening programmes and diagnostic tools of HCC. (5)

HCC is a complex disease with a variety of underlying pathogenic anomalies caused by multiple risk factors. The lack of ideal biomarkers for HCC diagnosis, prognosis, and therapy have posed a major challenge to HCC management. These complications highlight the need to identify valuable biomarkers for the diagnosis and treatment of HCC. Surgical resection, liver transplantation and local ablation are considered curative therapeutic practices for HCC. Other modalities, such as targeted therapy and trans-

arterial chemoembolization (TACE) are palliative treatments. Despite these curative or palliative treatments, prognosis is still poor due to underlying liver diseases and the unique biology of HCC. As a result, biomarkers that better predict patients who are at higher risk of recurrence and poorer prognosis would help guide their treatment. (6) In addition to imaging techniques including ultrasonography, magnetic resonance imaging, and computerized tomography, the assessment of circulating biomarkers is used in the diagnosis of HCC without pathologic confirmation.(7)

Some studies have shown that tumor angiogenesis occurs at the early stage of tumor formation, which plays a vital role in promoting progressive tumor growth. (8) Any tumor, including HCC, depends on the formation of a vascular network to provide its with oxygen and essential nutrients.(9) The tumor that has not acquired its own new blood supply can not grow to more than a few millimeters in size. (8)

The renin–angiotensin–aldosterone (RAS) axis is a system which involves many essential regulations in the human body for blood pressure, fluid and electrolyte balance. (10) Studies have demonstrated that angiotensin-converting enzyme (ACE), the regulator of the renin-angiotensin system, is overexpressed in several cancers including HCC, and directly involved in the process of cancer cell proliferation, differentiation, apoptosis, and angiogenesis. (11) It also plays a vital role in inducing liver fibrosis and developing HCC (12). In addition, ACE inhibitors (ACE-Is), conventional antihypertensive agents, were found to decrease the cancer risk, and attenuate tumor growth via the suppression of angiogenesis *in vitro* and *in vivo.*(13,14) Several studies have confirmed that ACE-Is may protect against the risk of HCC. (15,16)

The ACE gene is located on long arm of chromosome 17 (17q23.3). The gene is 21 kilo bases (kb) long and comprises 26 exons and 25 introns. More than 160 ACE gene polymorphisms have been reported so far and most of which are single nucleotide polymorphisms (SNPs). Only 34 of those polymorphisms are located in coding region of this gene. (17) **Rigat et al., [18]** were the first to report the insertion/deletion (I/D) polymorphism of ACE. This polymorphism is characterized by the presence (insertion) or absence (deletion) of a 287 bp AluYa5 element inside intron 16 producing three genotypes (II homozygote, ID heterozygote and DD homozygote). Although I/D polymorphism is located in a non-coding region (namely intron) of the ACE gene, several investigators have found that the D allele is related to the activity of ACE in blood. (19)

It was reported that the angiotensin-converting enzyme gene I/D polymorphism accounted for 30–50% of the ACE serum concentration variance. Some studies have showed a link between the ACE I/D polymorphism and the risk of various malignancies such as breast, gastric, and pancreatic cancer. (18,20,21,22) However, to our knowledge, the relationship between the ACE polymorphism and the risk of HCC has not been completely clarified.

Aim of the study:

The aim of this study was to study whether ACE insertion/deletion (I/D) gene polymorphism associated with risk of HCC in Egyptian HCV and HBV patients.

2. Patients and methods

1- Study population:

The present study was carried out at Clinical Pathology Department in collaboration with Internal Medicine Department, Faculty of Medicine, Menoufia and Benha University in the period between March, 2014 & September 2015.

The study was conducted on 150 subjects who were divided into three groups: 60 patients with chronic HCV and HBV (49 males and 11 females) with a mean age ±SD of 50.83±6.34, 60 patients with HCV & HBV related HCC (48 males and 12 females) with a mean age ±SD of 51.23±6.80. In addition to 30 gender and age matched subject (26 males and 4 females) with a mean age ±SD of 49.27±5.80.

Exclusion criteria included cases of autoimmune hepatitis, metabolic liver diseases (haemochromatosis, Wilson's disease, non alcoholic steatohepatitis), history of alcohol consumption or having any other cancer.

For all the subjects the followings were done: history and clinical examination, routine liver function tests, HBsAg, by third generation enzyme immunoassay and positive cases were further confirmed by real time PCR for HBV-DNA,HCV antibodies by ELISA technique and positive cases were further confirmed by real time PCR for HCV-RNA, AFP for all cases, HCC cases were further confirmed by abdominal Triphasic CT. Polymerase chain reaction was used to determine the distribution of allele and genotype frequencies of the I/D polymorphism in ACE gene. Written informed-consents were provided by all participants and accredited by our ethical committee.

Sampling:

Under complete aseptic conditions, 8 ml of venous blood were collected after 12 hour fasting & divided as follows: Tube A, 2 ml of blood collected in EDTA (to prevent clotting and DNA degradation) for DNA extraction and kept immediately at -20°C. Tube B, 2 ml of blood collected in Sodium Citrate for Prothrombin time, activity, concentration and INR.

Tube C, 2 ml were collected, left to clot and serum was separated and used for routine liver function tests (serum albumin, bilirubin, AST, ALT, Alkaline phosphatase) and AFP. Tube D, 2 ml were collected, left to clot and serum was separated and used for immediate assay of hepatitis viral markers.

Laboratory investigations:

Serum HBsAg, HCV Ab & AFP were done by VIDAS systems (bioMérieux, Marcy l'Etoile, France) which is an automated enzyme-linked fluorescent immunoassay (ELFA) based on a one-step immunoassay sandwich method and a final fluorescent detection step for the quantitative measurements, Biochemical tests for liver function tests were done on AU- 480 autoanalyser using kit supplied by Beckman Coulter (Beckman Instrument. Inc. Fullerton, California USA).

DNA analysis:

DNA extraction:

DNA was purified from whole blood using Axygen Prep Blood Genomic DNA Miniprep Kit for the purification of genomic DNA from whole blood. This method was based on the efficient release of genomic DNA from anti-coagulated whole blood by a special cell lysis and heme/protein precipitation buffer coupled with the selective adsorption of the genomic DNA to a special AxyPrep column. The purified genomic DNA was eluted in a low salt Tris buffer containing 0.5 mM EDTA which enhanced DNA solubility and helped to protect the high molecular weight DNA against subsequent nuclease degradation. The eluted genomic DNA was kept immediately at -20°C subjected to PCR.

PCR amplification:

The DNA was then subjected to nested PCR amplification of the insertion/deletion (I/D) polymorphic site of the ACE gene DreamTaq™ Green PCR Master Mix (Fermentas) was a ready to use solution containing DreamTaq™ DNA polymerase, optimized DreamTaq™ Green buffer, MgCl2 and dNTPs. The master mix was supplemented with two tracking dyes and a density reagent that allow for direct loading of the PCR product on a gel.

Reaction mixture:

For a total 50 µl reaction volume: DreamTaq™ Green PCR Master Mix (2X) 25 µl, GIIS Primer 2µl, GAS Primer 2 µl, Extracted DNA 10 µl, Water nuclease-free 11 µl.

Primers:

The lyophilized primers (Fermentas Life Sciences) were reconstituted by addition of sterile water to a final concentration of 50 pmoles/µl and distributed in aliquots and stored at –20°C. The primer sequences were as follow:

Forward primer:

5′ CTGGAGACCACTCCCATCCTTTCT-3′.

Reverse primer:

5 GATGTGGCCATCACATTCGTCAGAT-3′.

Protocol of amplification:

The PCR conditions consisted of an initial five-minute denaturation at 95°C, followed by 35 cycles of 30 s at 94°C, 90 s at 70°C, 90 s at 72°C, and 10 m at 72°C. Amplified ACE fragments were by electrophoresis on 2% agarose gel stained with ethidium bromide and visualized on a UV trans-illuminator. The 490-bp-long fragment amplification indicated presence of the I allele, while the 190-bp-long fragment amplification indicated presence of the D allele. The ID genotype commonly showed a double band at 490 bp and 190 bp. (Fig 1).

Statisticalanalysis:

The statistical analysis was undertaken using SPSS software (version 17; SPSS Inc., Chicago, IL, USA). Descriptive statistics in the form of mean and standard deviation for parametric data were used. Chi-square test ($\chi 2$) was used for qualitative variables. T-test for comparison between the two groups having quantitative variables normally distributed, man-witteny test for comparison between two groups having quantitative variables not normally distributed, ANOVA test for comparison between the three groups having quantitative variables normally distributed followed by LSD (least significant difference) and Kruskal-Wallis test for comparison between three groups not normally distributed having quantitative variables. Odd ratios (ORs) and 95% confidence intervals (CI) were calculated by logistic regression analysis. The significance level was set at 0.05 or less.

3. Results:

There was no significant difference in age and gender distribution between the cases and controls, suggesting that subject matching based on these variables was adequate as shown in table (1).

Comparison between the studied groups regarding liver functions and AFP serum level:

There was a highly significant difference regarding all measured liver function tests as well as Alpha-fetoprotein between healthy control group and HCC group (*P* value in all > 0.001). Also,there was a significant difference regarding all measured liver function tests except AST serum level as well as Alpha-fetoprotein between healthy control group and chronic HCA and HBV group (*P* value in all > 0.05). Comparing between chronic HCV & HBV group and HCC group; there was no significant statistical difference regarding serum albumin, PT, INR, bilirubin and alkaline phosphatase, while there was a significant statistical difference regarding AST and ALT serum level and a highly significant statistical difference with *P* value > 0.001 regarding serum alpha-fetoprotein level (table 2).

Gene polymorphism and allele among the studied groups:

The results showed that there were no significant statistical differences between Chronic HCV & HBV patients when compared with healthy controls regarding the genotype and allele frequencies of the ACE I/D polymorphism (table 3). However, there were a significant statistical difference between HCC patients and healthy controls regarding the genotype and allele frequencies of the ACE I/D polymorphism [DD (OR = 26, 95% CI, 2.61- 259.32, *P* = 0.002) and DI (OR = 6.0; 95% CI, 1.26 – 28.55; *P* = 0.02)], and the D allele was correlated with a significant increased HCC risk when compared with the I allele (OR = 6.91, 95% CI, 2.45– 19.5, *P*> 0.001) as shown in table (4). Similarly, in table (5)The DD genotype were more frequently increased in HCC patients than in HCV & HBV patients with significant statistical difference (OR = 5.2, 95% CI, 1.25 - 21.57, *P* = 0.02) between both groups and the D allele was correlated with a significant increased HCC risk when compared with the I allele (OR = 2.59, 95% CI, 1.24 – 5.41 *P*> 0.01).

Comparison between different genotypes as regards liver functions, alpha fetoprotein and tumor size in HCC group:

The results showed that there were significant statistical differences between different genotypes as regard tumor size *(P* = 0.01) but no significant statistical differences between different genotypes as regards liver functions, alpha fetoprotein in HCC group *(P* >0.05) table (6).

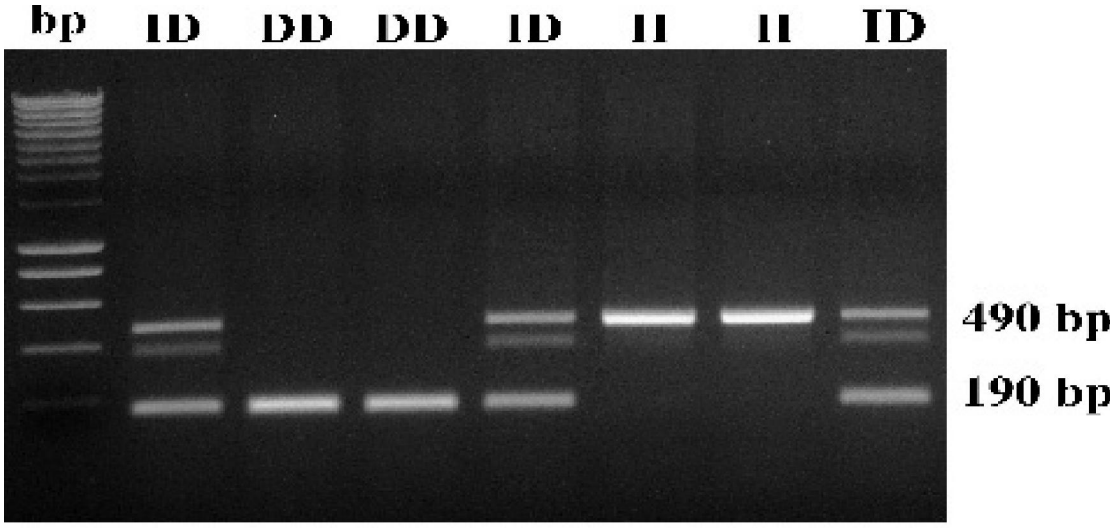

Fig 1: Determination of ACE genotypes by PCR amplification. Amplified ACE gene fragments were separated on 2% agarose gels and visualized by ethidium bromide staining. The 490-bp-long fragment amplification indicated presence of the I allele, while the 190-bp-long fragment amplification indicated presence of the D allele. The ID genotype commonly showed a double band at 490 bp and 190 bp..bp, molecular-weight markers.

Table 1: Comparison between the studied groups as regards age and sex

	The studied groups						**Test**	***P* value**
	Control N = 30		chronic HCV & HBV **N = 60**		HCC N = 60			
Age X ± SD Range	49.27±5.80 40 – 60		50.83±6.34 40 – 61		**51.23**±6.80 **36 – 65**		**ANOVA** 0.48	0.62
	No	%	No	%	No	%	X^2	
Sex Male Female	26 4	86.7 13.3	49 11	81.6 18.4	48 12	80.0 20.0	0.35	0.84

X= mean, SD = Standard deviation, X^2 = Chi squared test, P value >0.05 = non significant

Table 2: Comparison between the studied groups as regards liver functions and alpha fetoprotein (AFP) results

	The studied groups			U or t-Test	*P* value
	Control N = 30	chronic HCV & HBV **N = 60**	**HCC N = 60**		
ALT (U/L) X ± SD Range	28.53±3.56 23 – 35	53.53±24.33 15 – 92	69.6±14.57 34-90	U 3.07 5.37 2.45	0.002[1] <0.001[2] 0.01[3]
AST (U/L) X ± SD Range	28.53±4.79 21 – 37	48.17±22.26 15- 84	63.2±12.54 30 – 82	U 2.61 5.28 2.52	0.009[1] <0.001[2] 0.01[3]
Alkaline phosphatase (U/L) X ± SD Range	54.40±6.31 45 – 66	132.3±62.40 58 – 274	180.90±66.6 68 – 310	U 5.30 5.42 2.80	<0.001[1] <0.001[2] 0.005[3]
Prothrombin time (Sec.) X ± SD Range	12.83±0.23 12.5 – 13.3	14.26±1.52 12.5 – 18.2	14.73±1.61 12.8 – 18.2	t-test 5.05 6.33 1.14	<0.001[1] <0.001[2] 0.26[3]
INR X ± SD Range	1.03±0.05 1 – 1.1	1.33±0.27 1 – 2	1.44±0.32 1 – 2.1	t-test 5.91 6.83 1.44	<0.001[1] <0.001[2] 0.16[3]
Tot. bilirubin (mg/dl) X ± SD Range	0.58±0.14 0.4 – 0.90	1.78±0.53 1.1 – 2.9	1.86±0.54 0.5-2.8	t-test 11.62 12.25 0.58	<0.001[1] <0.001[2] 0.57[3]
Direct bilirubin(mg/dl) X ± SD Range	0.14±0.04 0.1 – 0.2	1.30±0.38 0.8 – 2.1	1.32±0.45 0.02 – 2.1	U 5.43 5.07 0.50	<0.001[1] <0.001[2] 0.62[3]
Albumin (g/dl) X ± SD Range	4.06±0.26 3.7 – 4.7	3.19±1.0 1.5 – 5.2	3.09±0.78 1.6 – 4.8	t-test 4.45 6.15 0.42	<0.001[1] <0.001[2] 0.68[3]
Alpha fetoprotein (ng/ml) **X ± SD** **Range**	3.99±0.81 2.9 – 5.5	124.73±64.75 4 – 223	468.10±364.7 200 – 2000	U 5.21 5.42 6.55	<0.001[1] <0.001[2] <0.001[3]

X = mean, SD = Standard deviation, t = t- test, U = Mann Whitney U 1 = Comparing between Control group and chronic HCV & HBV group 2 = Comparing between Control and HCC groups 3 = Comparing between chronic HCV & HBV group and HCC group

Table 3: Comparison between chronic HCV & HBV group**and control group regarding ACE genotypes and allele**

	The studied Cases				Test	*P* value	OR	95% CI
	Control N = 30		chronic HCV & HBV **N = 60**					
	No	%	No	%	X^2			
Polymorphism DD DI II	2 8 20	6.7 26.7 66.7	12 24 24	20.0 40.0 40.0	2.19 1.67 ----	0.20 0.31 -----	 5.6 5.0	 0.57 – 55.43 0.51 – 48.75
Allele D I	N=60 12 48	 20.0 80.0	N=120 48 72	 40.0 60.0	 3.60	 0.06	 2.67	 0.95 – 7.49

X^2 = Chi square test, P value >0.05 = non significant, P value < 0.05 = significant, OR =Odds ratio CI = confidence interval

Table 4: Comparison between HCC and control groups as regards ACE genotypes and allele

	The studied Cases				Test	*P* value	OR	95% CI
	Control N = 30		HCC N = 60					
	No	%	No	%	X^2			
Polymorphism								
DD	2	6.7	26	40.0	10.9	0.002	26.0	2.61- 259.32
DI	8	26.7	24	40.0	5.43	0.02	6.0	1.26 – 28.55
II	20	66.7	10	20.0	----	-----		
Allele	N=60		N=120					
D	12	20.0	76	63.3	15.30	<0.001	6.91	2.45– 19.5
I	48	80.0	44	36.7				

X^2 = Chi square test, P value >0.05 = non significant, P value <0.05 = significant, OR =Odds ratio CI = confidence interval

Table 5: Comparison between chronic HCV & HBV **and HCC groups as regards ACE genotypes and allele:**

	The studied Cases				Test	*P* value	OR	95% CI
	chronic HCV & HBV N = 60		HCC N = 60					
	No	%	No	%	X^2			
Polymorphism								
DD	12	20.0	26	40.0	5.46	0.02	5.20	1.25 - 21.57
DI	24	40.0	24	40.0	1.74	0.19	2.4	0.64 – 8.94
II	24	40.0	10	20.0	----	-----		
Allele	N=120		N=120					
D	48	40.0	76	63.3	6.54	0.01	2.59	1.24 – 5.41
I	72	60.0	44	36.7				

X^2 = Chi square test, P value >0.05 = non significant, P value <0.05 = significant, OR =Odds ratio, CI = confidence interval

Table 6: Comparison between different genotypes as regards liver functions, alpha fetoprotein and Tumor size in HCC group

	Genotypes in HCC			K test	*P* value
	DD N = 26	DI N = 24	II N = 10		
ALT (U/L)					
X ± SD	64.2±13.8	74.2±14.6	72.6±14.8	4.41	0.11
Range	35 – 86	34 – 88	56 – 90		
AST (U/L)					
X ± SD	59.4±10.5	66.8±14.6	64.6±11.6	4.17	0.13
Range	38 – 73	30 – 82	48 – 77		
Alkaline phosphatase (U/L)					
X ± SD	160.7±57.3	200.9±65.0	185.4±90.5	2.42	0.30
Range	68 – 245	92 – 310	85 – 274		
Prothrombin time (Sec.)					
X ± SD	13.9±0.9	15.2±1.5	15.8±2.4	4.63	0.10
Range	12.8 – 15.3	`12.8 – 17.3	12.9 – 18.2		
INR					
X ± SD	1.3±0.3	1.5±0.3	1.5±0.4	2.68	0.26
Range	1.1 – 2.1	1 – 2.1	1.1 – 2		
Tot. bilirubin (mg/dl)					
X ± SD	1.9±0.4	1.9 ±0.6	1.6 ±0.7	1.47	0.48
Range	1.2 – 2.5	0.5 – 2.8	1.1 – 2.7		
Direct bilirubin(mg/dl)					
X ± SD	1.3±0.3	1.3 ±0.6	1.3 ±0.6	0.35	0.84
Range	0.8 – 1.7	0.02 – 1.9	0.7 – 2.1		
Albumin (g/dl)					
X ± SD	3.2±0.9	3.0±0.7	3.1 ±0.8	0.43	0.81
Range	1.6 – 4.8	1.8 – 4.2	2.2 – 4.1		
Alpha fetoprotein (ng/ml)					
X ± SD	361.1±169.0	566.3±512.9	510.8±294.7	2.25	0.33
Range	200 – 860	200 – 2000	210 – 1000		
Tumor size					
X ± SD	20.46±30.89	61.75±44.28	45.2±33.70	8.77	0.01
Range	6 – 90	6 – 120	12 – 90		

K = Kruskal Wallis test

4. Discussion:

Hepatocellular carcinoma is one of the most frequently diagnosed cancers worldwide. The disease is predominant in Asia and Africa, but its incidence is steadily increasing throughout the rest of the world. Most HCC develop in patients with a history of chronic hepatitis or cirrhosis in which there is continuous inflammation and regeneration of hepatocytes. Unlike other solid malignancies, the coexistence of inflammation and cirrhosis makes the early diagnosis and prognostic assessment of HCC much more difficult. (23)

Early detection of liver malignancy makes it possible to provide the patient with the most optimal therapy. For a long time, serum tumor markers have been used as an effective method for detecting malignant tumors (24-26) and they could be valuable supplements to ultrasonography and computed tomography in the diagnosis of HCC (27-29).

HCV infection is characterized by continuous inflammation that slowly results in liver fibrosis that eventually may result in the development of hepatocellular carcinoma. Hepatic fibrosis in HCV and HBV affected patients has been attributed to increased cytokines production as a result of viral infection and uncontrolled activation of the immune system. Other factors independently associated with more rapid liver fibrosis progression in chronic HCV infection include: male gender, older age, longer duration of HCV infection and alcohol abuse Reports have revealed that the renin angiotensin system (RAS) plays an important role in the liver fibrosis development with RAS components significantly up-regulated during the liver fibrosis development. (30-31)

Furthermore, it has been reported that the combination treatment with IFN in HCV or Anti-virals in HBV and ACE blockers exerted a more potent inhibitory effect on murine liver fibrosis development than either single agent. Collectively these reports point to an important role that RAS system plays in the development of HCV and HBV complications. (31)

Much evidence indicates that ACE associated with the pathology of carcinoma. ACE is differentially expressed in several malignancies and influences tumor cell proliferation, tumor cell migration, angiogenesis, and metastatic behavior. (32-33)

Our study showed that DD carriers were associated with increased HCC risk compared with II carriers. This finding suggests that the DD genotype of ACE I/D polymorphism may alter the susceptibility to HCC. This in agreement with the finding of George et al (11) who provided support that the mutation of RAS components contribute to the risk of developing of certain malignancies. Also, Yong et al (34) show that the DD carriers are associated with increased HCC risk compared with II carriers in a Chinese Dai population.

Several new studies have been published to investigate the associations between ACE gene polymorphism with cancer risk. However, the results were inconsistent and conflicting. Liu et al. (35) reported the ACE DD genotype was related to a 5.46-fold higher risk of developing oral cancer than those with the II genotype in the Chinese Taiwan population. Ruiter et al. (36) reported a correlation between the D allele and an increased risk of prostate cancer and postmenopausal breast cancer in a meta-analysis. Also Yigit et al. (37) reported DD genotype and D allele may have statistically significant increase in cancer prostate. On the contrary, Jin-Fei et al. (38) found no significant association between the D/I polymorphism of ACE gene and digestive system cancer risk. Also, Gao et al.(39) did not detect any significant correlation between ACE I/D polymorphism and lung cancer risk in a meta-analysis of six studies of 1183 lung cancer patients and 1065 controls. Similarly, Zhang et al. (40) found no significant association between the I/D polymorphism and lung cancer, breast cancer, prostate cancer, colorectal cancer, gastric cancer risks through the comparison of DD and DI vs II genotype in a meta-analysis of 25 case-control studies comprising 3914 cancer patients and 11,391 controls.

The discrepancy of the results might be explained partially by cancer or ethnicity specific effects. Cancer is multifactorial disease with complex etiology, for which interplay of various genetic and non-genetic factors is characteristic. Lukic et al. (41) found that ACE I/D polymorphism may play a role in the development of pancreatic cancer through interaction with other genetic and environmental factors. The ACE I/D polymorphism have been found to influence serum ACE activity. (42) The presence of the I allele gives rise to lower ACE activity in serum and tissues, whereas DD carriers have increased ACE levels. The increased ACE level plays a important role in neovascularization of hepatocellular carcinoma via stimulating angiotensin II production. (12,43) Castellon et al. (44) supposed that the ACE I/D polymorphism were associated with cancer susceptibility through regulation of the ACE level and angiotensin-II activity. Angiotensin-II would in turn induce vascular endothelial growth factor (VEGF) expression in the tumor cells and vascular endothelial cells.

The ACE I/D polymorphism has also been identified as a factor for predicting cancer progression and clinical outcome. Rocken et al. (45) have found that the gene polymorphism influence the metastatic behavior of gastric cancer. On the contrary, De

Martino et al.(46) revealed that neither the ACE genotypes nor alleles were associated with the tumor stage or grade in renal cell carcinoma.

In summary, in spite of the limited sample size, our results still suggest that the DD carriers are associated with increased HCC risk compared with II carriers on top of HCV and HBV infection in Egyptian population. This study also implies the possibility of prevention of HCC by manipulation of ACE function once its effect is further confirmed.

Conclusion:

The results suggested The DD and DI genotypes were correlated with a significant increased HCC risk as compared with the II genotype and the D allele was correlated with a significant increased HCC risk.

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