Global Journal of Medical research
Volume 11 Issue 5 Version 1.0 December 2011
Type: Double Blind Peer Reviewed International Research Journal
Publisher: Global Journals Inc. (USA)
Online ISSN: 2249-4618 & Print ISSN : 0975-5888

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GJMR-N Classification: *NLMC Code: QU 135-14*

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Angiotensin Converting Enzyme Gene I/D polymorphism correlates with complications in HCV infected Egyptian Patients

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Aims: The current study aimed at investigating whether there is a difference in I/D ACE genotypes distribution in a cohort of HCV Egyptian patients compared to their healthy counterparts and whether there is a significant association between different I/D genotypes and markers of HCV disease severity.

Subjects and methods: The current study included 2 groups: Hepatitis C (HCV) patients' group comprised of 78 patients (56 men and 22 women) aged (Mean ☐SD) 47.5 ± 7.0 years and a sex and aged matched control group comprised of 42 control subjects (30 men and 12 women) aged 45.2±7.5 years.

Results: Data showed a significant diference in the distribution of Angiotensin converting enzyme between HCV patients and healthy controls (p: 0.021). The percentage of the I/I, D/I and D/D in the patients and controls were: 57.1 %, 33.3%, 9.5% and 23.1%, 46.2%, 30.8% in controls and HCV patients respectively. The D allele was associated with increased lecuocytic count, wider portal vein diameter, higher Child Pugh score, increased ALT and glucose levels.

Conclusion: Our data suggest a possible role for the D allele in the progression and development of complications in Egyptian HCV patients. Larger studies are needed to confirm this hypothesis.

Keywords : Angiotenisn Converting enzyme gene polymorphism, I/D polymorphism, HCV, PCR.

I. Introduction

Hepatitis C virus (HCV) infection is the leading cause of chronic liver disease worldwide[1]. HCV infection represents a major health problem in Egypt[2]. About 60 to 80% of patients develop chronic infection, which may progress to complications (e.g. cirrhosis, variceal bleeding and hepatocellular carcinoma)[3]. On the other hand some patients had HCV latent infection for years and others may have an eventual recovery with sero-positivity as the only indication of their past HCV infection[4]. Many factors, including age, gender, alcohol consumption[5], body mass index, steatosis[6], and concomitant other viral infections (e.g. human immunodeficiency virus (HIV), hepatitis B virus)[7] affect disease outcome but are insufficient to explain it. Immunologic and genetic factors may also play an important role and are believed to have an impact on the outcome of HCV infection. Studies among monozygotic twins suggest that host genetic factors may account for 50% or more of the variability in the major outcomes in infectious diseases,[8] [9]. Different studies have illustrated a genetic predisposition for viral infections [10, 11, 12].

The ACE gene insertion/deletion (I/D) polymorphism was first identified in 1990. The gene-encoding ACE (or dipeptidyl carboxy peptidase1: DCP1) is located on chromosome 17q35 and consists of 26 exons. A 250-bp deletion/insertion polymorphism exists in intron 16 of the ACE gene and the deletion variant is associated with higher serum levels of the enzyme. The ACE gene insertion/deletion (I/D) polymorphism has been investigated in several diseases [13,14,15]. The angiotensin converting enzyme (ACE) gene I/D polymorphism influences the production of angiotensin II (ANG II), whose role in the regulation of fibrosis in the liver and other organs is increasingly recognized[16]. Recently, an inflammatory role for ACE gene has been suggested[17]. A Finnish study revealed an association between the deletion variant (D) and certain granulomatous disease "sarcoidosis[18] with a possible role in altering the cytokines level during the inflammatory process. This is alteration and suseptability of disease progression is mainly evident in certain genotypes of the angiotensin converting enzyme gene[18]. Up to our knowledge, scanty data area available about the distribution of I/D ACE gene polymorphism in patients affected with hepatitis C. The current study aimed at investigating whether there is a difference in I/D ACE genotypes distribution in a cohort of HCV Egyptian patients compared to their healthy counterparts and whether there is a significant

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association between different I/D genotypes and the HCV disease severity.

II. Patients and Methods

The study was approved by Alexandria University Ethical committee. Patients included in this study were seen in the Internal Medicine Department of the Medical Research Institute Teaching Hospital of Alexandria University, Egypt. All patients and control subjects gave their written informed consent before participating in the study. The current study included 2 groups: Hepatitis C (HCV) patients' group comprised of 78 patients (56 men and 22 women) aged (Mean ±SD) 47.5 ± 7.0 years and a sex and aged matched control group comprised of 42 control subjects (30 men and 12 women) aged 45.2±7.5 years.

Exclusion criteria included cases of hepatitis B infection, autoimmune hepatitis, metabolic liver diseases (haemochromatosis, Wilson's disease, non alcoholic steatohepatitis), history of alcohol consumption or malignancy.

The followings were done for the patients and control subjects: full clinical examination including history taking, blood pressure measurement and abdominal ultrasound examination with evaluation of different hepatobillry parameters including portal, hepatic and mesenteric veins diameters. Complete urine and stool examination[19]. Venous blood samples were taken from each subject after an over night fast. Blood samples were collected in plain tubes, centrifuged and analyzed for fasting serum glucose, urea, creatinine, total serum protein, albumin, liver enzymes (Aspartate aminotransferase and alanine aminotransferase), gamma glutareyl transferase, alkaline phosphastase, total and direct bilirubin. These were measured using a Konelab Chemistry analyzer[20] (Thermo Electron Oy, Vantaa, Finland. https://www.thermo.com). Citrated and EDTA samples were taken for prothrombin activity and full blood count. The remaining serum was used for testing HCV antibodies. The presence of anti-HCV antibodies was determined in serum samples by enzyme linked immunosorbent assay (ELISA-II; Ortho Diagnostic Test Systems, Raritan, NJ, USA).

Genomic DNA was isolated from nucleated blood cells (separated from EDTA blood sample) using standard technique[21]. DNA samples were kept at -80 ^{0}C till analysed. The I/D polymorphism of the ACE gene was determined according to the method of Rigat et al[22]. Briefly, about 50 to 80 ng DNA samples were amplified in a final volume of 25 µL containing 1×PCR buffer with 1.5 mmol/L MgCl2, 2 unit Taq DNA polymerase, 100 µmol/L dNTP,and 0.5 µmol/L of each primer and 5% DMSO (dimethyl sulphoxide). The sequences of the sense and antisense primers were 5'-CTG GAG ACC ACT CCC ATC CTT TCT-3' and 5'-GAT GTG GCC ATC ACA TTC GTC AGA T-3', respectively. DMSO was included in the PCR to prevent underestimation of heterozygotes and overestimation of D/D genotype[23]

PCR was performed in a GeneAmp, thermocycler (Biorad, USA). Samples were denatured for 1 minute at 94°C and then cycled 30 times through the following steps: 45 seconds at 94°C, 1 minute at 62°C, and 1 minute at 72°C. PCR products were electrophoresed in 1.6% agarose gel and visualized directly with ethidium bromide staining. The insertion allele (I) was detected as a 490-bp band, and the deletion allele (D) was detected as a 190-bp band. While The I/I genotype was detected as a single band of 490 Bp, the D/D genotype was detected as a single band of 190-bp while the I/D was detected by the presence of two bands a 490-bp and 190 –bp. To ensure quality control, genotyping was performed with blinding to case/control status, and random samples of cases and controls were tested twice by different persons, and the results were concordant for all masked cases.

III. Statistical Analysis

Prevalence of alleles and genotype among cases and control subjects were counted and compared with Hardy–Weinberg predictions[24]. Chi-square test (Fisher's exact test) was used to test the distribution of the different genotypes in the different groups. P value of < 0.05 was considered statistically significant. Statistical analysis was performed using SPSS 11.5 statistical Package.

IV. Results

Clinical and biochemical data of the studied groups are illustrated in table1. There was no significant age difference between the different groups enrolled in this study. Ultrasound findings showed a significantly enlargement of the liver right lobe and spleen diameters and portal vein diameters in HCV patients compared to the health controls ($P<0.05$) Spleenic vein diameter correlated positively with portal vein diameter and longitudinal spleen length (r: 0.649 P: 0001 & r:0.37 &P:0.02).

The different genotypes were in agreement with Hardy–Weinberg equilibrium. Analysis of the I/D angiotensin gene polymorphism revealed a significant difference for the different genotypes between the different groups. ($P<0.021$). Figure 1 shows an illustration of the different genotypes of I/D Ace gene polymorphism. HCV patients group showed a higher percentage of D/I and DD genotypes than the control group. Table II shows the frequency of each genotype in the different groups. Multivariate analysis did not show a significant confounding effect of age, sex, history of schistosmoal infection on the ACE genotyping results.

HCV patients were stratified according to the different I/D genotype and the different parameters were analyzed (table III). There was a significant difference

within the three groups, namely I/I, D/I and those with D/D genotype for total leucocytic count, Child Pugh score, portal vein diameter, ALT and plasma glucose glucose($P<0.05$).

Patients with D/I and those with D/D had significantly higher total leucocytic counts, Child Pugh scoring, portal vein diameter, ALT (alanine amino transferase) and plasma glucose (P values: 0.032, 0.027, 0.0495, 0.029 and 0.043 respectively).

V. Discussion

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 affected patients has been attributed to increased cytokines production as a result of HCV infection and uncontrolled activation of the immune system. Other factors that may contribute in the progression of hepatic fibrosis, include male sex, older age, longer duration of HCV infection, high levels of alcohol consumption and HIV co-infection. These factors have been associated with more severe liver damage in patients with chronic hepatitis C and accelerated HCV-related liver fibrosis.

Recent 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. Furthermore, it has been recently reported that the combination treatment with IFN 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 complications.

The current study centered on exploring the possibility of the presence of genetic factors affecting the RAS specially the I/D polymorphism of the Angiotensin Converting Enzyme gene, that might influence the susceptibility to HCV infection and development of complications. The study evaluated the distribution of I/D polymorphism of the angiotensin converting enzyme gene in HCV patients and an age and sex matched control group. There was a significantly higher percentage of HCV Egyptian patients having the I/D and DD genotypes than the healthy controls. In HCV patients, the D allele carriers had a significantly higher total leucocytic counts, alanine aminotransferase, plasma glucose and had a higher Child Pugh scoring. Our results are in agreement with Fabris et al who found a carriers of the D allele especially female patients have a poor outcome with increased complication post hepatic transplantation[32].

The current study also showed an association between the D allele and increased plasma glucose level in HCV positive patients. Insulin resistance is a known complication of HCV patients. Previously, increased insulin resistance has been documented in HCV positive patients that correlated with the HCV infectivity and was attributed to increase cytokines production in chronic HCV patients.[33] Recently, tudies have also demonstrated that ACE insertion/deletion (I/D) polymorphism is associated with development of insulin resistance and eventually diabetes mellitus complications in a different ethnic populations.[34]

The findings of increased blood glucose in D allele carriers of HCV patients is in keeping with the findings of Mittal et al who clearly demonstrated an association of the components of metabolic syndrome especially fasting glucose and the D allele of ACE gene polymorphism[35]. Similarly in Iranians, the D allele of the angiotensin converting enzyme gene seemed to be associated with Diabetes mellitus and poor glycemic control.[36]Thus the association found in the current study between elevated blood glucose and the D allele may offer an important rationale for the increased insulin resistance commonly seen in chronic HCV patients. The adverse effect on glycemic control that is seen in our HCV patients may possibly be through end organ damage, fibrosis, and poor inflammatory response36 and control of microvascular blood flow[37]and free radical levels[38]secondary to modulated ACE gene expression.

In summary, in Egyptians our results shows an association between D allele of the Angiotenisn Converting Enzyme gene and the different complications of HCV infections (namely; higher Child Pugh scoring, portal vein diameter and poor glycemic control. Our findings may be important in detecting HCV who may need more intensive treatment to prevent complications.

Future work may concentrate on evaluating whether Angiotensin gene polymorphism may be a factor in determining the response of different antiviral therapies used in HCV infection.

VI. Limitation of the Current Study

This is a pilot study involving only 78 HCV positive Egyptian patients an 42 controls. The small sample size may have been a limiting factor in the detection of other possible association between ACE gene polymorphism and other clinical variables. ACE activity was not performed as the study aimed mainly at evaluation I/D ACE genotype distribution in the studied group of Egyptians.

Acknowledgements

This work was supported by a research fund from Medical Research Institute, Alexandria University, Egypt. The authors declares no conflict of interests.

References References Referencias

1. Boyer, N., and P. Marcellin. 2000. Pathogenesis, diagnosis and management of hepatitis C. J. Hepatol. 32(Suppl. 1):98-112.

2. Darwish NM, Abbas MO, Hady SI, Mohammed TA. Study of the high prevalence of HCV in Egypt. J Egypt Public Health Assoc. 1995;70:397-414.
3. Bostan N, Mahmood T .An overview about hepatitis C: a devastating virus. Crit Rev Microbiol. 2010;36:91-133..
4. Guobuzaite A, Chokshi S, Balciūniene L, Voinic A, Stikleryte A, Zagminas K, Ambrozaitis A, Naoumov N. Viral clearance or persistence after acute hepatitis C infection: interim results from a prospective study. Medicina (Kaunas). 2008;44:510-20.
5. Chen CM, Yoon YH, Yi HY, Lucas DL.Alcohol and hepatitis C mortality among males and females in the United States: a life table analysis. Alcohol Clin Exp Res. 2007;31:285-92.
6. Cholet F, Nousbaum JB, Richecoeur M, Oger E, Cauvin JM, Lagarde N, Robaszkiewicz M, Gouérou H .Factors associated with liver steatosis and fibrosis in chronic hepatitis C patients. Gastroenterol Clin Biol. 2004;28:272-8.
7. De Bona A, Galli L, Gallotta G, Guzzo A, Alagna L, Lazzarin A, Uberti-Foppa C.Rate of cirrhosis progression reduced in HIV/HCV co-infected non-responders to anti-HCV therapy. New Microbiol. 2007;30:259-64.
8. Lin TM, Chen CJ, Wu MM et al. Hepatitis B virus markers in Chinese twins. Anticancer Res 1989; 9: 737–741.
9. Alric L, Fort M, Izopet J et al. Genes of the major histocompatibility complex class II influence the outcome of hepatitis C virus infection. Gastroenterology 1997; 113: 1675–1681.
10. Ying-Li He, Ying-Ren Zhao, Shu-Lin Zhang, Shu-Mei Lin. Host susceptibility to persistent hepatitis B virus infection. World J Gastroenterol 2006; 12: 4788-4793.
11. Krzysztof Kiryluk, Jeremiah Martino, and Ali G. Gharavi. Genetic Susceptibility, HIV infection and the Kidney. Clin J Am Soc Nephrol 2007; 2: S25-S35,
12. Stark JM, McDowell SA, Koenigsknecht V, Prows DR, Leikauf JE, Le Vine AM, Leikauf GD Genetic susceptibility to respiratory syncytial virus infection in inbred mice. J Med Virol. 2002;67:92-100.
13. Baudin B. New aspects on angiotensin-converting enzyme: from gene to disease. Clin Chem Lab Med 2002;40:256-65.
14. Pietinalho A, Furuya K, Yamaguchi E, et al. The angiotensin- converting enzyme DD gene is associated with poor prognosis in Finnish sarcoidosis patients. Eur Respir J 1999;13:723-6.
15. Beohar N, Damaraju S, Prather A, et al. Angiotensin-I converting enzyme genotype DD is a risk factor for coronary artery disease. J Investig Med 1995;43:275-80.
16. Moreno M, Gonzalo T, Kok RJ, Sancho-Bru P, van Beuge M, Swart J, Prakash J, Temming K, Fondevila C, Beljaars L, Lacombe M, van der Hoeven P, Arroyo V, Poelstra K, Brenner DA, Ginès P, Bataller R. Reduction of advanced liver fibrosis by short-term targeted delivery of an angiotensin receptor blocker to hepatic stellate cells in rats. Hepatology. 2010 ;51:942-52.
17. Tousoulis D, Koumallos N, Antoniades C, Antonopoulos AS, Bakogiannis C, Milliou A, Marinou K, Kallikazarou E, Stefanadi E, Mentzikof D, Stefanadis C. Genetic polymorphism on type 2 receptor of angiotensin II, modifies cardiovascular risk and systemic inflammation in hypertensive males. Am J Hypertens. 2010;23:237-42.
18. Pietinalho A, Furuya K, Yamaguchi E, Kawakami K, Selroos O. The angiotensin-converting enzyme DD gene is associated with poor prognosis in Finnish sarcoidosis patients. Eur Respir J 1999;13:723–726.
19. Peters PA, El Alamy M, Warren KS, Mahmoud AA. Quick Kato smear for field quantification of Schistosoma mansoni eggs. Am J Trop Med Hyg. 1980;29:217-9.
20. Burtis CA, Ashwood ER. Tietz Fundamental of Clinical Chemistry, 4th edn Philadelphia: W.B. Saunders, 1996.
21. Ausubel FM, Brent R, Kingston RE, Moore DD, Seidman JG, Smith JA, Struhl K. Current Protocols In Molecular Biology, 2nd ed. New York: Wiley Interscience. 1987.
22. Rigat B, Hubert C, Corvol P, Soubrier F. PCR detection of the insertion/deletion polymorphism of the human angiotensin-converting enzyme gene (DCP1 dipeptidyl carboxypeptidase). Nucl Acids Res. 1992;20:1433.
23. Fogarty DG, Maxwell AP, Doherty CC, Hughes AE, Nevin NC. ACE gene typing. Lancet. 1994;343:851.
24. Emery AEH. Hardy–Weinberg equilibrium and the estimation of gene frequencies. In: Emery AEH, editor. Methodology in medical genetics: an introduction to statistical methods. Edinburgh: Churchill Livingstone.1976:3 - 9.
25. McGlynn KA, London WT The global epidemiology of hepatocellular carcinoma: present and future. Clin Liver Dis. 2011 May;15(2):223-43, vii-x.
26. Eurich D, Bahra M, Boas-Knoop S, Lock JF, Golembus J, Neuhaus R, Neuhaus P, Neumann UP Transforming growth factor β1 polymorphisms and progression of graft fibrosis after liver transplantation for hepatitis C virus--induced liver disease. Liver Transpl. 2011 Mar;17(3):279-88.
27. Peters L, Rockstroh JK. Biomarkers of fibrosis and impaired liver function in chronic hepatitis C: how well do they predict clinical outcomes? Curr Opin HIV AIDS. 2010;5:517-23.
28. Yoshiji H, Kuriyama S, Fukui H. 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 2002; 23: 348-356.

29. Bataller R, Sancho-Bru P, Gines P, Brenner DA. Liver fibrogenesis: a new role for the renin-angiotensin system. Antioxid Redox Signal 2005; 7: 1346-1355.
30. Yoshiji H, Yoshii J, Ikenaka Y, Noguchi R, Tsujinoue H, Nakatani T, Imazu H, Yanase K, Kuriyama S, Fukui H. Inhibition of renin-angiotensin system attenuates liver enzyme-altered preneoplastic lesions and fibrosis development in rats. J Hepatol 2002; 37: 22-30
31. Rimola A, Londono MC, Guevara G, Bruguera M, Navasa M, Forns X, Garcia-Retortillo M, Garcia-Valdecasas JC, Rodes J. Beneficial effect of angiotensin-blocking agents on graft fibrosis in hepatitis C recurrence after liver transplantation. Transplantation 2004; 78: 686-691.
32. Fabris C, Toniutto P, Bitetto D, Minisini R, Fornasiere E, Smirne C, Pirisi M. . Sex-related influence of angiotensin-converting enzyme polymorphisms on fibrosis progression due to recurrent hepatitis C after liver transplantation. J Gastroenterol. 2007;42:543-9.
33. Elsammak M, Refai W, Elsawaf A, Abdel-Fattah I, Abd Elatti E, Ghazal A. Elevated serum tumor necrosis factor alpha and ferritin may contribute to the insulin resistance found in HCV positive Egyptian patients. Curr Med Res Opin. 2005;21:527-34.
34. Wang Y, Ng MC, So WY, Tong PC, Ma RC, Chow CC, Cockram CS, Chan JC: Prognostic effect of insertion/deletion polymorphism of the ace gene on renal and cardiovascular clinical outcomes in Chinese patients with type 2 diabetes. Diabetes Care.2005; 28: 348–354.
35. Mittal G, Gupta V, Haque SF, Khan AS. Effect of angiotensin converting enzyme gene I/D polymorphism in patients with metabolic syndrome in North Indian population. Chin Med J (Engl). 2011;124:45-8.
36. Nikzamir A, Nakhjavani M, Golmohamadi T, Dibai L.Association of angiotensin-converting enzyme gene insertion/deletion polymorphism with metabolic syndrome in Iranians with type 2 diabetes mellitus. Arch Iran Med. 2008 ;11:3-9
37. Xia H, Lazartigues E. Angiotensin-converting enzyme 2: central regulator for cardiovascular function. Curr Hypertens Rep. 2010;12:170-5
38. San José G, Fortuño A, Moreno MU, Robador PA, Bidegain J, Varo N, Beloqui O, Díez J, Zalba G. The angiotensin-converting enzyme insertion/deletion polymorphism is associated with phagocytic NADPH oxidase-dependent superoxide generation: potential implication in hypertension. Clin Sci (Lond). 2009 ;116:233-40.

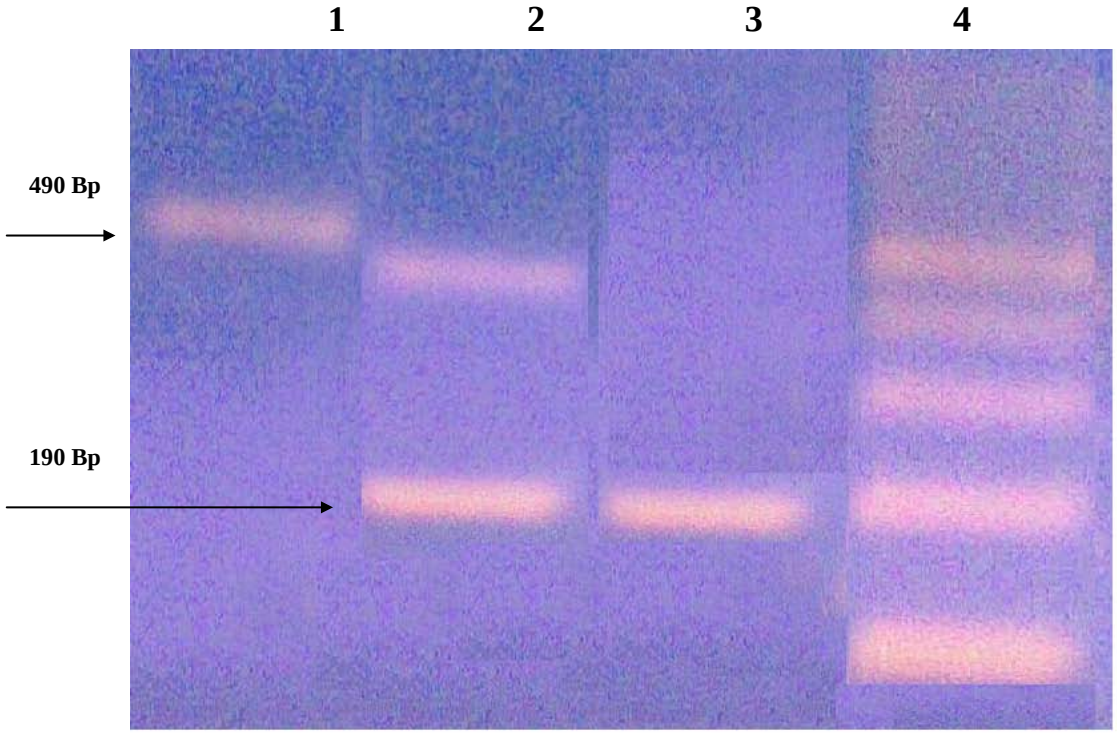

Figure 1 : Products of PCR amplification of the I/D polymorphism in the Angiotensin converting enzyme gene.

PCR products were electrophoresed in 1.6% agarose gel and visualized directly with ethidium bromide staining. The insertion allele (*I*) was detected as a 490-bp band, and the deletion allele (*D*) was detected as a 190-bp band. DNA samples were amplified using PCR and digested with *Taq1b* restriction enzyme. The presence of the Taq1B restriction site produces the B1 allele pattern in electrophoresis with 2 DNA fragments at 361 and 174 Bp respectively. The lack of Taq1B restriction site results in the B2B2 pattern with only one band at 535 Bp. The B1B2 genotypes resulted in 3 bands at 535, 361 and 174 Bp.

Lane 1: I/I genotype showing as one DNA band at 490 Bp
Lane 2: I/D genotype with 2 DNA bands at 490 and 190 Bp
Lane 3: D/D genotype showing as one band at 190 Bp
Lane 4: Ladder marker.100 base pairs (Bp)

Parameter	Controls (n: 42)	HCV patients (n:78)
AGE (years)	45.2±7.5	47.5 ± 7.0
Males/females	30/12	56/22
History of Encephalopathy	0	20 % (16cases)* *
Liver Right lobe (cm)	12.9±0.22	14.8±1.9**
Spleen (Cm)	10.8 ± 0.92	15.7 ± 2.4**
History of Ascites	No	46 No ascites 22 Mild 4 Moderate 6 Severe
Spleenic vein Diameter (mm)	7.6±0.8	10.2 ± 2.8**
Superior Mesenteric vein Diameter (mm)	7.6 ± 0 .71	10.6 ± 2.5**
Portal Vein Diameter (mm)	10.92 ± 0.7	15.65 ± 2.3**
Prothrombin activity (%)	92.9±6.8	61.9 ±16.5**
Haemoglobin (gm/dl)	14.3 ± 0.5	11.8 ± 1.5
Leucocytic count $X10^3$	4. 2 (2.3 -14.9.)	6.2 (4.5 – 9.0) *
Platelets $X10^6$	286.0(174.0-432.0)	115.0(110.0- 303.0)* *
Child Pugh score	0	7.1± 2.6 10 cases score 5 18 cases sscore:6 8 Cases scores 7 14 Cases scores 8 6 Cases scores 9 4 Cases scores 10 8 Cases scores 11 4 Cases scores 12 4 Cases scores 13 2 Cases scores 14
ALT (U/L)	23.0(6.0- 41.(	49.0(12.0-122.0)*
AST (U/L)	21.08.0-38.(	47.0(21.0- 157.0)*

GGT(U/L)	21.0(16.5-61.0	77.0(20- 625.0)*
ALP (U/L)	78.0(47.0-117.(	112.0(57.0- 589.0)*
Serum Protein (g/dl)	7.9 ± 0.7	7.03 ± 0.7*
Serum Albumin (g/dl)	4.4 ± 0.5	3.5 ± 0.7*
Total bilirubin (umol/L)	13.0 ± 4.0	32.0 ± 21.0*
Direct bilirubin (umol/L)	4.0 ± 2.0	15.0 ± 5.0*
Urea (mmol/L)	4.5 (2.0- 8.2)	4.3 (2.7 – 31.3)
Creatinine (umol/L)	76.9 ± 20.3	97.2 ± 34.4*
FPG (mmol/L)	4.8 (3.7-6.3)	6.2 (3.6- 14.7)*

Table1 : Clinical and biochemical criteria of the studied groups:

Data are presented as Mean ±SD for normally distributed variables for non-normally distributed variables results are presented as Median (range)

ALT: Alanine amino transferase

AST: Aspartate amino transferase

ALP: Alkaline phosphatase

GGT: Gamma Glutaryl transferase

FPG: Fasting plasma glucose

* = Significant difference versus the control groups (P<0.05)

** = Significant difference versus the control groups (P<0.05)

Group			Gene			Total	P value
			D / D	D / I	I / I		
	Controls	Number	4	14	24	42	
		% within Controls	9.5%	33.3%	57.1%	100.0%	
		% of Total	3.3%	11.7%	20.0%	35.0%	
	HCV (Patients)	Number	24	36	18	78	0.021*
		% within patients	30.8%	46.2%	23.1%	100.0%	
		% of Total	20.0%	30.0%	15.0%	65.0%	
Total		Count	28	50	42	120	
		% of Total	23.3%	41.7%	35.0%	100.0%	

Table II : Frequency of the different D/I genotypes in HCV patients and healthy controls and the result of the Chi square testing in the different studied groups. Table shows the frequency and the results of the cross tabulation of the different genotypes in the studied groups. The number in each subgroup is shown and the (percentage). Chi square test was used in calculation.

* : Significant P with a value <0.05

Parameter	I /I (n:18)	D /I (n:36)	D /D (n:24)	*P* value
Liver Right lobe (cm)	15.3 ±1.4	14.4 ±1.99	14.9 ± 2.0	0.227
Spleem (Cm)	14.4 ±1.82	16.01 ± 2.55	16.2 ± 2.4	0.097
Spleenic vein Diameter (mm)	9.16 ± 1.6	10.57 ± 3.21	10.48 ± 2.98	0.74
Superior Mesenteric vein Diameter(mm)	10.1 ± 2.45	10.33 ± 2.62	11.45 ± 2.3	0.32
Portal Vein Diameter (mm)	14.67 ± 0.68	15.95 ± 2.51	15.91 ± 2.83	0.038*
Prothrombin activity (%)	70.0 ± 17.6	60.76 ± 15.84	53.68 ± 12.78	0.049
Haemoglobin (gm/dl)	12.7 ± 1.6	11.4 ±1.4	11.5 ±1.2	0.35
Leucocytic count X10^3	3.4 (2.3 -7.6)	4.3 (3.0 .- 14.90.0)	4.3 (3.0 .- 9.0)	0.032*
Platelets count X 10^6	115.0 (75.0 -176.0)	102.5 (110.0 – 303.0)	148.0 (77.0 – 212.0)	0.62
Child Pugh score	6.75 ±1.8	8.9 ± 2.6	8.6 ± 2.8	0.04*
ALT (U/L)	34.0(12-76)	45.0(16-92)	57.50(17.0-122.0)	0.029
AST (U/L)	40.0(21-72)	51.5(26-112)	49.50(25-157.0)	0.81
GGT(U/L)	77.0(35.0-457)	68.0(20-625)	116.0(29.0-248.0)	0.05
ALP (U/L)	112.0(88.0-589)	112.0(66-226)	105.0(57.0- 188.0)	0.459
Serum Protein (g/dl)	6.9 ± 0.8	7.2 ± 0.7	7.05 ± 0.5	0.245
Serum Albumin (g/dl)	3.41 ± 0.9	3.27± 0.73	3.73 ± 0.5	0.211
Total bilirubin (umol/L)	22.0 ±19.0	40.0 ±26.0	24.0 ± 9.0	0.332
Urea (mmol/L)	4.5(3.5-18.8)	4.2(3.2-19.6)	4.3(2.7-15.6)	0.561
Creatinine (umol/L)	105.2 ± 40.7	91.1 ± 30.9	86.6 ± 33.6	0.446
Glucose (mmol/L)	4.22.(2.85-12.9)	5.4(4.1-14.7)	5.6(4.5- 14.1)	0.04*

Table III : Clinical and biochemical parameters in HCV patients included in the study according to their ACE genotype. Data are presented as Mean ±SD for normally distributed variables for non-normally distributed variables results are presented as Median (range)

ALT: Alanine amino transferase

AST: Aspartate amino transferase

ALP: Alkaline phosphatase

GGT: Gamma Glutaryl transferase

FPG: Fasting plasma glucose

* = Significant difference versus the control groups ($P<0.05$)

$P<0.05$= Significant difference