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

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Abstract

Hepatitis C (HCV) infection represents a major health problem in Egypt with a reported prevalence of more than 20

Index terms— Angiotenisn Converting enzyme gene polymorphism, I/D polymorphism, HCV, PCR

1 INTRODUCTION

epatitis 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, **??** 9 . Different studies have illustrated a genetic predisposition for viral infections **??**0 , 11 ,12 . The ACE gene insertion/deletion (I/D) polymorphism was first identified in 1990. The geneencoding 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 H Global Journal of Medical 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 association between different I/D genotypes and the HCV disease severity.

2 II.

3 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 ?L containing 1×PCR buffer with 1.5 mmol/L MgCl2, 2 unit Taq DNA polymerase, 100 ?mol/L dNTP,and 0.5 ?mol/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.

4 III.

5 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.

6 IV.

7 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 **??**I 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 **??**II). 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.

8 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 reninangiotensin 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 ACEblockers 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 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 .

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. 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.

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 . Similarly in Iranians, the D allele of the angiotensin converting enzyme gene seemed to be associated with Diabetes mellitus and poor glycemic control. 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 and free radical levels 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.

9 VI. LIMITATION OF THE CURRENT STUDY

1 2 3 4 5

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Figure 1: Figure 1 :

Effect of angiotensin converting enzymegene 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

Figure 2:

.1 ACKNOWLEDGEMENTS

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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.

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