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Impact of Apo E gene polymorphism on HCV therapy related outcome in a cohort of HCV Egyptian patients

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ABSTRACT

The functional apolipoprotein E (Apo E) gene polymorphism could be used as a determinant of outcome of HCV infection. This study aimed to demonstrate the impact of Apo E genotype on the response to HCV combined therapy. Material and methods: The study has been implemented on 125 individuals with persistent HCV infection and 120 cases with sustained virologic response (SVR). All participants were genotyped for ApoE gene polymorphism by a real-time quantitative PCR (qPCR). Results: Statistically significant differences were demonstrated regarding the Apo E genotypes between the two groups (P-value < .001) where the frequency of E3E3 was significantly higher among the chronic HCV-patients while E3E4 and E4E4 genotypes frequencies were higher among the SVR-subjects group and E3E3 genotype was associated with increased risk of chronicity (OR 4.7; 95% CI 1.9–12.1, P-value < .001). Moreover, There were statically significant differences regarding E3 and E4 alleles frequencies, where E3 allele display a higher frequency among the chronic HCV-patient group while the SVR-subjects group showed higher frequency of E4 allele and the carriers of E3 allele have 1.4 times more risk to develop chronicity than those with E4 allele (OR 1.4; 95% CI 1.0–2.0, P-value < .05). Meanwhile the protective E2 allele was absent in all infected participants. Conclusion: This study supports the hypothesis of the protective impact of Apo E4 allele that favors viral clearance of HCV infection and its recovery after combined therapy, while the Apo E3 allele is considered as a particular risk factor for the chronicity in HCV patients and resistance to therapy. Whereas the Apo E2 allele confers a resistance to HCV infection at a time of exposure.

Contents

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1. Introduction

A unique feature of HCV is that both cholesterol metabolism and fatty acid biosynthetic pathways are among the fundamental stones for both RNA replication and virion assembly in host cells [1]. Apolipoprotein E (Apo E), a ligand for low density lipoprotein receptor (LDLr), is now prescribed as an irreplaceable component of the HCV–host lipid interaction through involvement at several stages in the viral lifecycle, including intracellular viral assembly [2,3]. Apo E has been well described as a main regulator of many steps of lipid (cholesterol and triglyceride) and lipoprotein metabolism, including synthesis and secretion of very low density lipoproteins (VLDL), VLDL hydrolysis to produce low density lipoproteins (LDL) and receptor mediated removal of triglyceride-rich lipoprotein remnants (VLDL and Chylomicrons remnants) by the liver. Lipoviral Particles (LVP) had been identified as low-density HCV virions packaged as LVPs with densities similar to that of the very-low-density lipoprotein (VLDL) and abundance of Apo E on their surface detected by electron microscopy [4], shedding the light on the implication of the Apo E in mediating HCV infectivity via lipoprotein receptors [5]. Furthermore, Apo E have been detected in the low-density fractions of the HCV RNA-containing particles which had been declared as the fractions carries the probability of being infectious [6]. Endocytosis of HCV is mediated by low density lipoprotein receptor (LDLr) and Scavenger receptor B1 (Sc-B1). LDL r normally transports 2 different classes of cholesterol containing lipoprotein particles (LDL & VLDL) which contain multiple copies of Apo E [7]. While Sc-B1 which is expressed primarily in the liver recognizes a broad variety of lipoprotein ligands (HDL, LDL, VLDL and oxidized LDL) [8].

Apo E is defined as a polymorphic protein arising from three alleles. The human Apo E gene was widely studied and described to be located on chromosome 19, closely linked to the Apo C-I/C-II gene complex [9]. The three major alleles, termed Epsilon-2, Epsilon-3 and Epsilon-4 have been reported [10]. The apoE2, apoE3 and apoE4 protein isoforms, corresponding products of these alleles, differ only by a single amino acid at two residues. Where Apo E2 contains cysteine at two residue 112 and 158 and Apo E4 has arginine at both positions while apoE3 has cysteine at residue 112 and arginine at residue 158 [11]. Less than 0.1% of the population have additionally, two minor alleles of the gene, ε1 and ε5. Three homozygous (E2/E2, E3/E3, E4/E4) and three heterozygous (E2/E3, E2/E4, E3/E4) genotypes will be determined by these three major alleles [12].

This study was conducted to determine the relevance of the Apo E gene polymorphism on the outcome of HCV infection after combined therapy and whether these allelic variants could be used as risk biomarkers of HCV infection prognosis.

2. Material and methods

2.1. Patients

Patients with HCV-genotype 4 infection attended for a morning fasting blood sample. Individuals that were alcohol dependent, on concurrent lipid lowering or co-infected with hepatitis B, schistosoma, or human-immunodeficiency virus, were excluded from the start. A total of 245 patients were included in this study, 125 chronic HCV cases (non responders to Interferon and ribavirin therapy), 120 patients who achieved a SVR (those had a negative HCV-RNA after 6 months of completing 48 weeks therapy) [13].

The participants were recruited from hepatology clinic of National Research Centre and Liver Institute of Cairo University. The study protocol conformed to the ethical guidelines of the 1975 Declaration of Helsinki and was approved by the National Research Centre (Egypt) ethical committee and patients provided written informed consents to participate.

2.2. Laboratory investigations

A peripheral blood sample was obtained from all participants. An immediate centrifugation was done for 10 min at 5000 rpm at 4 °C. The centrifuged serum was transferred into sterile tubes. All samples were stored at −20 °C until assay. One ml of venous blood sample was collected in EDTA vials for the extraction of genomic DNA.

Total cholesterol, HDL-cholesterol (HDL-C), triglycerides and very low density cholesterol, were measured by standard automated enzymatic methods using an Olympus AU400 analyser (Olympus, Diagnostica, Japan). LDL cholesterol (LDL-C) was calculated using the Friedewald equation [14]. The serum LDL-c was calculated by this formula [15] as TG level did not exceed 400 mg/dl: LDL-c = total cholesterol − (TG/5 + HDL-c).

2.3. Hepatitis C virus detection and genotyping

The presence of HCV antibodies in serum was detected by third-generation enzyme linked immunosorbent assay ELISA; CTK-Bioteck-USA).

Viral RNA was extracted from patient's plasma using the QIAamp Viral RNA Kit (Qiagen Hilden, Germany) according to the manufacturer's protocol. HCV RNA was determined by Toyobo RNA-direct real time PCR kit on SLAN Real Time PCR Detection System, LG Lifescience, Korea.

The HCV genotype was defined by the reverse line probe assay (INNO-LIPA v.1.0, innogenetics, Ghent, Belgium) according to the manufacturer's instructions. This is the most widely used methods for HCV genotyping where the 5′ UTR of HCV is amplified with biotinylated primers, after which the PCR product is hybridized to a membrane impregnated with genotype-specific probes and detected with streptavidin linked to a colorimetric detector [16]. The LiPA-I kit included 17 probes: generic 1 (2 probes), 1a, 1b, generic 2 (2 probes), 2a (2 probes), 2b (2 probes), 3a (4 probes), and 4/5 (3 probes) [17].

2.4. Apo E genotyping

A real-time qPCR strategy with SYBR** Green I was used that rapidly genotype human Apo E haplotype alleles according to the methodology described by Andre et al., 2004 [18] with slight modifications. Apo E haplotype determination based on the differential amplification of alleles using designed primer sets that contain specific terminal bases for SNP interrogation. Amplification is performed on Applied Biosystems 7500 Instrument.

Primers used in the reaction: the 3′ terminal base of each primer corresponds to a specific SNP allele

Forward primers correspond to the SNP at position 3937:

F(T):5′GGA-CAT-GGA-GGA-CGT-G(T)-3′
F(C):5′GGA-CAT-GGA-GGA-CGT-G(C)-3′

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