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# The potential impact of P53 and APO-1 genetic polymorphisms on hepatitis C genotype 4a susceptibility

Emad F. Eskander [a], Ahmed A. Abd-Rabou [a,*], Mervat S. Mohamed [b], Shaymaa M.M. Yahya [a], Ashraf El Sherbini [c], Olfat G. Shaker [d]

[a] Hormones Department, Medical Research Division, National Research Centre, Cairo, Egypt
[b] Chemistry Department, Biochemistry Specialty, Faculty of Science, Cairo University, Egypt
[c] Internal Medicine Department, Medical Research Division, National Research Centre, Cairo, Egypt
[d] Medical Biochemistry and Molecular Biology Department, Faculty of Medicine, Cairo University, Egypt

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ABSTRACT

The hepatitis C virus (HCV), the main cause of morbidity and mortality, is endemic worldwide. HCV causes cirrhosis and other complications that often lead to death. HCV is most common in underdeveloped nations, with the highest prevalence rates in Egypt. Tumor suppressor gene (P53) induces the expression of apoptotic antigen-1 gene (APO-1) by binding to its promoter for mediating apoptosis; an important mechanism for limiting viral replication. This study aims at investigating the impact of P53 72 Arg/Pro and APO-1 −670 A/G polymorphisms on HCV genotype 4a susceptibility. Two hundred and forty volunteers were enrolled in this study and divided into two major groups; 160 HCV infected patient group and 80 healthy control group. HCV patients were classified according to Metavir scoring system into two subgroups; 72 patients in F0/1-HCV subgroup (patients with no or mild fibrotic stages) and 38 patients in F3/4-HCV subgroup (patients with advanced fibrotic stages). Quantification of HCV-RNA by qRT-PCR and fibrotic scores as well as genotyping of HCV-RNA, P53 at 72 Arg/Pro, and APO-1 at −670 A/G were performed for all subjects. It was resulted that F0/1-HCV patients have significant differences of P53 at 72 (Pro/Pro and Arg/Arg) genotypes and dominant/recessive genetic models as well as APO-1 −670 A/A genotype and dominant genetic model as compared to F3/4-HCV patients. Moreover, HCV patients have significant differences of P53 at 72 (Pro/Pro) genotype and recessive genetic model as well as APO-1 −670 A/A genotype and dominant genetic model as compared to those of healthy individuals. Finally, it was concluded that P53 rs 1042522 (Pro/Pro and Arg/Arg) genotypes and APO-1 rs 1800682 A/A genotype may be potentially used as sensitive genetic markers for HCV genotype 4a susceptibility.



## 1. Introduction

The hepatitis C virus (HCV), the silent killer, is identified 25 years ago (Choo et al., 1989). Since then, this endemic disease has spread widely, affecting 2–3% of the population (Division of Viral Hepatitis NCfHA, Viral Hepatitis, STD, and TB Prevention, 2011; Mohd Hanafiah et al., 2013; Prevention CfDCa, 2014). The distribution of HCV infection varies broadly among various geographic areas with the highest prevalence rates in Africa and Asia, while lower rates in industrialized nations like North America, Northern and Western Europe, and Australia (Zidan et al., 2012).

Egypt has the highest prevalence of HCV infections with a worse situation, where approximately 13% of the Egyptians are HCV positive (Elkady et al., 2009). In Egypt, the number of infections increased from 1920 to 1980 owing to use of unsterile glass syringes for injection of parenteral antischistosomal therapy (PAT) during the early campaign to treat schistosomiasis (Tanaka et al., 2006). Intriguingly, eleven genetically distinct genotypes of the virus and more than fifty subtypes have been recently identified worldwide (Czepiel et al., 2008). Genotypes 1a, 2a, and 2b have a global distribution, while genotype 4 is mainly concentrated in Egypt (Simmonds et al., 2005).

HCV infection is hardly ever diagnosed during the acute phase. Nevertheless, clinical manifestations of acute hepatitis including; jaundice, malaise and nausea can occur in some patients, usually within 8 weeks after infection (Lisker-Melman and Walewski, 2013). The early stage of chronic infection is typically characterized by a prolonged asymptomatic period. Once chronic infection has been established, spontaneous clearance of viremia is rare. Most chronic infections lead to hepatitis and frequent

*Abbreviations:* HCV, hepatitis C virus; APO-1, a member of the TNF-R family of receptors; TNF-R, tumor necrosis factor receptor; P53, tumor suppressor gene; mRNA, messenger ribonucleic acid; ROS, reactive oxygen species; DISC, death-inducing-signaling-complex; DNA, deoxyribonucleic acid; Arg/Pro, arginine proline substitution; HCC, hepatocellular carcinoma; SNP, single nucleotide polymorphism; ALT, alanine transaminase; AST, aspartate aminotransferase; T. Bil, total bilirubin; D. Bil, direct bilirubin; AFP, alpha fetoprotein; GH, growth hormone; ELISA, enzyme-linked immunosorbent assay; EIA, ENZYME immunoassay; F, fibrosis score; A, hepatic activity; qRT-PCR, quantitative real time reverse transcription polymerase chain reaction; RFLP, restriction fragment length polymorphism.

* Corresponding author at: Hormones Department, Medical Research Division, National Research Centre, Cairo 12622, Egypt.
*E-mail address:* ahmedchemia87@yahoo.com (A.A. Abd-Rabou).

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fibrosis. Annually, 10–20% of those infected chronically develop liver cirrhosis and 1–4% at this stage of the disease has the risk of developing hepatocellular carcinoma (HCC) (Tsukuma et al., 1993).

P53 gene, the guardian of genome, is induced by HCV and in turn activates the cell-surface receptor APO-1, a member of the tumor necrosis factor receptor (TNF-R) family, as a key element of the extrinsic pathway enhancement (Nagata and Golstein, 1995). P53 gene induces APO-1 mRNA expression by binding to elements found in the promoter and the first intron of the APO-1 gene (Bouvard et al., 2000; Müller et al., 1998). Scientists reported that HCV damages the human genome directly or indirectly, which in turn associated with the activation of the P53 network, through the enhancement of reactive oxygen species (ROS) production (Pal et al., 2010). P53 is involved in the induction of two distinct apoptotic signaling pathways that lead to mediating apoptosis through formation of the death-inducing-signaling-complex (DISC) via extrinsic pathway (Ashkenazi and Dixit, 1998; Ko and Prives, 1996; Nicholson, 1997).

Matlashewski and his colleagues discovered the primary polymorphic structure of human P53 Arg72Pro over 25 years ago based on mobility differences of electrophoresis (Matlashewski et al., 1987). There are many observations investigated the role of P53 Arg72Pro polymorphism on HCC and HCV-associated HCC incidences. Minouchi et al. (2002) have suggested that there was a significant correlation between P53 72Pro homozygote and HCV type 1b infection (Minouchi et al., 2002). However, they added that there was no significant statistical difference between the P53 polymorphism and HCV genotypes 2a and 2b. Okada et al. (2001) investigated that P53 genetic polymorphism was frequently found in cirrhotic livers compared with patient livers with chronic hepatitis, suggesting that P53 polymorphism at the stage of cirrhosis may be a causative factor that may potentially lead to HCC (Okada et al., 2001). On the contrary, Leveri et al. (2004) found that there was no association between P53 codon 72 genotypes and disease severity or liver cancer (Leveri et al., 2004).

APO-1-expressing hepatocytes in accordance with the severity of liver inflammation become susceptible to the apoptotic death signal and seem to play a critical role in liver cell injury caused by HCV infection (Fischer et al., 2007; Hiramatsu et al., 1994). Moreover, it was found that APO-1 −670A/G polymorphism was potentially associated with significant fibrosis and cirrhosis in chronic HCV patients (Deghady et al., 2012). It was added a higher frequency of the A/A genotype of the APO-1 gene in spontaneously recovered patients from HCV infection as compared to patients with persistent HCV infection. In addition, authors deduced that the A/A genotype of the APO-1 gene influences the outcome of HCV infection in patients on hemodialysis (Ksiaa Cheikhrouhou et al., 2011). Furthermore, there was an association between the APO-1 −670 A/G polymorphism and the grade of necrosis in periportal areas in chronic HCV diseased patients (Aguilar-Reina et al., 2005). Conversely, others found that there was no correlation between APO-1 promoter genotypes and fibrotic stages. However, APO-1 gene polymorphism may account for some of the histopathological variability in HCV infection (Mcllroy et al., 2005).

The lack of information concerning a possible association between the P53 Arg72Pro and APO-1 −670 A/G single nucleotide polymorphisms and the HCV genotype 4a infections among Egyptian population encouraged us to study a cohort of Egyptian HCV genotype 4a infected patients to evaluate the impact of P53 and APO-1 polymorphisms on the disease susceptibility. In addition, this study aims at investigating the association between hormonal profile and these genetic polymorphisms in Egyptian patients infected with HCV genotype 4a.

## 2. Material and methods

### 2.1. Study population

Two hundred and forty volunteers were enrolled in the current study and divided into two groups; 160 HCV infected patient group and 80 healthy control group. HCV patients were classified into two subgroups: 72 patients in F0/1-HCV subgroup and 38 patients in F3/4-HCV subgroup. One hundred and sixty HCV infected patients visited outpatient clinics of the Tropical Medicine and Hepatology Department, El-Kasr El-Aini Hospital, Cairo University, Egypt and diagnosed as chronically infected with HCV after signing a consent form.

Patients participated in the study fulfilled the inclusion criteria included: age 19–54 years, elevated alanine transaminase (ALT) and aspartate transaminase (AST) (>37 IU/L); within 6 months prior to entry of the study, positive HCV antibodies, detectable HCV-RNA, HCV genotype 4a, liver biopsy showing histological evidence of chronic hepatitis and they were never previously treated with interferon. While, patients presented with hepatocellular carcinoma (HCC), hemoglobin (<11 g/dL), total leucocyte count (<3000/mm$^3$), neutrophil (<1500/mm$^3$), platelets (< 100,000/mm$^3$), prothrombin time out of normal range (9.8–13.8 s), presence of antinuclear antibodies (ANA titre > 1/160), liver diseases other than hepatitis C such as hepatitis B surface antigen (HBsAg) seropositivity or autoimmune hepatitis or co-contamination with the human immunodeficiency virus (HIV) or active schistosomiasis were excluded from this study. The study protocol and informed consent were approved by the Ethics Committee of Cairo University.

### 2.2. Biochemical and Metavir scoring histopathological tests

Liver function tests including; hepatic enzymes (ALT, and AST), total bilirubin (T. Bil), direct bilirubin (D. Bil), albumin, and alpha fetoprotein (AFP) as standard biochemical tests were performed on Integra-400 (Roche-Germany) for HCV genotype 4a infected patients and healthy controls. Human prolactin, total testosterone, and growth hormone were quantified in all HCV infected patients as well as healthy controls using the Enzyme-Linked Immunosorbent Assay (ELISA). Enzyme Immunoassay (EIA) for the quantitative determination of prolactin and total testosterone concentrations in human sera were measured using ELISA kits purchased from (Immunospec Corporation, USA). While, human growth hormone levels were measured using ELISA kits supplied by (DRG International, Inc., USA).

Chronic hepatitis C infected patients were subjected to abdominal ultrasound and liver biopsy was taken from each patient to estimate the grades of activity and fibrosis according to Metavir scoring system. All biopsies were classified according to Metavir scoring system (Bedossa and Poynard, 1996) into 5 stages of fibrosis (F0: no fibrosis; F1: enlargement of portal tract without septa formation; F2: enlargement of portal tract with rare septa formation; F3: numerous septa without cirrhosis; F4: established cirrhosis and 4 grades of histological activity (A0: none; A1: mild; A2: moderate; A3: severe) based on the intensity of necroinflammatory lesions.

### 2.3. Qualitative and quantitative assessment of HCV

The assessment of the circulating HCV-antibody and HCV-RNA were qualitatively and quantitatively determined in all subjects enrolled in the prospective study. The presence or absence of HCV antibodies was investigated by third generation ELISA (DiaSorin, Torino, Italy). Viral load was measured to confirm the seropositive results obtained from standard viral antibody tests by real time reverse transcription polymerase chain reaction (qRT-PCR) using a LightCycler system (Roche Diagnostics GmbH, Mannheim, Germany) after RNA extraction from sera using a Qiagen Viral RNA kit (Hilden, Germany). Amplification primers for HCV were 5′ primer K78F (CAAGCACCC TATCAGGCAGT) and 3′ primer K80R (AGCGTCTAGCCATGGCGT). Hybridization probes FL 5′(GCAGCCTCCAGGACCCCCC)3′ and LC 5′(CCCGGGAGAGCCATAGTG GTCTG)3′ were used to detect the product. Reaction mixtures included 7.5 μL of Lightcycler RNA Master HybProbe, 3.25 mM $Mn(OAc)_2$, 0.5 μM concentration of each primer, 0.4 μM of hybridization probe mix and 1 μL of the RNA template in a total volume of 20 μL. HCV RNA was first reverse-transcribed at 61 °C for 20 min. Following denaturation