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REVIEW

## HCV Genotype 4; A Brief Review

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### ABSTRACT

Hepatitis C virus (HCV) is a known leading cause of chronic liver diseases and hepatocellular carcinoma (HCC). The current available treatment depends on the co-administration of peginterferon and ribavirin. Current treatment drugs are extremely expensive, not effective in all patients with different genotypes and are associated with serious side effects that may lead even to death in some cases. Phylogenetic analysis has led to the classification of hepatitis C virus (HCV) into more than 7 genotypes depending on several criteria, amongst them is their worldwide distribution, transmission and disease progression. The lack of availability of an effective vaccine allowed education programs to be the most suitable interventions in the control of HCV spread. Here in this review we will discuss the advanced tools to study HCV genotype 4, and current treatments and vaccination trials of HCV and finally the progress of HCV into hepatocellular carcinoma.

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**Key words:** HCV; Genotype 4; Treatment; Vaccine trials

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### INTRODUCTION

Hepatitis C virus (HCV) is a complex liver disease. Its medical importance and the need to rapidly identify new therapeutic approaches has resulted in intensive study of its causative agent. HCV infection is a major public health problem with more than 160 million people infected worldwide, this figure represents nearly 3% of the world's total population according to the estimates of the World Health Organization (WHO), and 2-3 million new cases per year<sup>[1,2]</sup>.

HCV is one of the main health problems particularly in Egypt, where HCV genotype 4 predominates, as it infects more than 20% of its general population according to WHO estimates. The highest prevalence of antibodies to hepatitis C virus (HCV) in the world was reported in Egypt, where HCV genotype 4 predominates<sup>[3]</sup>.

There are many possible reasons that lie behind the wide transmission of HCV in Egypt. Amongst these is dental treatment as a possible source for transmission of HCV infection<sup>[4]</sup>. Patients undergoing hemodialysis were also found to be at great risk of HCV transmission<sup>[5]</sup>. Moreover, the use of glass syringes was reported before in south Italy to be one of the old modes of transmission of infection<sup>[6]</sup>. And finally, major risk factors for HCV infection in Korea were mostly related to inappropriate healthcare procedures such as blood transfusion, needle stick injury, surgery, dental procedure, and tattooing<sup>[7]</sup>.

### HCV PARTICLE

HCV is a small enveloped virus, classified in the Hepacivirus genus within the Flaviviridae family. The 5'-UTR of the HCV genome contains a large structured domain that serves as an internal ribosomal entry site (IRES)<sup>[1,8,9]</sup>. Upon HCV infection, the normal cap-dependent translation process is strongly inhibited, and infected cells instead shift most of their protein synthesis capacity to viral polyprotein production. The HCV IRES element recruits the 40S ribosomal subunit directly to initiate viral RNA translation bypassing the requirement for several translation initiation factors<sup>[10,11]</sup>. HCV positive-strand RNA genome then encodes a single polyprotein which is processed by cellular and viral proteases into 10 mature proteins (4 structural and 6 non-structural proteins)<sup>[12]</sup>.

Our understanding of the HCV morphogenesis process is still in its infancy. Different viral components were identified as players in the morphogenesis process. As expected, the structural proteins are essential in the virus makeup. Among these components, HCV envelope glycoproteins, E1 and E2, are of particular interest since in addition to their role in assembly, they are also the major players during the entry process. HCV envelope glycoproteins E1 and E2 are type-I transmembrane proteins that consist of a C-terminal transmembrane domain and a large N-terminal ectodomain, which is heavily modified by N-linked glycans<sup>[13]</sup>. Importantly, entry and assembly functions need to be tightly controlled in order to occur at the appropriate location and time<sup>[14]</sup>. These two proteins assemble as a non-covalent heterodimer within the endoplasmic reticulum (ER) of infected cells, whereas they form large disulfide-linked complexes on the virion surface<sup>[15]</sup>. They are indeed involved in receptor binding, and mediate the fusion process between the viral envelope and an endosomal host cell membrane. E1 and E2 glycoproteins are deeply involved in the vaccination trials against HCV.

## HCV GENOTYPES

HCV is further classified into at least seven major genotypes that differ by about 30 percent in their nucleotide sequence. These genotypes (1, 2, 3, 4, 5, 6 & 7) show differences based on their worldwide distribution, transmission and disease progression (as mentioned above). There are a lot of differences amongst these genotypes in their nucleotide substitutions in the viral genome (31-33% nucleotide sequence difference between different genotypes) and more than 100 subtypes (20-25% difference between different subtypes of the same genotype) present in different regions of the world<sup>[16]</sup>.

There exist a big debate on the classification of different genotypes of HCV. Some investigators proposed up to 11 different HCV genotypes, and according to others HCV is only divided into 6 major genotypes, genotypes 6-11 being variants of genotype 6. According to this theory, genotypes 6-11 should be classified as a single genotype i.e. type 6<sup>[17]</sup>. As shown in recently, genotypes 1-3 are distributed worldwide, and types 1a and 1b are the most prevalent globally. Genotype 1b is most prevalent in Japan (70%)<sup>[18-20]</sup>. Genotype 1 is more prevalent in Europe<sup>[21,22]</sup>. In South-East Asia genotype 3 is most common up to 90 % patients<sup>[23-25]</sup>. Whereas genotype 4 is most common in central Africa, Egypt and Middle East<sup>[26]</sup>. It is noteworthy that genotype 4 is quasi-exclusive (91%) in Egypt<sup>[27]</sup>. Vietnamese population harbors geographically isolated genotypes 7, 8 and 9 and these genotypes so far identified from this region only<sup>[28]</sup>. Genotypes 10 and 11 are only identified in Indonesian HCV infected patients<sup>[29]</sup>.

## DEVELOPMENT OF ADVANCED RESEARCH TOOLS TO STUDY HCV GENOTYPE 4

HCV circulates in infected patients at relatively low titers and forms complexes with serum components such as lipoproteins<sup>[30,31]</sup>. For reasons that remain partly unknown, serum-derived HCV cannot be propagated in cell culture. HCV research has been hindered by these restrictions and has largely depended on surrogate systems. For instance, replication-deficient retroviruses pseudotyped with HCV envelope glycoproteins (HCVpp)<sup>[32]</sup> were used to study HCV entry<sup>[33]</sup>. Similarly, replicon systems allowed investigation of HCV replication (for a review, see<sup>[34]</sup>). However, until 2005, there was no system available to reproduce HCV assembly and secretion, which remained mostly elusive. Likewise, 21 years after HCV discovery, its structure remains unknown. Nevertheless, in 2005, several teams finally

succeeded in propagating HCV in cell culture (cell-cultured HCV, HCVcc)<sup>[35-37]</sup>, which allowed to confirm most of the previous data on viral entry and which facilitated antiviral testing. Moreover, this system allowed for the first time the investigation of virus assembly.

After its identification, there were many attempts to define HCV genotype 4 by analyzing the partial sequences of variants. HCV genotype 4 variants were amplified from patients' sera. 5' UTR, core, E1, E2, NS3, NS4, NS5 and 3'UTR regions were sequenced and analyzed<sup>[38-41]</sup>. In 1997 Chamberlain *et al* reported the complete nucleotide sequence of the HCV genotype 4a Egyptian variant, ED43 (GenBank accession no. Y11604)<sup>[42]</sup>. Furthermore, in 2007 Timm *et al* described the full-length sequence of HCV genotype 4d, the most prevalent subtype in sub-Saharan African infections<sup>[43]</sup>. HCV genotype 4 and its subtypes reference sequences can be found in the (LosAlamos HCV database) (<http://hcv.lanl.gov/content/hcv-db/classification/genotable.html>).

## SUB-GENOMIC REPLICON

In 1999, HCV replicon system was developed for genotype 1b. This system was developed by trimming the viral genome necessary for viral replication i.e. NS3-NS5B along with 3', 5' UTR regions<sup>[44]</sup>. Due to smaller size than authentic viral genome it was possible to insert a heterologous dominant selectable marker (e.g., Neomycin Phosphotransferase, neo) without exceeding the natural length of the HCV genome. Replicons were often bicistronic constructs, with Neomycin Phosphotransferase translation driven by the HCV IRES, followed by the HCV NS3-3' UTR under the control of a second IRES derived from encephalomyocarditis virus (EMCV).

Previously HCV replicon systems have been successfully established for only wild type or adapted genotypes 1a<sup>[44-56]</sup>. Very recently, Charles Rice lab developed replicon systems for 3a and 4a genotypes<sup>[57]</sup>. The prototype strain ED43 (4a) was used to generate G418-selectable subgenomic replicons. To make these replicons more useful for high-throughput screening and evaluation of antiviral compounds, they were modified to express a chimeric fusion protein of firefly luciferase and neomycin phosphotransferase to yield stable replicon-expressing cells.

## PSEUDOTYPED RETROVIRAL PARTICLES

In the absence of HCV cell culture system for studying entire viral life cycle, surrogate models to study the role of HCV glycoproteins in virus entry were developed. The most successful model to investigate early steps of HCV infection was the establishment of retroviral pseudotypes bearing unmodified HCV glycoproteins (HCVpp)<sup>[58,59]</sup>. HCVpp is based on the co-transfection of 293T kidney cells with expression vectors encoding HCV E1 and E2, gag-pol proteins of either murine leukemia virus (MLV) or human immunodeficiency virus (HIV) and finally a retroviral genome encoding a reporter gene (luciferase). The HCVpp can be used for antibody neutralization studies as the attachment and receptor interaction of these retroviral pseudotypes is governed by the functional HCV E1-E2 protein complex incorporated into the envelope of these particles. It is worth noting that the HCVpp system for HCV genotype 4 were developed by three independent groups<sup>[60-62]</sup> which were widely used for viral entry and receptor binding investigation studies.

## MODELS TO STUDY INDIVIDUAL HCV GENOTYPE 4 PROTEINS IN CONTEXT OF FULL LENGTH VIRUS

The development of efficient culture systems for studying the important genotype specific antiviral targets NS3, NS5A, in the context of complete virus, allowed the investigation of small direct acting antiviral compounds. Indeed, NS5A is considered one of the promising targets for small anti HCV compounds which are already in clinical trials<sup>[63]</sup>. Much is still to be learned about the role of the highly diverse NS5A functions in genotype 2a; and; furthermore for most other genotypes, NS5A has not been studied at all. Recently, infectious genotype-specific HCV NS5A cell culture systems for HCV genotype 4 along with other HCV genotypes was developed by replacing the complete NS5A of the J6/JFH1 recombinant<sup>[64]</sup> with NS5A from ED43 (4a)<sup>[65]</sup>.

Another very important target is the HCV non-structural protein NS3. The recently developed full-length replication-competent clone Jc1 (pFK JFH1/J6/C-846), which showed very high replication efficiency in Huh7.5 cells, was used to develop recombinant virus expressing the NS3 protease of HCV genotype 4 and all other genotypes<sup>[66]</sup>. This recombinant virus allowed the direct monitoring of antiviral drug inhibition for specific proteases through assessment of reduction in both supernatant infectivity and replication kinetics. This genotype specific protease system also provided a phenotypic assay for rapid assessment of emerging resistance during therapy and the influence of specific mutations on treatment outcome.

### RECOMBINANT VIRUS (4a/JFH1)

The year 2005 was a mile stone in HCV research when Wakita *et al*<sup>[67]</sup> developed the genotype 2a JFH1 cell culture system from a Japanese Fulminant Hepatitis patient. JFH1 is an efficient *in vitro* system and allows the investigation of the complete infectious virus life cycle. JFH1 has unique replication capacity in Huh7 cells. In the same year, Lindenbach *et al*<sup>[64]</sup> developed the more efficient HCV 2a cell culture system (J6/JFH1) by replacing the Core-NS2 region from another 2a isolate. Recently, intergenotypic recombinants (4a/JFH1) cell culture systems were developed containing the structural genes (Core, E1, and E2), p7, and all or part of NS2 of the 4a prototype strain ED43. These Chimeric viruses allowed genotype-specific functional analyses of Core-NS2 and screening of neutralizing antibodies and inhibitors targeting this region<sup>[68]</sup>.

Very recently, Gottwein *et al* (2011) developed reporter viruses using JFH1-based recombinants expressing core-NS2 of genotype 1 to 7 prototype isolates, Core-NS2 region of ED43 was used to generate the reporter recombinant system. Enhanced GFP (EGFP) or RLuc was inserted into C-terminal domain III of JFH1 NS5A at a site described previously by Moradpour *et al* 2004 (downstream of aa 2356 or 2390, numbering with reference to H77, Genotype 1b). These reporter viruses can be used for high-throughput fluorescence- and luminescence-based genotype-specific functional studies of HCV-receptor interactions, serum-neutralizing antibodies and therapeutics interfering with HCV entry or assembly.

### CHIMPANZEE MODEL AS THE ONLY RELIABLE MODEL FOR THE STUDY OF HCV GENOTYPE 4

Chimpanzees are the only model for studying HCV infection mirroring immuno-pathogenesis and viral persistence observed in human infections. Chimpanzees can be infected with intravenous inoculation of HCV particles and by intrahepatic transfection with RNA transcripts from full-length HCV cDNA clones. Molecular infectious clones of genotypes 1a (strains H77, HCV-1, HC-TN), 1b (HC-J4, Con1,

HCV-N), and 2a (HC-J6 and JFH1) were used to initiate monoclonal infections in chimpanzees (reviewed in Gottwein *et al*<sup>[69]</sup>), in order to study the function of certain genome regions by reverse genetic studies, and to study HCV natural history and protective immunity. Furthermore, plasma pools from monoclonally infected chimpanzees were used for virus challenge in vaccine studies and antivirals in chimpanzees<sup>[70]</sup> or SCID-uPA mice engrafted with human hepatocytes<sup>[71]</sup>. In 2010, Gottwein and Scheel *et al* generated consensus cDNA clones of strains ED43 (genotype 4a) which were fully functional in chimpanzees<sup>[70]</sup>. With the development of HCV genotype 4 chimpanzee model, it was possible to analyze certain viral genome regions by reverse genetics, host immune responses during the acute infection, including peripheral and intrahepatic T-cell responses, vaccines and antiviral studies particular for this important viral genotype.

### HUMAN LIVER CHIMERIC MICE MODEL

Human hepatocytes were transplanted into uPA/scid mice; a transgenic mice in which the urokinase gene is driven by the human albumin promoter/enhancer, these human transplanted cells proliferated and replaced the apoptotic mice liver cells<sup>[72]</sup>. These chimeric mice were shown to be susceptible to HCV infection. Replication levels of the virus is proportional to the repopulation index of humanized cells<sup>[73]</sup>. Successful viral replication is achieved in these chimeric mice for HCV genotype 2a<sup>[74]</sup>, 1a<sup>[75]</sup>, 1b<sup>[76]</sup>, and JFH1 intergenotypic chimeric viruses<sup>[74]</sup>. In the recently developed modified form of chimeric mice (Fah-/-Rag2-/-Il2rg-/- mouse) HCV is shown to persist for more than half a year and most likely does not have any impact on the animal's health. HCV can also be passaged from one chimeric mouse to another with viral titers and dynamics similar to those achieved with inoculation with patient serum. These chimeric animals was used to test the antiviral drugs like interferons<sup>[74]</sup>.

### PROGRESS IN HCV 4a TREATMENT AND VACCINATION

Treatment of HCV with pegylated interferon (PEG-IFN) and ribavirin (RBV) is becoming the standard of care (SOC) for the management of most of HCV genotypes where dual therapy is the state of the art only for non-genotype 1 infections<sup>[77]</sup>. However, several factors affect the efficacy of the PEG-INF/RBV combination regimens. Similar to the variation of treatment efficacy by viral genotype, the predictors of sustained virologic response (SVR) varies by genotype. The comparatively high cost of treatment with PEG-IFN/RBV combination regimens and the cruel side effects strengthen the importance of evaluation and optimization of the factors affecting the treatment before its initiation. Up to date, little is known about factors affecting PEG-INF/RBV treatment outcome in HCV-4. Some of these factors are virus related and others are host related factors. Virus-related factors are HCV genotype, baseline viral load and HCV quasispecies<sup>[78]</sup>. The host-related factors are single nucleotide polymorphisms (SNPs) in interleukin (IL)-28B and NS5A genes, pretreatment serum anti-E1E2 and interferon-gamma-inducible protein-10 kDa (IP-10 or CXCL10), ethnicity and liver histopathology.

### VIRAL FACTORS

HCV-4 is a difficult-to-treat genotype<sup>[78]</sup>. Moreover, high baseline viral load increases the resistance to therapy. Cut-off value of 400,000 IU/mL is used in most studies<sup>[79,80]</sup>. Another major cause of

the treatment difficulty is the presence of multiple HCV-4 species<sup>[81]</sup>. The species diversity allows the virus to escape from the antiviral therapy.

## HOST FACTORS

A recent cohort study detects an association of the IL-28B polymorphism and the response to interferon treatment<sup>[82]</sup>. This study included 129 HCV-4 patients from three tertiary healthcare centers in Saudi Arabia. In these patients, five Single nucleotide polymorphisms (SNPs) were identified and correlated with SVR. These data showed that the IL-28B rs12979860 CC genotype and rs12980275AA were associated with SVR. Moreover, IFN/RBV resistance-determining region (IRRDR) was identified as a new region located near the C terminus of NS5A gene. SNPs in this region showed correlation with PEG-IFN/RBV treatment outcome in both HCV-2a and -2b infections. Recently, polymorphisms in this region showed a correlation with PEG-IFN/RBV treatment outcome in HCV-4 infected Egyptian patients<sup>[83]</sup>.

A recent report by Shaker *et al.*, from Egypt showed that SNPs in IL-28B (rs8099917 T/G and rs12979860 C/T) and the serum levels of IL-10 and IL-28 may be promising predictors for HCV genotype 4 pegylated interferon/ribavirin-therapy outcome<sup>[84]</sup>. A recently published meta analysis regarding the implications of IL-28B polymorphisms (rs12979860 and rs8099917) in spontaneous and treatment-related clearance for patients with hepatitis C showed that these polymorphisms have strong predictive association of therapy response for HCV genotype 4<sup>[85]</sup>. Fortunato *et al.* (2008)<sup>[86]</sup> reported that genetic variants in the IRF-1 and Stat1 genes of the IFN pathway are also associated with HCV infection.

Pretreatment serum anti-E1E2 and IP-10 are also other factors affecting the treatment response. Pretreatment serum anti-E1E2 response predicts better HCV RNA clearance and treatment outcome. The combination of anti-E1E2 and IP-10 significantly improves the prediction of treatment response. This warrants further investigation and validation on larger cohorts of patients in the context of new therapeutic strategies. Data on the correlation between ethnicity and PEG-IFN/RBV treatment outcome on HCV-4 patients are still controversial. Two studies evaluated the effect of ethnicity between Egyptians and Europeans on the therapeutic outcome of PEG-IFN/RBV treatment and showed no significant difference between the two different ethnic groups<sup>[87,88]</sup>. On the contrary, recent study concluded that Egyptian ethnicity is a favorable factor for clinical outcome<sup>[77,89]</sup>. In Egypt, a study concluded that severe fibrosis, severe steatosis, treatment with standard interferon are predictors of low SVR<sup>[90]</sup>. This study suggests that the routine assessment of factors predictive of a treatment response should consider pretreatment serum alpha fetoprotein (AFP) level.

Currently, hepatitis C patients are treated with interferon alpha (IFN- $\alpha$ ) given either alone or in combination with the nucleoside analog ribavirin. IFN system is reported to mediate antiviral, antiproliferative, immune, and other cellular effects<sup>[91]</sup>. A hallmark of all IFNs is their ability to enhance the expression of numerous genes. Some of these genes encode effector proteins like PKR protein with remarkable antiviral activities. In humans, IFN antiviral action is mediated by the induction of at least two major proteins, protein kinase R, and MxA<sup>[92]</sup>. PKR (protein kinase RNA-regulated) is involved in the pathogenesis of HCV genotype 4 related HCC by inhibiting viral and cellular proteins related to cell growth and proliferation. PKR gene expression is considered a reliable marker to predict HCC with high sensitivity, specificity and diagnostic accuracy<sup>[93]</sup>.

## HCV VACCINATION TRIALS

The development of a protective vaccine against HCV has proven to be an extremely challenging task. Extensive research in this area suggests that a successful HCV vaccine will need to stimulate the production of neutralizing antibodies (perhaps against E1 and or E2 HCV envelope glycoproteins) and potent HCV-specific T cell responses.

E1 and E2 proteins are present on the surface of viral particles and thus are recognized by neutralizing antibodies. E2 glycoprotein is considered the major target of HCV neutralizing antibodies, and it is also the HCV receptor-binding protein, which has been shown to interact with CD81 tetraspanin and scavenger receptor BI (SRB1), two HCV co-receptors.

Most of the antibodies identified to date target receptor-binding epitopes within E2 glycoprotein. Targeting host factors may represent an alternative approach to preventing HCV entry. These antibodies do not target the virus particle itself, but rather block viral-host receptor interaction. Indeed a number of anti-receptor antibodies targeting CD81 and SR-BI have been shown to block viral entry.

## HCV NEUTRALIZING EPITOPES AND ANTIBODIES

Identification of mechanisms of immune protection is a crucial step in HCV vaccine design. Indeed, the identification of human antibody clones with broad neutralizing activity is important for the design of an effective vaccine against most known human viruses. It has been shown before that induction of neutralizing antibodies following vaccination comprise a major component of protection, provided by adaptive immune response, against a number of viruses. However, in the case of hepatitis C virus, the infection can persist even in the presence of a broadly neutralizing antibody in over 50% of cases, where many patients fail to clear the virus and instead develop chronic infection in the presence of HCV neutralizing antibodies which are usually detectable within 1-3 months of HCV infection. This can result in various forms of chronic hepatitis, liver cirrhosis and can eventually lead to hepatocellular carcinoma and death. Various mechanisms underlying this phenomenon have been suggested, and one of the most recently proposed ones is the presence of interfering antibodies that stops antibody neutralization responses. It has been reported before that the majority of chronically infected patients display cross-reactive neutralizing antibodies in their serum. Neutralizing antibodies have not been detected in several cases of acute resolving infections, and the detection of neutralizing antibodies in acutely infected individuals did not seem to be associated with viral clearance. On the contrary, another study reported that some patients had a progressive emergence of a relatively strong neutralizing response in correlation with a decrease in viral load in blood. In line with this study, it has been also demonstrated that neutralizing antibodies with high-titer serum were detected in individuals resolving infection. Furthermore, most individuals progressing to chronic infection showed low-titer neutralizing antibodies during early acute infection where virus escape might have occurred. Together, these observations suggest the clinical importance of neutralizing antibodies.

## E1 AND E2 PROTEINS ARE TWO MAIN TARGETS FOR SYNTHESIZING HCV VACCINE

HCV E1 glycoprotein displays a relatively high degree of conservation than E2. It has been proven to be a difficult target for monoclonal antibody neutralization. This can be explained on the basis of its proposed low immunogenicity comparable to E2 protein<sup>[94,95]</sup>. Despite this challenge, two monoclonal antibodies directed against HCV glycoprotein E1 were identified (IGH 505, and IGH 526)<sup>[96]</sup>. These antibodies strongly neutralize HCVpp bearing E1 envelope glycoprotein of different genotypes such as 1a, 2a, 1b, 4a, 5a, and 6a, whereas they failed to neutralize HCV genotype 3a. The epitopes for both monoclonal antibodies were mapped to the region encompassing amino acids 313 to 327. However, the mechanism by which these E1-directed monoclonal antibodies neutralize HCV infection remains unclear<sup>[96]</sup>. In addition to these two antibodies, H-111 antibody was reported to react in ELISA with expressed E1 proteins from genotypes 1a, 1b, 2b, and 3a. H-111 was reported to bind to 192YEVNRNVSGVYH211 region of E1. Furthermore, another identified E1 epitopic region was considered as universally conserved region of E1 comprising aminoacids 315-328.

E2 encodes clusters of highly immunogenic overlapping epitopes. An epitope involved in HCV virus neutralization located within the HCV E2 protein and comprising amino acids 412-419 have been previously identified. Another main target of neutralizing antibodies is the hypervariable region (HVR1) in the HCV E2 envelope protein<sup>[97]</sup>. The apparent variability of E2 hypervariable region (HVR1), the first 27 amino acids of the E2 ectodomain, seems to be driven by antibody selection of immune-escape variants. Despite the sequence variability of HVR1, the physicochemical properties of the residues at each position and the conformation of HVR1 are highly conserved among various species. E2 HVR1 is also reported to be responsible for virus binding and entry. It has been proposed that HVR1 plays the role of a neutralizing epitope since this region shows marked sequence variability. It has also been reported before that resolved infection is associated with stable HVR1 sequences, whereas persistent HCV infection is associated by HVR1 sequence change. Altogether, this suggests that HVR1 is playing an important role in targeting the antibody response to HCV E2 glycoprotein. Unfortunately, HVR1 immunogenic epitopes tend to be isolate specific with no recognition for other genotypes, leading to viral escape. Several HCV escape mutants were reported in the literature, among these is the Q412H was reported in chronic HCV carriers. Recently, it has been shown that the sequences of E1 and E2 glycoproteins are driven by neutralizing antibody responses where escape mutant strains shortly become the dominant ones.

## PROGRESS OF PATIENTS WITH HCV GENOTYPE 4 TO HEPATOCELLULAR CARCINOMA

HCV is often initially diagnosed in a late stage when the therapeutic options are already limited. Due to slow and silent onset, many patients are unaware of their infection and about 50% of cases remain undetected<sup>[98]</sup>. In particular, HCV has been given increasing attention because of its wide and deep penetration in the community, coupled with a very high incidence of HCC in chronic HCV infected patients. Indeed, hepatocellular carcinoma (HCC) has become the major cause of death in individuals persistently infected with HCV<sup>[99]</sup>. Once liver cirrhosis is established in hosts infected with HCV, HCC develops at a yearly rate of about 6%<sup>[91]</sup>. HCC accounts for about 2% of total cancers, and is the most lethal form of cancer, and its incidence has been rising over the last 30 years<sup>[1,100]</sup>. In line with these reports, an Indian study found that HCV infection (genotype 4) increases the risk

of developing HCC by three folds compared to other genotypes<sup>[101]</sup>. On the contrary, in Egypt, where HCV genotype 4 predominates, it was reported that there is no association between infection with genotype 4 and the development of HCC<sup>[102]</sup>.

## MECHANISMS OF PROGRESSION OF HCV TO HCC

The mechanism of carcinogenesis is not fully understood. Some studies demonstrate that when inflammation is induced in the liver in the presence of HCV core protein, the production of oxidative stress is triggered to an extent that cannot be stopped by a normal physiological antagonistic agent<sup>[103]</sup>. Moreover, Hepatic steatosis, a common and serious feature of hepatitis C virus (HCV) infection, may enhance oxidative stress overproduction<sup>[104]</sup>. Other possible pathways involving HCV viral core protein would be the alteration of the expression of some cellular genes and modulation of intracellular signaling pathways. For an example, tumor necrosis factor (TNF)- $\alpha$  and interleukin-1  $\beta$  have been found to be transcriptionally activated at the protein and mRNA levels in HCV infected mice<sup>[105]</sup>. Furthermore, it has been reported before that liver disease progression from chronic liver disease to HCC due to HCV genotype 4 infection is associated with an imbalance between certain types of cytokines<sup>[106]</sup>. In addition to these causes, the HCV core protein was reported to modulate the intracellular signaling pathways of some proteins such as retinoid X receptor (RXR)- $\alpha$ , which play essential roles in cell proliferation and metabolism<sup>[107]</sup>.

## CONFLICT OF INTERESTS

There are no conflicts of interest with regard to the present study.

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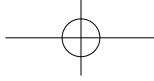
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