MOLECULAR DIAGNOSTICS OF HEPATITIS C VIRUS INFECTION

Essay

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بسم الله الرحمن الرحيم

اقرأ باسم ربك الذي خلق (١) خلق الإنسان من علق (٢)

اقرأ وربك الأكرم (٣) الذي علم بالقلم (٤)

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Abstract

Viral hepatitis has been increased and became endemic in many countries all over the world as acute or chronic, causing burden on human healh. HCV is the most famous virus that infects the liver, as it affects 3% of the world's population. Egypt has a very high prevalence of HCV and a high morbidity and mortality from chronic liver disease, cirrhosis and HCC. It was found that the most important risk factor for the prevalence of HCV is using the same unsterilized syringe for many patients as antischistosomal injection treatment, transfusion of blood and its components and sharp objects. Positive ELISA test indicates that patient had been exposed to HCV, but it can be false positive, so confirmatory test should be done. Seroconversion is delayed for several weeks. Test could be negative in early stages of infection and should be repeated after several months if enzymes are elevated. Sensitive HCV RNA test should be done to confirm infection with HCV. There are qualitative tests which can detect the virus 1-2 weeks from infection and the quantitative test to measure the viral load, but the viral load doesn't indicate the severity of the disease or response for treatment.

Key words: molecular, HCV RNA, PCR, quantitative , qualitative

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List of Abbreviations

ALT	Alanine aminotransferase
bDNA	branched-chain DNA
CAP/CTM	COBAS Ampliprep/COBAS TaqMan HCV viral load test
CIA	Chemiluminescence Immunoassay
CTL	Cytotoxic T lymphocyte
COBAS	Complete bioanalytical system
DNA	Deoxyribonucleic acid
ELISA	Enzyme Liked immunoassays
EIA	Enzyme immunoassays
FDA	Food and drug administration
FRET	Fluorescence resonance energy transfer
FT-AT	Fibro-Test Acti-Test
HCV	Hepatitis C virus
HCC	Hepatocellular carcinoma
HIV	Human Immunodeficiency Virus
HGV	Hepatitis G virus
HBV	Hepatitis B virus
HCVcAg	HCV core antigen
HVR	Hypervariable regions
IFN	Interferon
MHC-I	Major histocompatibility complex class I
NANB	Non-A, non-B
NAT	Nucleic acid testing

NCR	Noncoding region
NK	Natural killer
PCR	Polymerase chain reaction
Peg-IFN	Pegylated-interferon
PKR	Protein kinase
Q	Quencher
QS	Quantitation standard
R	Reporter
RFLP	Restriction fragment length polymorphism
RNA	Ribonucleic acid
RLU	Relative light units
RBV	Ribavirin
RIBA	Recombinant immunoblot assay
SOD	Superoxid dismutase
SVR	Sustained virological response
TTV	Torque Teno virus
UTR	Untranslated region
WHO	World Health Organization

INTRODUCTION AND AIM OF THE WORK

Hepatitis C virus (HCV) is an enveloped RNA virus, classified in the *Flaviviridae* family, in the genus *Hepacivirus*. Globally, HCV is estimated to infect 170 million people and creates a huge disease burden from chronic progressive liver disease. In the 1990s, at least 10,000 deaths annually were directly attributable to hepatitis C, with a projection of a tripling of the hepatitis C related deaths by 2020 **(Choo et al., 1991, CDC, 1998 and Deuffic-Burban, 2007).**

HCV is one of the major public health problems facing Egyptian population. Studies in Egypt have demonstrated a strikingly high prevalence of HCV among healthy blood donors and patients with chronic liver diseases **(Frank et al., 2000 and Theodore and Jamal, 2006**).

It has been classified into six major genotypes and more than 100 subtypes. These genotypes, numbered 1 through 6, have unique geographic niches and important clinical implications **(Simmonds et al., 1993 and Higashi et al., 2005).**

Currently, the diagnosis of HCV infection is more reliable. Enzyme-linked immunosorbent assay (ELISA) should be confirmed by recombinant immunoblot assay (RIBA) to eliminate false positivity. Molecular virological techniques play a key role in diagnosis and monitoring of treatment. Because it is difficult to culture the virus, molecular techniques were instrumental in first identifying HCV, making it one of the first pathogens to be identified purely by molecular diagnostics. HCV infection is confirmed by the detection of viral RNA through nucleic acid tests (NATs), and these tests are used to monitor the response to antiviral therapy. HCV genotype test results provide important prognostic information related to therapeutic response and are used for selecting treatment regimens **(John and David, 2007).**

New standards of therapy for chronic hepatitis C have greatly improved in recent years. Rates of sustained virological response (SVR) have increased significantly in the late 1990s with the addition of ribavirin (RBV) to interferon (IFN) and have further improved with the use of pegylated-IFNs (PEG-IFNs) in combination with RBV **(Alfredo and Luisa, 2003).**

Although progress has been made in developing a vaccine to prevent HCV infection, there is no vaccine against HCV infection until now **(Frey et al., 2005 and Annemarie et al., 2008**).

Subsequently, the aim of this work is to review the currently available molecular diagnostic tests for HCV, their clinical application and how these tests shed light on the natural history of HCV.

Hepatitis C Virus

History:

Since its discovery in 1989, HCV has been the subject of intense research and clinical investigations due to its major role in human hepatitis especially post-transfusion hepatitis **(Richter, 2002 and Ismail et al., 2004).**

Clinical and epidemiologic studies and cross-challenge experiments in chimpanzees had suggested that there were several non A-non B hepatitis (NANBH) agents which based on serologic tests that were not related to hepatitis viruses A and B (HAV & HBV) **(Koretz et al., 1973 and Feinstone et al., 1975).**

Progress towards identifying the cause of NANBH was hampered by the difficulty in culturing the agent in cell or organ culture, and the eventual characterization of the infectious agent of NANBH was made possible after the successful transmission of the agent into chimpanzees **(Bradley et al., 1979 and Bradley and Maynard 1986).**

The chimpanzee is the only animal model for HCV with demonstrated susceptibility to HCV infection, and which show a course of infection resembling that in humans. In particular, exposed chimpanzees may become infected with incubation periods, seroconversion profiles and elevation of liver enzymes broadly similar to that of human infections. Unfortunately, the later events in HCV infection in humans, such as the development of cirrhosis and hepatocellular carcinoma(HCC) typically occurring over periods of 20-30 years, make it difficult to conduct natural history studies of the main disease outcomes of HCV infection in chimpanzees **(Walker, 1997).**

One frequently observed difference between humans and chimpanzees lies in the immune response to infection. These differences complicate the use of the chimpanzee as a model for vaccine development. **(Mikkelsen and Bukh, 2007).**

General Properties:

HCV is a single positive-stranded RNA virus, classified as family *Flaviviridae*, genus *Hepacivirus*. This virus can be differentiated by RNA sequence analysis into at least six major genotypes and more than 100 sub-types. Genotypes differ from each other by 25-35%

at the nucleotide level, and subtypes differ from each other by 15-25% .The genome is 9.5 kb in size and encodes a core protein, two envelope glycoproteins, and several nonstructural proteins . Most cases of post-transfusion NANB hepatitis were caused by HCV **(Alter, 1999, Richter, 2002 and Ismail et al., 2004).**

HCV forms virus particles of diameter 55-65 nm with 6-nm spike like projections that may correspond to the envelope glycoproteins on the virion surface. The addition of relatively large carbohydrate groups contribute structurally to the outer surface of the virion, and influence the ability of antibody to neutralize infectivity **(Kaito et al., 1994)**.

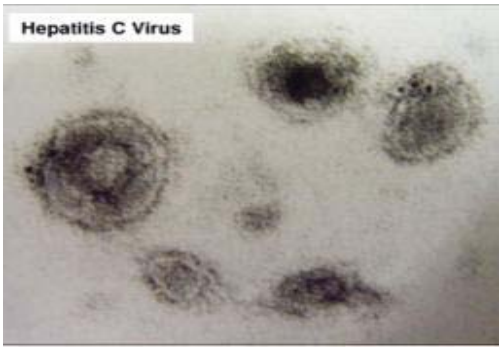

Fig. (1) HCV by electron microscopy **(Petit et al., 2005)**

HCV is inactivated by exposure to chloroform, ether, and other organic solvents, detergents, and by dry heat treatment at 80°C or wet heat at 60°C **(Mannucci, 1993).**

Genetic Organization of HCV:

HCV genome consists of a single, open reading frame and 2 highly conserved untranslated regions (UTR); 5'- and 3'-UTR, at both ends of the genome. The genome has approximately 9500 base pairs and encodes a single polyprotein of 3011 amino acids that are processed into 10 structural and regulatory proteins. The 5`-UTR is highly conserved with 92% homology among different HCV types **(Shimizu et al., 1994).**

The genome of HCV encodes at least ten proteins of which three are structural and six non-structural. HCV also expresses P7, a membrane associated ion channel that may function during virus assembly or infection (**Lin et al., 1994)**.

1. Structural proteins:

The structural proteins of HCV are encoded by sequences at the 5' end of the genome and so are translated first namely core, envelope E1 and E2 **(Santolini et al., 1994 and Anzola and Burgos, 2003).**

The core protein has a size of 21-22 kDa. It has been proposed that the core protein of HCV can be functionally divided into three domains; domain 1 forms the nucleocapsid of the virus, while domain 3 represents the final 10-12 amino acid hydrophobic leader sequence that is cleaved after translation. Domain 2 has been shown to be the region of core responsible for its targeting to lipid droplets, a process which potentially interferes with lipid metabolism within the infected hepatocyte and which may play a role in the development of steatosis as a complication of HCV infection **(Hope and McLauchlan, 2000 and Perlemuter et al., 2002).**

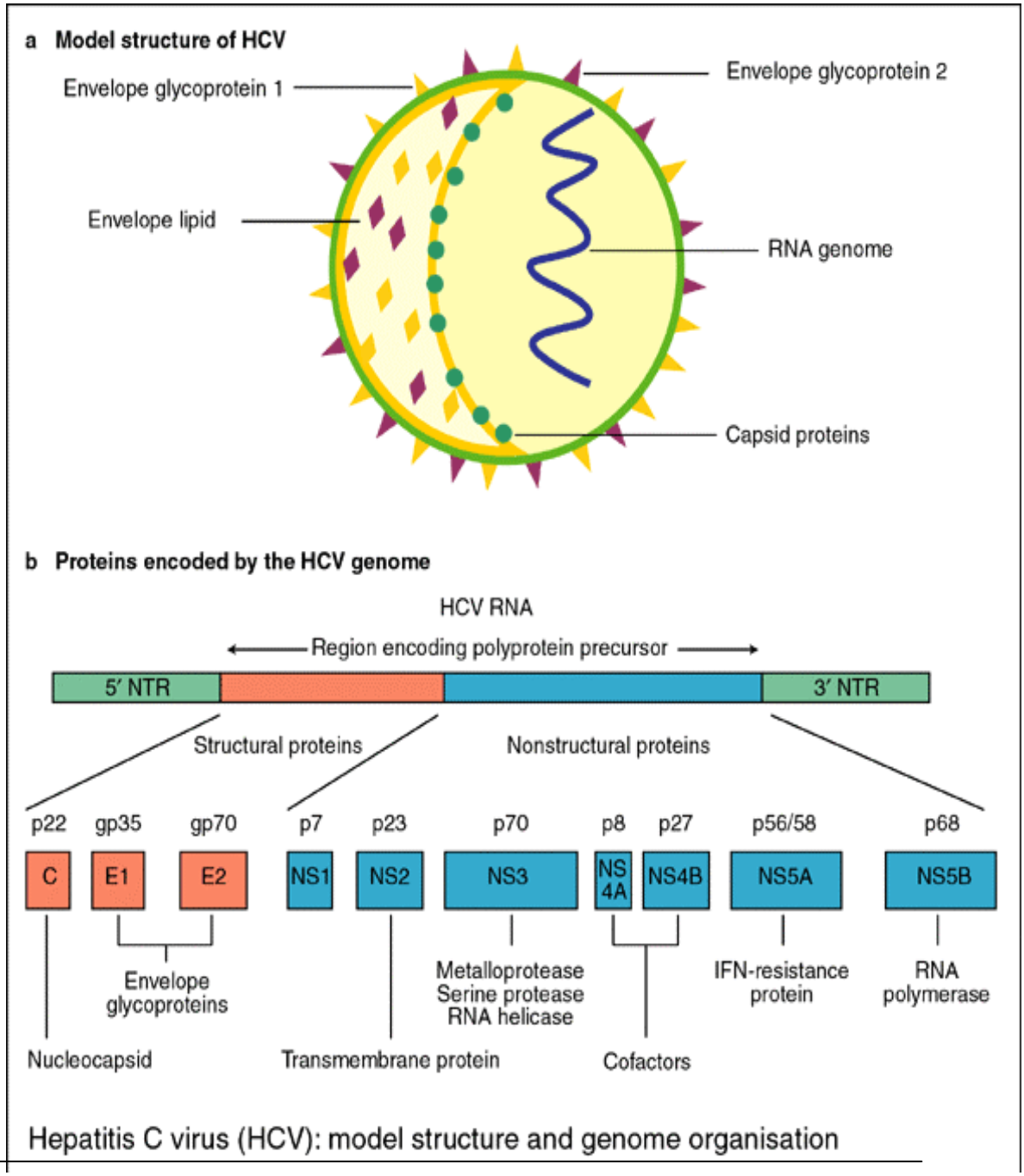

Fig. (2) Model structure and genome organization **(Anzola and Burgos, 2003)**

E1 and E2 of HCV are synthesized in mammalian cells as proteins with sizes ranging from 31- 35 kDa and 68 -72 kDa. Both are thought to form heterodimeric structures in the virus envelope through a non-covalent interaction. They form the principle target of antibody-mediated neutralization of virus infectivity **(Logvinoff et al., 2004).**