INTRODUCTION

Hepatitis C virus is a hepatotropic but not a directly cytopathic virus. It is capable of provoking acute and chronic necro-inflammatory liver injury and it is the most common cause of liver disease worldwide *(Guidotti et al., 2006)*. Fibrosis is a central event in chronic viral hepatitis C leading to cirrhosis and associated morbidity and mortality caused by decompensated liver functions *(Soha et al., 2008)*. The stage of liver fibrosis provides useful information not only for diagnosis, but also for therapeutic decisions in chronic viral hepatitis *(Ubina et al., 2006)*. We rely on liver biopsies with their associated risks, cost, and sampling errors to detect, grade, and monitor hepatic pathology in hepatitis C virus (HCV) infections and other chronic liver diseases *(Halfon et al., 2006)*. It is difficult to justify serial liver biopsies to diagnose and monitor patients with chronic HCV when there are limited options for managing their disease, as it is the usual case in Egypt *(Strickland, 2006)*. Despite its importance, liver biopsy presents some limitations. The risk of a disease underestimation is the most significant one, as hepatic lesions are often irregularly located within the liver. Parallel to the limitations of liver biopsy, clinical needs for an early identification of progressive fibrosis require additional non-invasive techniques to be developed *(Desmet et al., 2003)*. During

chronic liver disease (CLD) as chronic hepatitis C, the extracellular matrix undergoes a process of remodeling, leading to new collagen formation and deposition. Tissue remodeling is regulated by fine molecular mechanisms, involving proteases, inhibitors and growth factors. The major role in matrix degradation is played by matrix metalloproteinases (MMPs), a class of zinc and calcium-dependent enzymes, and their tissue inhibitors (tissue inhibitor of metalloproteinases) TIMPs *(Consolo et al., 2009)*.

The activity of MMPs is tightly regulated by the amount of active protein and the concentration of tissue inhibitors of metalloproteinases (TIMPs). Extensive studies have identified that TIMPs play a key role in the progression of fibrosis.TIMP-1 is the first discovered tissue inhibitor of TIMPs in the liver and it can inhibit most interstitial collagenases *(Qing et al, 2006)*. TIMP-1 is produced by kupffer cells, stellate cells and myofibroblasts in the liver. It promotes fibrosis by inhibition of collagen degradation *(Yoshiji et al, 2002)*. It was shown that the serum and liver TIMP-1 levels correlate with each other *(Murawaki et al, 1999)*. Serum level of TIMP-1 could reflect the change of liver TIMP-1 in patients with chronic liver disease and liver TIMP-1 concentration increases with progression of the liver disease *(Mrawaki et al, 2001)*. So, TIMP-1 has been considered as a useful diagnostic index of hepatic fibrosis *(Afdhal, 2005)*.

AIM OF THE WORK

The aim of the present study is to evaluate serum tissue inhibitor of metalloproteinase type 1 (TIMP-1) as a non invasive serum biomarker of liver cell injury and fibrosis development compared to liver biopsy in patients with chronic hepatitis C.

Chapter 1

Chronic Hepatitis C

I- Structure of Hepatitis C Virus:

HCV is a small (50nm in size), enveloped, positive sense single strand ribonucleic acid (RNA) virus of the family Flaviviridae *(Beeck and Dubuisson, 2003)*. Other members of the family include Yellow fever virus, West Nile virus and Dengue Fever virus (*Dufour, 2005).*

HCV consists of a core of genetic material "RNA", surrounded by an icosahedral protective shell of protein, and further encased in a lipid envelope of cellular origin. Two viral envelope glycoproteins, E1 and E2, are embedded in the lipid envelope (*Beeck and Dubuisson, 2003)*.

HCV RNA genome consists of a single open reading frame of 9600 nucleotide bases *(Kato, 2000).* At the 5' and 3' ends of the RNA there are untranslated regions (UTR) that are not translated into proteins but are important for translation and replication of the viral RNA. The 5' UTR has a

ribosome binding site (*Jubin, 2003*) that starts the translation of a 3011 amino acid containing protein that is later cut by cellular and viral proteases into 10 active structural (virion-associated) and non-structural (NS) smaller proteins *(Dubuisson, 2007)*.

The 3'UTR is composed of a non-conserved variable region, a poly-U element and a conserved 98-nucleotide sequence, termed the 3´-X region, which is believed to be essential for replication *(Song et al., 2006)*.

The NS2 peptide is a metalloprotease essential for the cleavage of the viral NS2/NS3 junction. The amino-terminus region of NS3 encodes a viral serine protease that is important in post translational cleavage of viral peptides from NS3 to NS5. The carboxyl two-third portion of NS3 functions as a helicase, which is essential for unwinding of viral RNA during replication. The NS5B protein functions as a viral RNA- dependent RNA polymerase *(Lau et al., 2006)*.

The NS proteins then recruit the viral genome in to an RNA replication complex, which is associated with rearranged cytoplasmic membranes. RNA replication

takes places via the viral RNA-dependent RNA polymerase of NS5B, which produces a negative-strand RNA intermediate. The negative strand RNA then serves as a template for the production of new positive-strand viral genomes. New virus particles are assembled and released at the cell surface *(Lindenbach and Rice, 2005)*.

In addition to the HCV polyprotein, a recently described 17-kDa HCV protein designated F, is produced by a ribosomal frameshift around codon 11 of the core protein and is recognized by serum antibodies from infected individuals but its role in infection is undetermined (Fig. 1) *(Xu, 2007)*.

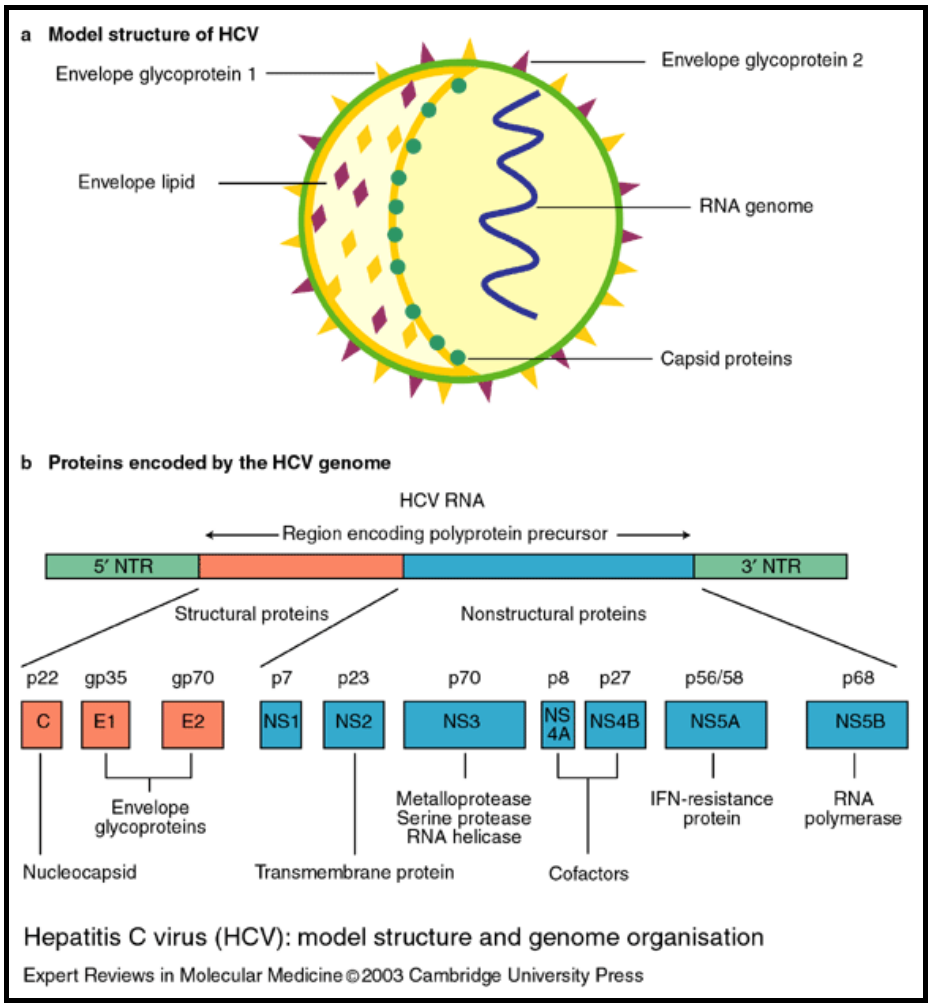

Fig. (1): HCV: model structure and genome organisation *(Mónica and Juan, 2003)*.

II- Structure and function of the viral proteins:

1. ***Core:*** The first structural protein encoded by the HCV open reading frame is the core protein which forms the viral nucleocapsid. An internal signal sequence located between the core and E1

sequences targets the nascent polypeptide to the endoplasmic reticulum (ER) membrane for translocation of the E1 ectodomain into the ER lumen *(McLauchlan et al., 2002).*

The N-terminal hydrophilic domain (D1) of the core protein contains a high proportion of basic amino-acid residues and has been implicated both in RNA binding and homo-oligomerization. The core protein is an α-helical protein that is found on membranes of the ER, in membranous webs and on the surface of lipid droplets. The association with lipid droplets, which is mediated by the central, relatively hydrophobic domain (D2) and ensures its folding, may have a role during viral replication and/or virion morphogenesis *(Boulant et al., 2006).*

In addition, it has been speculated that the interaction of the core protein with lipid droplets might affect lipid metabolism, contributing to the development of liver steatosis, which is the collection of excessive amounts of triglycerides and other fats inside liver cells and is often seen in hepatitis C, particularly in patients infected with genotype 3 *(Asselah et al., 2006).*

2. *Envelope glycoproteins:* The envelope proteins E1 and E2 are glycosylated and form a non-covalent complex, which is believed to be the building block for the viral envelope. HCV glycoprotein maturation and folding is a complex process that involves the ER machinery and depends on disulphide bond formation as well as glycosylation. The transmembrane domains of E1 and E2, located at their C-termini, are involved in heterodimerization and have ER retention properties. Each of them is composed of two stretches of hydrophobic amino-acid residues that are separated by a short polar segment. The second hydrophobic patch functions as an internal signal peptide for the downstream E2 and p7 proteins. Before signal sequence cleavage, the E1 and E2 transmembrane domains are proposed to adopt a hairpin structure at the translocon. After cleavage, the signal sequence is reorientated towards the cytosol, resulting in a single transmembrane passage. Determination of the three-dimensional structures of E1 and E2 will be key to elucidating the receptor binding and fusion processes that are mediated by these proteins *(Dubuisson et al., 2002)*.

3. ***p7:*** p7 is a 63-amino-acid polypeptide that is often incompletely cleaved from E2. It has two transmembrane segments connected by a short cytoplasmic loop, and the N terminus and C terminus are oriented toward the ER lumen ***(Carrère-Kremer et al., 2002).*** HCV p7 is not required for RNA replication in vitro but is essential for productive infection in vivo ***(Sakai, 2003).*** It has been reported to form oligomers and to have cation channel activity suggesting that it belongs to the viroporin family, and could have an important role in viral particle maturation and release. It might therefore be an attractive target for antiviral intervention ***(Griffin et al., 2003)***.

4. ***NS2-3 protease:*** The NS2-3 protease, also known as the autoprotease, has been one of the difficult-to-study HCV proteins. It is dispensable for RNA replication in vitro but is essential for the complete replication cycle in vitro and in vivo ***(Pietschmann et al., 2006).*** The catalytic activity of the NS2-3 protease resides in the C-terminal half of NS2 and the N-terminal one-third of NS3. Site-directed mutagenesis has shown that amino

acids His143, Glu163 and Cys184 are essential for proteolytic activity. As with all of the HCV proteins, NS2 is associated with intracellular membranes. Its N-terminal domain contains at least one (possibly three) transmembrane segments. Recombinant proteins lacking this domain retain enzymatic activity *(Pallaoro et al., 2001).*

The crystal structure of the NS2 protease domain (amino-acid residues 94-217, NS2pro) has recently been solved. NS2pro forms a dimer that has two composite active sites. Each active site is composed of residues from the two monomers, that is, residues His143 and Glu163 are contributed by one monomer and Cys184 by the other *(Lorenz et al., 2006).*

5. ***NS3-4A complex***: NS3 is a multifunctional protein, with a serine protease located in the N-terminal one-third and an RNA helicase/NTPase (nucleoside triphosphatase) located in the C-terminal two thirds of the protein. Both enzyme activities have been well characterized and high-resolution structures have been solved *(Yao et al., 1999).*

The NS4A polypeptide functions as a cofactor for the NS3 serine protease. Its central portion is incorporated as an integral component into the enzyme core, and its N-terminal portion is responsible for membrane association of the NS3-4A complex *(Wölk et al., 2000)*

6. ***NS4B:*** NS4B is a relatively poorly characterized 27-kDa protein. One of its functions is to induce the formation of the membranous web, the specific membrane alteration that serves as a scaffold for the HCV replication complex. It is predicted to contain four transmembrane segments *(Yu et al., 2006)*.

7. ***NS5A:*** NS5A is a monotopic protein anchored to the membrane by an N-terminal amphipathic α-helix embedded in-plane into the cytosolic leaflet of the membrane bilayer *(Penin et al., 2004).*

This helix has a hydrophobic, tryptophan-rich face that is embedded in the cytosolic membrane interface, whereas the polar, charged face is exposed to the cytosol and is probably involved in specific protein-protein interactions that are essential for the formation of a functional HCV replication complex *(Penin et al., 2004).*

NS5A is a phosphoprotein that can be found in basally phosphorylated (56kDa) and hyperphosphorylated (58 kDa) forms. Biochemical and functional studies suggest that the α-isoform of protein kinase may be responsible for NS5A hyperphosphorylation *(Quintavalle et al., 2006).*

Phosphorylation of NS5A is a conserved feature among hepaciviruses and pestiviruses and is also found in flavivirus. NS5 proteins, arguing that it has an important role in the HCV lifecycle *(Reed et al., 1998).* Indeed, cell culture adaptive mutations often affect centrally located serine residues that are required for hyperphosphorylation, suggesting that the phosphorylation state of NS5A modulates the efficiency of HCV RNA replication *(Appel et al., 2005)*.

8. *NS5B:* HCV replication proceeds by the synthesis of a complementary negative-strand RNA using the genome as a template and the subsequent synthesis of genomic positive-strand RNA from this negative-strand RNA template. The key enzyme responsible for both of these steps is the NS5B RdRp (RNA-dependent RNA polymerase) *(Bressanelli et al., 2002).*

Membrane association is mediated by the C-terminal 21 amino-acid residues, which are dispensable for polymerase activity in vitro but indispensable for RNA replication in cells *(Moradpour, 2004)*.

9. ***ARFP/F proteins:*** An alternative reading frame (ARF) was identified in the HCV core coding region that has the potential to encode a protein of up to 160 amino acids, designated ARFP (alternative reading frame protein) or F (frameshift) protein *(Branch et al., 2005).*

Detection of antibodies and T cells that are specific for the ARFP/F proteins in patients with hepatitis C suggests that these proteins are expressed during HCV infection. Yet the ARFP/F proteins are not required for HCV RNA replication in vitro or in vivo *(McMullan, 2007).*

III- Epidemiology of Hepatitis C Virus:

HCV is considered the most common blood-borne infection and is one of the leading causes for liver transplantation among adults. After the HCV antibody test became available, epidemiological

studies were performed to ascertain the incidence of the infection *(Yilmaz et al., 2007).*

The World Health Organization has declared hepatitis C a global health problem, with approximately 3% of the world's population (roughly 170-200 million people) infected with HCV *(Burban et al., 2006).*

Based on genome sequence similarity, international standardization of nomenclature recently classified HCV in to 6 major genotypes (1-6) and more than 70 sub-genotypes. Their prevalence and distribution are linked to geographical location and mode of transmission *(Roman et al., 2008).*

Information supporting geographical differences in the prevalence of HCV infection using population-based survey is not available in many parts of the world. Therefore, global prevalence rates are estimated based on HCV infection in the blood donor population. The reported prevalence rates for HCV infection are lowest in the United Kingdom and Scandinavia (0.01-0.1%); marginally higher in the Americas, Western Europe, Australia, and South Africa (0.2-0.5%); and intermediate in Brazil, Eastern