Introduction

Chronic hepatitis C is a public health problem, it has an estimated worldwide prevalence of 3% ***(Antwerp et al., 1999).***

Hepatitis C virus (HCV) is a leading cause of liver cirrhosis and cancer, and Egypt has possibly the highest HCV prevalence worldwide ***(Pybus et al., 2003).***

The prognosis and management of a patient with chronic hepatitis C virus (HCV) infection depend very much on the severity of liver fibrosis ***(Saadeh et al., 2001).***

At present, the golden standard to assess liver fibrosis is a liver biopsy using the Ishak ***(Ishak et al., 1995)*** or Metavir ***(Bedossa et al., 1996).***

However, biopsy is prone to sampling error and substantial intra and inter observer variability, leading to over or under staging of fibrosis ***(Regev et al., 2005).***

This procedure also has significant morbidity, including infections, major bleeding, ascites leakage and postprocedure pain, and can lead to mortality ***(Afdahal et al., 2004).***

It is contraindicated for patients with a coagulation disorder.

Moreover, liver biopsy is not a perfect golden standard because it sometimes results in false positive and false negative diagnoses ***(Bedossa et al., 2003).***

Needle biopsy of the liver is also clearly unsuitable for monitoring disease progression, especially in untreated patients.

For these reasons, non-invasive methods of assessing the severity of fibrosis which may someday completely replace LB, and are constantly being searched for. Among the non-invasive tests, the best results were obtained with liver stiffness measurement (LSM) by means of transient elastography (TE) (FibroScan). This non-invasive method is expensive and or requires equipment that is not widely available ***(Ziol et al., 2005).***

Therefore simpler, cheaper methods for the prediction of hepatic fibrosis were sought for.

The FIB-4 index is a simple, non invasive and inexpensive algorithm consisting of age and the routine laboratory tests (AST, ALT and platelets) ***(Sterling et al., 2006).***

Aim of the Work

To evaluate the utility of FIB-4 index as a simple inexpensive non invasive marker to assess liver fibrosis in chronic HCV infection in comparison to transient elastography (TE) (FibroScan).

Chapter 1

Hepatitis C Infection

Historical background:

In the mid-1970s, Harvey J. Alter, Chief of the Infectious Disease Section in the Department of Transfusion Medicine at the National Institutes of Health, and his research team demonstrated how most post-transfusion hepatitis cases were not due to hepatitis A or B viruses. Despite this discovery, international research efforts to identify the virus, initially called non-A, non-B hepatitis (NANBH), failed for the next decade. In 1987, Michael Houghton, Qui-Lim Choo, and George Kuo at Chiron Corporation, collaborating with Dr. D.W. Bradley at the Centers for Disease Control and Prevention, used a novel molecular cloning approach to identify the unknown organism and develop a diagnostic test ***(Boyer et al., 2001).*** In 1988, the virus was confirmed by Alter by verifying its presence in a panel of NANBH specimens. In April 1989, the discovery of HCV was published in two articles in the journal Science ***(Choo et al., 1989)*** **and** ***(Kuo et al., 1989).*** The discovery led to significant improvements in diagnosis and improved antiviral treatment ***(Boyer et al., 2001).*** In 2000, Drs. Alter and Houghton were honored with the Lasker Award for Clinical Medical Research for "pioneering work leading to the discovery of the virus that causes hepatitis C and the development of screening methods that reduced the risk of blood transfusion-associated hepatitis in

the U.S. from 30% in 1970 to virtually zero in 2000" ***(Lasker foundation, 2008).***

Epidemiology:

It is estimated that 130–170 million people, or ~3% of the world's population, are living with chronic hepatitis C ***(Fung et al., 2008).*** About 3–4 million people are infected per year, and more than 350,000 people die yearly from hepatitis C-related diseases ***(Fung et al., 2008).*** Rates have increased substantially in the 20th century due to a combination of intravenous drug use and intravenous medication or poorly sterilized medical equipments ***(Mueller et al., 2009).***

Among those chronically infected, the risk of cirrhosis after 20 years varies between studies but has been estimated at ~10%-15% for men and ~1-5% for women. The reason for this difference is not known. Once cirrhosis is established, the rate of developing hepatocellular carcinoma is ~1%-4% per year ***(WHO, 2011).***

In the United States, about 2% of people have hepatitis C ***(Maheshwari et al., 2008).*** Rates have decreased in the Western world since the 1990s due to improved screening of blood before transfusion ***(Alter et al., 2007).***

Prevalence is higher in some countries in Africa and Asia ***(Blatt et al., 2004).*** Countries with particularly high rates of infection include Egypt (22%), Pakistan (4.8%) and China (3.2%) ***(Fung et al., 2008).*** It is believed that the high prevalence in

Egypt is linked to a now-discontinued mass-treatment campaign for schistosomiasis, using improperly sterilized glass syringes ***(Mueller et al., 2009).***

Figure (1): Estimated HCV prevalence by region ***(Alter, 2007)***

Virology

1. Taxonomy

The hepatitis C virus belongs to the genus Hepacivirus a member of the family Flaviviridae.

2. Viral Structure

Structural analyses of HCV virions are very limited because for a long time the virus was difficult to cultivate in cell culture systems, a prerequisite for yielding sufficient virions for electron microscopy. Moreover, serum-derived virus particles are associated with serum low-density lipoproteins ***(Thomssen et al., 1992)***, which makes it difficult to isolate virions from serum/plasma of subjects via centrifugation.

It has been shown that HCV virions isolated from cell culture have a spherical envelope containing tetramers (or dimer of heterodimers) of the HCV E1 and E2 glycoproteins ***(Heller et al.,2005), (Wakita et al., 2005 & Yu et al 2007).*** Inside the virions a spherical structure has been observed ***(Wakita et al., 2005)*** representing the nucleocapsid (core) that harbours the viral genome.

3. Genome Organization

The genome of the hepatitis C virus consists of one 9.6 kb single stranded RNA molecule with positive polarity. Similar to other positive-strand RNA viruses, the genomic RNA of hepatitis C virus serves as messenger RNA (mRNA) for the translation of viral proteins. The linear molecule contains a single open reading frame (ORF) coding for a precursor polyprotein of approximately 3000 amino acid residues flanked by two regulatory nontranslated regions (NTR) ***(Mauss et al., 2013).***

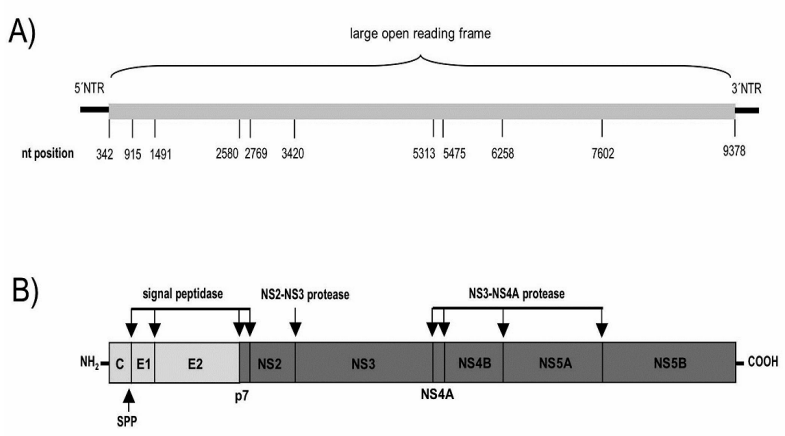

Figure (2): Genome organisation of Hepatitis C virus ***(Mauss et al., 2013)***

Table (1): Size and main function of HCV proteins

Protein	MW	Function
Core	21 kd	Capsid-forming protein. Regulatory functions in translation, RNA replication, and particle assembly.
F-protein or ARFP	16-17 kd	Unknown.
Envelope glycoprotein 1 (E1)	35 kd	Transmembrane glycoprotein in the viral envelope. Adsorption, receptor-mediated endocytosis.
Envelope glycoprotein 1 (E2)	70 kd	Transmembrane glycoprotein in the viral envelope Adsorption, receptor-mediated endocytosis.
p7	7 kd	Forms an ion-channel in the endoplasmic reticulum. Essential formation of infectious virions.
NS2	21 kd	Portion of the NS2-3 protease which catalyses cleavage of the polyprotein precursor between NS2 and NS3
NS3	70 kd	NS2-NS3 protease, cleavage of the downstream HCV proteins. ATPase/helicase activity, binding and unwinding of viral RNA.
NS4A	4 kd	Cofactor of the NS3-NS4A protease.
NS4B	27 kd	Crucial in HCV replication. Induces membranous web at the ER during HCV RNA replication.
NS5A	56 kd	Multi-functional phosphoprotein. Contains the IFN α sensitivity-determining region (ISDR) that plays a significant role in the response to IFN α-based therapy.
NS5B	66 kd	Viral RNA-dependent RNA polymerase. NS5B is an error-prone enzyme that incorporates wrong ribonucleotides at a rate of approximately 10-3 per nucleotide per generation.

(Mauss et al., 2013)

HCV Proteins

Translation of the HCV polyprotein is initiated through involvement of some domains in the NTRs of the genomic HCV RNA. The resulting polyprotein consists of ten proteins that are co-translationally or post-translationally cleaved from the polyprotein. In addition, the F (frameshift) or ARF (alternate reading frame) protein has been explored ***(Walewski et al., 2001).*** During translation ARFP is the product of ribosomal frame shifting within the core protein-encoding region.

Viral lifecycle

a- Adsorption and viral entry

A cascade of virus-cell interactions is necessary for the infection of hepatocytes. The precise mechanism of viral entry is complex and still not completely understood. The current model of viral adsorption assumes that HCV is associated with low-density lipoproteins (LDL). The binding step includes binding of the LDL component to the LDL-receptor (LDL-R) on the cell surface ***(Agnello et al., 1999)*** and simultaneous interaction of the viral glycoproteins with cellular glycosaminoglycans (GAG) ***(Germi et al., 2002).*** This initiation step is followed by consecutive interactions of HCV with scavenger receptor B type I (SR-BI) ***(Scarselli et al., 2002)*** and the tetraspanin CD81 ***(Pileri et al., 1998).*** More recent findings indicate subsequent transfer of the virus to the tight junctions, a protein complex located between

adjacent hepatocytes. Two components of tight junctions, Claudin-1 (CLDN1) and occludin (OCLN) have been shown to interact with HCV ***(Evans, 2007).*** Although the precise mechanism of HCV uptake in hepatocytes is still not clarified, these cellular components may represent the complete set of host cell factors necessary for cellfree HCV entry. Interaction of HCV with CLDN1 and OCLN seems to induce the internalisation of the virion via clathrin-mediated endocytosis ***(Hsu et al., 2003).*** Subsequent HCV E1-E2 glycoprotein mediation fuses the viral envelope with the endosome membrane ***(Meertens et al., 2006).***

Despite having identified several host factors that probably interact with the viral glycoproteins, the precise mechanisms of interaction still need to be investigated.

b- Translation and posttranslational processes

As a result of the fusion of the viral envelope and the endosomic membrane, the genomic HCV RNA is released into the cytoplasm of the cell (uncoating). The viral genomic RNA possesses a nontranslated region (NTR) at each terminus. It contains an internal ribosome entry site (IRES) involved in ribosome binding and subsequent initiation of translation ***(Tsukiyama-Kohara et al., 1992).*** The synthesized HCV precursor polyprotein is subsequently processed by at least four distinct peptidases. The cellular signal peptidase (SP) cleaves the N-terminal viral protein's immature core protein, E1, E2, and p7 ***(Hijikata et al., 1991),*** while the cellular signal peptide peptidase (SPP) is responsible for the cleavage of the E1 signal

sequence from the C-terminus of the immature core protein, resulting in the mature form of the core ***(McLauchlan et al., 2002).*** The E1 and E2 proteins remain within the lumen of the ER where they are subsequently N-glycosylated with E1 having 5 and E2 harbouring 11 putative N-glycosylation sites ***(Duvet et al., 2002).*** The remaining HCV proteins are posttranslationally cleaved by the viral NS2-NS3 and the NS3- NS4A protease, respectively.

c- HCV RNA replication

The process of HCV RNA replication is poorly understood. The key enzyme for viral RNA replication is NS5B, an RNA-dependent RNA polymerase (RdRp) of HCV. After the RdRp has bound to its template, the NS3 helicase is assumed to unwind putative secondary structures of the template RNA in order to facilitate the synthesis of minus-strand RNAthe synthesis of minus-strand RNA ***(Jin et al., 1995).*** In turn, the newly synthesized antisense RNA molecule serves as the template for the synthesis of numerous plus-stranded RNA. The resulting sense RNA may be used subsequently as genomic RNA for HCV progeny as well as for polyprotein translation. Another important viral factor for the formation of the replication complex appears to be NS4B, which is able to induce an ER derived membranous web containing most of the non-structural HCV proteins including NS5B ***(Egger et al., 2002).***

d- ***Assembly and release***

After the viral proteins, glycoproteins, and the genomic HCV RNA have been synthesized these components have to be arranged in order to produce infectious virions. Viral assembly is a multi-step procedure involving most viral components along with many cellular factors. Recent findings suggest that viral assembly takes place within the endoplasmic reticulum ***(Gastaminza et al., 2008)*** and that lipid droplets are involved in particle formation ***(Miyanari et al., 2007).*** However, the precise mechanisms for the formation and release of infectious HCV particles are still unknown.

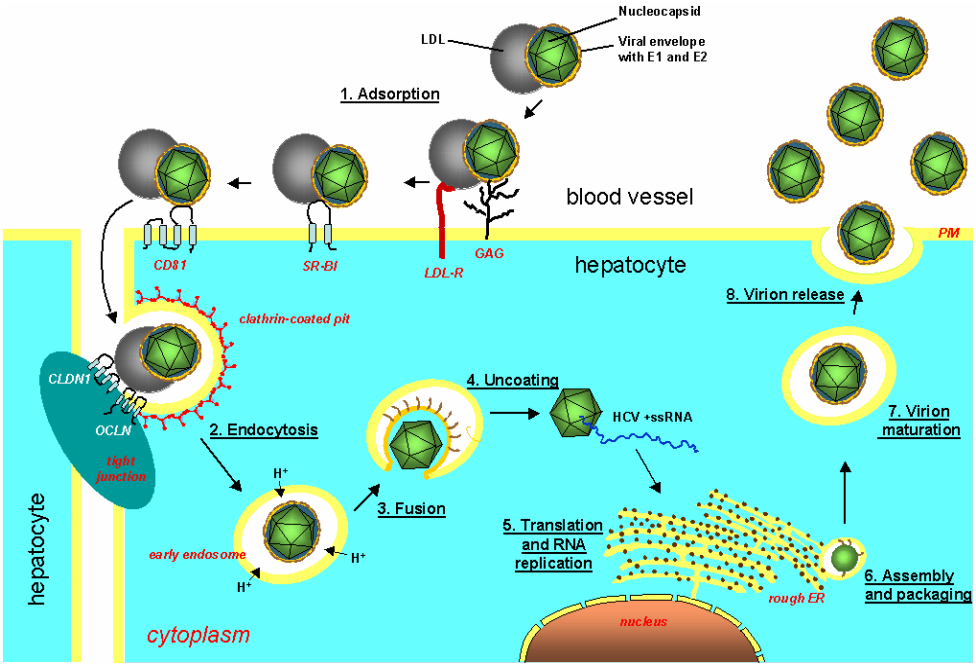

Figure (3): A simplified diagram of the HCV replication cycle ***(Pawlotsky et al., 2007)***

Transmission

The primary method of transmission in the developed world is intravenous drug use (IDU), while in the developing world the main methods are blood transfusions and unsafe medical procedures ***(Maheshwari et al., 2010).***

1. Intravenous drug use:

IDU is a major risk factor for hepatitis C in many parts of the world ***(Xia et al., 2008).*** Of 77 countries reviewed 25 (including the United States) were found to have prevalences of hepatitis C in the intravenous drug user population of between 60% and 80% ***(Nelson et al., 2011 & Xia et al., 2008).*** While twelve countries had rates greater than 80% ***(Nelson et al., 2011).*** It is believed that ten million intravenous drug users are infected with hepatitis C, China (1.6 million), the United States (1.5 million), and Russia (1.3 million) have the highest absolute totals ***(Nelson et al., 2011).*** Occurrence of hepatitis C among prison inmates in the United States are ten to 20 times that of the occurrence observed in the general population, this has been attributed to high-risk behavior in prisons such as intravenous drug use and tattooing with nonsterile equipment ***(Imperial et al., 2010 & Vescio et al., 2006).***

2. Healthcare exposure

Blood transfusion, transfusion of blood products, or organ transplantation without HCV screening carry significant risks of infection ***(Wilkins et al., 2010).*** The United States

instituted universal screening in 1992 and the risk subsequently has decreased from one in 10,000 to 10,000,000 per unit of blood ***(Springer et al., 2011). (Pondé et al., 2011)*** down from a risk of one in 200 units of blood ***(Marx et al., 2010).*** This low risk remains as there is a period of about 11–70 days between the potential blood donor acquiring hepatitis C and their blood testing positive depending on the method ***(Pondé et al., 2011).*** Some countries do not screen for hepatitis C due to the cost ***(Alter et al., 2007).***

Those who have experienced a needle stick injury from someone who was HCV positive have about a 1.8% chance of subsequently contracting the disease themselves ***(Wilkins et al., 2010).*** The risk is greater if the needle in question is hollow and the puncture wound is deep ***(Alter MJ et al., 2007).*** There is a risk from mucosal exposures to blood, but this risk is low, and there is no risk if blood exposure occurs on intact skin ***(Alter MJ et al., 2007).***

Hospital equipment has also been documented as a method of transmission of hepatitis C including: reuse of needles and syringes, multiple-use medication vials, infusion bags, and improperly sterilized surgical equipment, among others ***(Alter et al., 2007).*** Limitations in the implementation and enforcement of stringent standard precautions in public and private medical and dental facilities are known to be the primary cause of the spread of HCV in Egypt, the country with highest rate of infection in the world ***(Al Bawaaba, 2010).***

3. Sexual intercourse

Whether hepatitis C can be transmitted through sexual activity is controversial ***(Tohme and Holmberg, 2010).*** While there is an association between high-risk sexual activity and hepatitis C, it is not known whether transmission of the disease is due to drug use that has not been admitted to or sex as a risk factor ***(Wilkins et al., 2010).*** The majority of evidence supports there being no risk for monogamous heterosexual couples ***(Tohme et al., 2010).*** Sexual practices that involve higher levels of trauma to the anogenital mucosa, such as anal penetrative sex, or that occur when there is a concurrent sexually transmitted infection, including HIV or genital ulceration, do present a risk ***(Tohme et al., 2010).*** The United States government only recommends condom use to prevent hepatitis C transmission in those with multiple partners.

4. Body piercings

Tattooing is associated with two to threefold increased risk of hepatitis C ***(Jafari et al., 2010).*** This can be due to either improperly sterilized equipment or contamination of the dyes being used ***(Jafari et al., 2010).*** The risk also appears to be greater for larger tattoos. It is rare for tattoos in a licensed facility to be directly associated with HCV infection ***(CDC, 2012).***