INTRODUCTION

Hepatitis C Virus (HCV)

Hepatitis C is an <u>infectious disease</u> affecting primarily the <u>liver</u>, caused by the <u>hepatitis C virus</u> (HCV) which is the leading cause of chronic liver disease worldwide. It is estimated that about 170 million people are chronically infected with HCV. Chronic hepatitis C is a major cause of cirrhosis, hepatocellular carcinoma and HCV-related end stage liver disease, in many countries it is the first cause of liver transplantation. The infection is often <u>asymptomatic</u>, but chronic infection can lead to scarring of the liver and ultimately to <u>cirrhosis</u>, which is generally apparent after many years.[1]

Hepatitis C causes acute infection in 10-15% of cases. Symptoms are generally mild and vague, including a <u>decreased appetite</u>, fatigue, <u>nausea</u>, <u>muscle</u> or <u>joint pains</u>, and rarely <u>acute liver failure</u> results. Most cases of acute infection are not associated with <u>jaundice</u>. The infection resolves spontaneously in 10–50% of cases.[2]

About 85% of those exposed to the virus develop a chronic infection,[3] which is defined as the presence of detectable viral replication for at least six months. Most experience revealed minimal or no symptoms during the initial few decades of the infection. It is estimated that up to 25% of patients with chronic hepatitis C will develop cirrhosis within 20 years. Hepatocellular carcinoma associated with HCV occurs almost in those with cirrhosis. Once cirrhosis is established, hepatocellular carcinoma develops at an annual rate of 1%-5 %. [4]

HCV Structure

The HCV is a small, enveloped, single-stranded, positive-sense, approximately 9500-nucleotide RNA virus classified as a separate genus (Hepacivirus) within the Flaviviridae family. (Figure 1) [5]
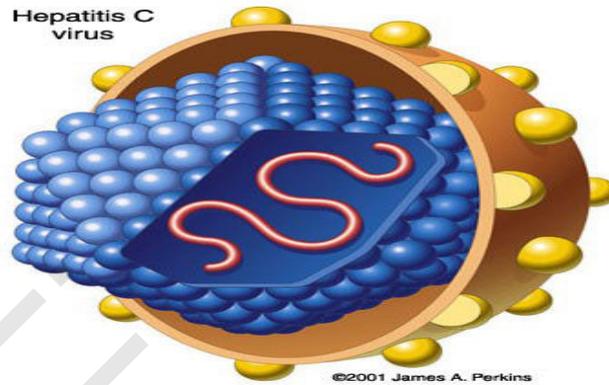
Figure (1): Schematic design of HCV [5]

The HCV genome contains a single large open reading frame (ORF) that codes for a virus polyprotein of approximately 3000 amino acids (Figure 2).[6] The 5´ end of the genome consists of an untranslated region adjacent to the genes for structural proteins, the nucleocapsid core protein and two envelope glycoproteins, E1 and E2/NS1. The 5´ untranslated region and core gene are highly conserved among genotypes. The envelope glycoproteins, E1 and E2, are coded for by the hypervariable region, which is responsible for the genetic diversity. It may allow the virus to prevent recognition by antibodies and so it is able to evade host immunologic containment directed at accessible virus-envelop proteins.[6] The 3´ end of the genome contains the genes for nonstructural (NS) proteins. The NS2 (250 amino acids), NS3 (500 amino acids), and NS4a proteins interact to mediate the processing of the presumed NS region of the polyprotein. NS3 (500 amino acids) is both a proteolytic cleavage enzyme and a helicase to facilitate unwinding of the viral genome for replication. NS5b is the RNA-dependent RNA polymerase needed for viral replication. [6]
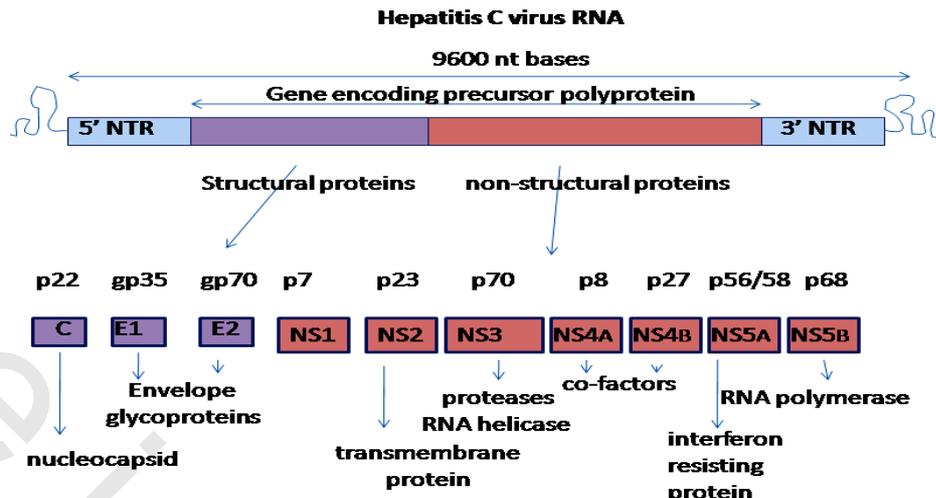
Figure (2): Genome of HCV [6]

At least six distinct genotypes and a number of subtypes have been identified by nucleotide sequencing. Because of divergence of HCV isolates within a genotype or subtype and within the same host, it makes it difficult to define a genotype, these intra genotypic differences are referred to as quasispecies. The genotypic and quasispecies diversity of HCV, resulting from its high mutation rate, interferes with effective humoral immunity. [7] Neutralizing antibodies to HCV have been demonstrated, but they tend to be short-lived and HCV infection has not been shown to induce lasting immunity against reinfection with different virus isolates or even with the same virus isolate. [7]

HCV is inactivated by exposure to lipid solvents or detergents, heating at 60°C for 10 hours or 100°C for 2 minutes in aqueous solution, formaldehyde (1:2000) at 37°C for 72 hours, ß-propriolactone and UV irradiation. HCV RNA is relatively unstable to the storage at room temperature and to the repeated freezing and thawing for quantification. [8]

Epidemiology

It is estimated that 150–200 million people, or approximately 3% of the world's population, are living with chronic hepatitis C. [9]

About 3–4 million people are infected per year, and more than 350,000 people die yearly from hepatitis C-related diseases. [10]Rates of infection are high (>3.5% of population are infected) in Central and East Asia, North Africa and the Middle East, they are intermediate (1.5%-3.5%) in South and Southeast Asia, sub-Saharan Africa, Andean, Central and Southern Latin America, Caribbean, Oceania, Australasia, Central, Eastern and Western Europe; and they are low (<1.5%) in Asia Pacific, Tropical Latin America and North America. [9] Some countries have massive HCV problems, especially Egypt where about 15-20% of the population are infected.[11]

Transmission

HCV is spread primarily by direct contact with infected human blood. Blood transfusion, transfusion of blood products, or organ transplants without HCV screening carry significant risks of infection. [12] 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. [11] 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.[13] Transmission through needle-sharing among intravenous drug users is well documented.[14] Needle stick injuries are also responsible for transmission of pathogens, the estimated risk of transmission through one needle stick injury is 1 - 3% for HCV, 0.3% for human immunodeficiency virus (HIV) and 6-30% for hepatitis B virus (HBV).

(12) Transmission through sexual activity is controversial there is no conclusive evidence that hepatitis C can be transmitted by sexual activity, The majority of evidence supports there is no risk for monogamous heterosexual couples. 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 increase HCV transmission risk. (15) The vertical transmission risk is estimated at 4 - 7%, it rises with HIV-1 co infection and a high maternal HCV viral load, transmission may occur both during gestation and at delivery, a prolonged labor is associated with a greater risk of transmission. There is no evidence that breast-feeding spreads HCV; however, to be cautious, an infected mother is advised to avoid breastfeeding if nipples are cracked and bleeding, or her viral loads are high.(16) Other modes of transmission such as social, cultural and behavioral practices using percutaneous procedures (e.g., piercing, circumcision, tattooing) may occur if inadequately sterilized equipment is used. (17) HCV is not usually spread by sneezing, hugging, coughing, food or water, sharing eating utensils, and casual contact.(18)Finally it is believed that the primary route 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. The cause of transmission remains unknown in 20% of cases; however, many of these are believed to be accounted for by IDU.(19)

HCV Replication

HCV has a high rate of replication with approximately one trillion

particles are produced each day in the infected individual. HCV also has an exceptionally high mutation rate, a factor that may help it elude the host's immune response. HCV mainly replicates within hepatocytes in the liver, although there is controversial evidence for replication in lymphocytes or monocytes by mechanisms of host tropism. It is well established that the envelope glycoprotein (E1 and E2) complex is obviously a candidate ligand for receptors. Several candidates for the receptor have recently been detected. Probably, a receptor complex consisting of CD81- and scavenger receptor class B type I is responsible for binding to HCV.[20]

After possible receptor mediated endocytosis, the envelope glycoproteins are thought to mediate fusion. The viral nucleocapsid is thought to be uncoated by unknown mechanism after the release into the cytoplasm. HCV RNA serves as a template and the mRNA is coded for the viral proteins. Polyprotein translation is mediated by binding of the ribosomal 40S subunit to the internal ribosome entry site and of the HCV 5` UTR and the first 30-40 nucleosides to the core-encoding region. The polyprotein precursor is then co- and posttranslational processed by cellular and viral proteases to the mature proteins and later released. [21]
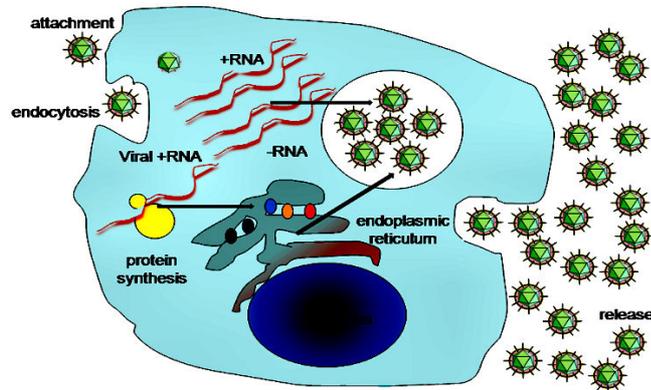
Figure (3): **HCV life cycle** [20]

Pathogenesis and Immune response

HCV infection is characterized by its propensity to evolve into chronicity. About 85% of patients infected by HCV will develop chronic infection and resolution of acute hepatitis C is observed in only 15%. The severity of the liver disease varies widely from asymptomatic chronic infection, with normal liver tests and nearly normal liver, to severe chronic hepatitis, leading rapidly to cirrhosis and hepatocellular carcinoma. The mechanisms responsible for the persistence of HCV infection and for the liver lesions are not well understood. The lack of an efficient in vitro replication system or an animal model (the chimpanzee model is limited) has greatly hampered the study of these mechanisms.[22]

The cellular immune response is crucial for the elimination or the persistence of HCV infection.CD4+ T cells and their cytokines seem to play an important role in the immunopathogenesis of chronic HCV infection. CD4+ T cell responses are polarized into type 1 and type 2 helper T cell (Thl and Th2) responses. The Thl cells secrete interleukin 2 (IL-2) and interferon gamma, which are important stimuli for the development of the host antiviral

immune responses, including cytotoxic T-lymphocyte (CTL) generation and natural killer cells (NK-cell) activation. The Th2 cells produce IL-4 and IL-10, which enhance antibody production and down regulate the Thl response.[23] It is hypothesized that the imbalance between the Thl and the Th2 responses is implicated in disease progression and the inability to clear infection.

Patients with acute HCV infection who clear the virus and have a self-limited acute hepatitis develop a strong Thl response, but a weak or absent Th2 response. Conversely, patients who develop a chronic infection show a predominant Th2 response, but a weak Th 1 response. [23]

The presence of HCV infection has been shown in peripheral blood mononuclear cells, monocytes and lymphocytes. Furthermore, the detection of minus strand RNA in hematopoietic cells suggests that this is a possible extra hepatic site of replication for HCV.[24]These extrahepatic sites of HCV infection might also play a role in the persistence of HCV infection, possibly by altering the immune response or by favoring infection of liver cells. Interestingly, a cellular protein that binds E2 has recently been identified, called CD81, which is expressed on the surface of several cell types, including lymphocytes and hepatocytes, and is currently believed to be an HCV receptor or co-receptor. Antibodies that neutralize infection by HCV appear to do so by preventing E2 binding to CD81. [25]

Antibody production is critical for neutralization of free viral particles and for preventing virus entry into the host. Studies showed that neutralizing antibodies are produced during natural HCV infection despite the high rate of chronic evolution. The most likely explanation for the ineffectiveness of the antibody response against HCV is that rapid occurrence of viral mutations within the epitopes recognized by neutralizing antibodies can abrogate antibody recognition of the new variant virus.[26]

Consequently, within any given individual infected with HCV, the viral population consists of a heterogeneous mixture of closely

related virions, termed quasispecies.[27] The biological consequences of quasispecies include the development of escape mutants to humoral and cellular immunity, variable cell tropism (lymphotropic versus hepatotropic), vaccine failure and rapid development of drug resistance. Indeed, HCV has a high genetic variability, especially in the E2 and the NSl regions of the genome, with two hypervariable regions within E2 called HVRl and HVR2.[28] This view is consistent with the extremely rapid viral production rate in patients with chronic hepatitis C, in the order of 10^{10}-10^{12} virions per day and a short in vivo half-life of the virus in the order of 47 hours. The high turnover rate of HCV explains the rapid generation of viral diversity and the opportunity for viral escape from the host immune response.[29]

Figure (4): Components of antiviral immune response to HCV [30]

Mechanisms involved in liver lesions

HCV is not directly cytopathic. The hepatitic process appears to result from the immune recognition and destruction of infected hepatocytes. Persistent HCV infection in the liver is continuously triggering an active T cell response, which is probably the main mechanism responsible for the liver lesions. Studies of the intrahepatic immune processes implicated in the pathogenesis of chronic hepatitis C are limited. However, HCV specific helper and cytotoxic T cells able to recognize structural and non structural HCV proteins (especially core and NS4 proteins) have been detected within the liver infiltrates. The predominant production of Thl cytokines is believed to play a role in enhancing necro - inflammatory process. This continuous necro-inflammatory process, inefficient viral clearing are probably the main cause of the fibrogenesis mechanisms responsible for the progression of the liver disease.[23]

The evolution of liver fibrosis varies widely from one patient to the other. The progression of liver fibrosis is influenced by many factors, some of these factors are virus related and the others are host related.

Regarding the virus related factors, the role of viral load and the genotype is still being debated. Most studies argue for the absence of correlation between serum HCV RNA levels and the severity of the liver lesions.[31] In many studies patients with chronic infection and normal serum aminotransferase levels and nearly normal liver histology may have high serum HCV RNA levels.[22]

Regarding the host related factors it was found that several factors such as immune status, genetic factors, age and alcohol could be considered as cofactors associated with the progression of liver disease. Recent studies showed that liver graft recipients generally have a more progressive liver disease than immunocompetent patients with higher incidence of cirrhosis, which might be responsible for an increased mortality. In such patients, high levels of serum HCV RNA have been demonstrated and direct cytopathic mechanisms of HCV can not be ruled out. It is believed that the more rapid progression of fibrosis is related to immunosuppressive therapy and, although the rationale is not clearly demonstrated, it is generally recommended to decrease immunosuppressive therapy as much as possible.[32]

In another population of immunocompromised patients, such as patients with HIV co infection, progression to cirrhosis is more rapid than in HIV-negative patients, although the severity of the liver disease varies considerably between these patients. In this population, cofactors such as drugs used against HIV might play an important role. Also viral co infection, with HBV, aggravates chronic hepatitis C and increases the risk of development of hepatocellular carcinoma.[33]

The detrimental role of alcohol in accelerating the evolution of chronic hepatitis C is well recognized. Several studies have shown that alcohol consumption accelerates the progression of fibrosis and the development of cirrhosis. In addition, heavy alcohol consumption is probably associated with a higher risk of hepatocellular carcinoma. The mechanisms may involve alterations of the immune response and direct toxicity of alcohol.[34]

Many studies have emphasized the role of the age in the outcome of chronic hepatitis C. Patients infected at a higher age have more severe histologic lesions and a more rapid evolution to cirrhosis. [35]

Also it is well known that the genetic constitution and immune status of the host are important factors in the persistence and progression of the virus as they influence antigen recognition and presentation as well as Th response.[36] Some Major Histocompatibility Complex (MHC) class II alleles have been associated with a lower incidence of cirrhosis in individuals chronically infected with HCV.[37] Rehermann et al. identified that Cytotoxic T lymphocytes (CTLs) restricted by histocompatibility leukocyte antigen A2 in 97% of chronic hepatitis C patients, compared with 2% of anti-HCV-negative controls.[38] It is speculated that the MHC class II molecule presentation of antigens is deficient in HCV-infected cells because some viral proteins inhibit the presentation of the antigen through IFN-induced negative immunoregulation .[39] Some pro-inflammatory cytokines appear to be associated with the viral infection response as well as with the expression of specific haplotypes, such as IL-10 haplotypes, which can be predictors of spontaneous elimination of HCV.[40]

Regarding gender the male sex is more prevalent in most studies on hepatitis C and was associated with the progression of the disease to cirrhosis.[41]

Some studies suggest that Afro-Americans are more resistance to treatment and higher tendency for development of hepatocellular carcinoma than Caucasian-Americans.[42]

Pathogenesis of liver fibrosis

Hepatic fibrosis is the result of the wound-healing response of the liver to repeated injury [43] (Figure 5, 6). After an acute liver injury (e.g., viral hepatitis), parenchymal cells regenerate and replace the necrotic or apoptotic

cells. This process is associated with an inflammatory response and a limited deposition of ECM. If the hepatic injury persists, then eventually the liver regeneration fails, and hepatocytes are substituted with abundant ECM, including fibrillar collagen. The distribution of this fibrous material depends on the origin of the liver injury. In chronic viral hepatitis and chronic cholestatic disorders, the fibrotic tissue is initially located around portal tracts, while in alcohol-induced liver disease, it locates in pericentral and perisinusoidal areas.[44] As fibrotic liver diseases advance, disease progression from collagen bands to bridging fibrosis to frank cirrhosis occurs.

Liver fibrosis is associated with major alterations in both the quantity and composition of ECM. In advanced stages, the liver contains approximately 6 times more ECM than normal, including collagens (I, III, and IV), fibronectin, undulin, elastin, laminin, hyaluronan, and proteoglycans. Accumulation of ECM results from both increased synthesis and decreased degradation. [45]

HSCs are the main ECM-producing cells in the injured liver.[46] In the normal liver, HSCs reside in the space of Disse and are the major storage sites of vitamin A. Following chronic injury, HSCs activate or transdifferentiate into myofibroblast-like cells, acquiring contractile, proinflammatory, and fibrogenic properties.[47] Activated HSCs migrate and accumulate at the sites of tissue repair, secreting large amounts of ECM and regulating ECM degradation. Collagen synthesis in HSCs is regulated at the transcriptional and posttranscriptional levels.[48] Interestingly, HSCs express a number of neuroendocrine markers (e.g., reelin, nestin, neurotrophins, synaptophysin, and glial-fibrillary acidic protein) and bear receptors for neurotransmitters, while activated HSCs express myogenic markers (α smooth muscle actin, c-myb, and myocyte enhancer factor–2). [49]

Different cell types other than HSCs may also have fibrogenic potential.[50]Culture of CD34$^+$CD38$^-$ hematopoietic stem cells with various growth factors has been shown to generate HSCs and myofibroblasts of bone marrow origin that infiltrate human livers

undergoing tissue remodeling. [51] These data suggest that cells originating in bone marrow can be a source of fibrogenic cells in the injured liver.

A complex interplay among different hepatic cell types takes place during hepatic fibrogenesis. Hepatocytes are targets for most hepatotoxic agents, including hepatitis viruses. Damaged hepatocytes release Reactive Oxygen Species (ROS) and fibrogenic mediators and induce the recruitment of inflammatory cells. Apoptosis of damaged hepatocytes stimulates the fibrogenic actions of liver myofibroblasts. Inflammatory cells, either lymphocytes or polymorphonuclear cells, activate HSCs to secrete collagen. Activated HSCs secrete inflammatory chemokines, express cell adhesion molecules, and modulate the activation of lymphocytes. Therefore, a vicious circle in which inflammatory and fibrogenic cells stimulate each other is likely to occur. Kupffer cells are resident macrophages that play a major role in liver inflammation by releasing ROS and cytokines. [52,53]
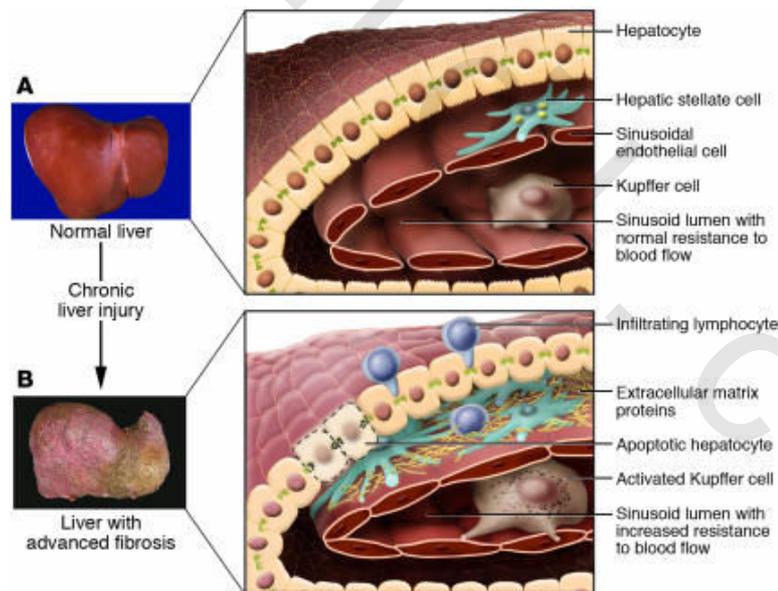
Figure (5): Changes in the hepatic architecture due to fibrosis [44]
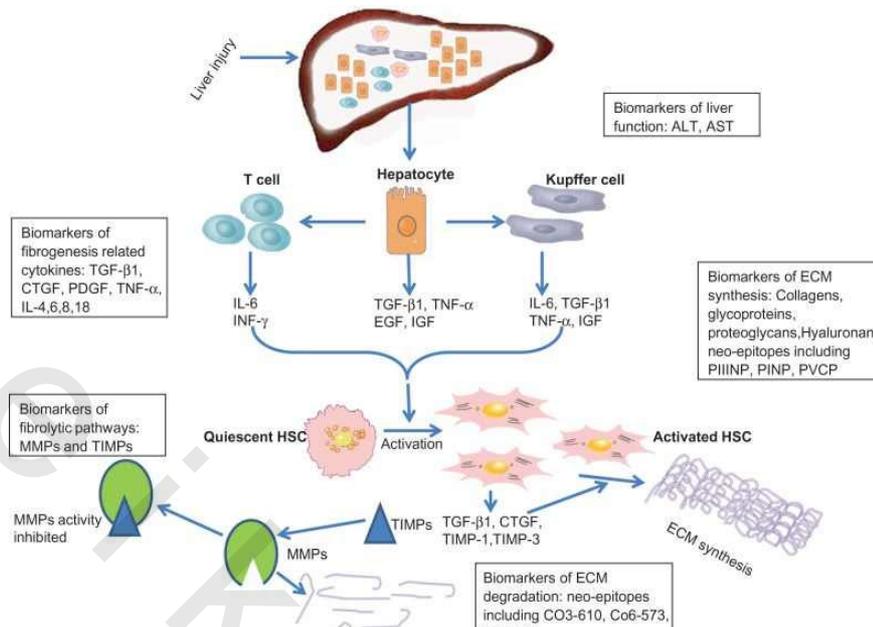
Figure (6): Mechanisms of hepatic fibrogenesis and possible molecular serum biomarkers. Some molecular serum biomarkers may reflect the pathogenesis of liver fibrosis: neo-epitopes, are related to basement membrane degradation; pro-collagen, is related to extracellular matrix (ECM) synthesis; MMPs and TIMPs are relate to ECM fibrolytic processes; ALT and AST are related to liver function and injury; other serum markers are fibrogenesis-related cytokines. [44]

Signs and symptoms

Acute viral hepatitis occurs after an incubation period of 15 - 60 days, the vast majority of acute HCV infections do not show any symptoms. In few patients the disease presented with mild flu-like symptoms, jaundice may be observed in about 25% of patients. Fulminant hepatitis is very rare in the acute infection stage.[2]

Chronic hepatitis C is often asymptomatic until evidence of liver failure becomes clinically apparent. Some patients with chronic infection experience malaise, nausea, abdominal pain and pruritus. Fluctuating alanine aminotransferase (ALT) levels is characteristic. In the late stage of chronic HCV infection, the physical examination may reveal signs of liver disease such as spider angiomata, palmar erythema and telangiectasia. Chronic infection causes chronic inflammation of the liver, ongoing cycles of inflammation, necrosis, and apoptosis leading to progressive fibrosis and finally to severe bridging fibrosis with nodular regeneration and cirrhosis. If advanced cirrhosis has developed, jaundice, splenomegaly, ascites, portal hypertension, esophageal varices, and hepatic encephalopathy may be observed. The rate of progression to cirrhosis is usually slow, with 20 or more years elapsing between infection and the development of serious complications.[54] After cirrhosis has developed, the risk of hepatocellular carcinoma is 1 – 4% each year.[55]

Extrahepatic manifestations of Hepatitis C

Several studies have found that between 70-74% of HCV patients experience extrahepatic manifestions.[56] Some of the most common symptoms and conditions reported include:

Fatigue: is one of the most common symptoms of hepatitis C and can range from mild to moderate to severe. It is thought that the fatigue is caused both by the body's immune response to hepatitis C as well as by the direct affect of the virus on the body.[56]

Arthralgia and Arthritis: which is estimated to affect about 4% of the HCV population, and is classified into two groups, polyarthritis which is similar to rheumatoid arthritis but is less serious and mono-oligoarthritis affects the medium and large-sized joints typically the ankles.[56]

Behcet's Disease: is a disease that presents as ulcerations in the eyes, mouth and genitals but can also affect any organ of the body and is caused by coagulation and destruction of arteries and veins.[57]

Sjogren's Syndrome (SS) is an autoimmune disease that affects the eyes and mouth, making them dry. Although hepatitis C has not been directly linked to SS, it is found more often in people with hepatitis C than in the general population.[57]

Cryoglobulinemia: is one of the most common disorders associated with hepatitis C. Cryoglobulinemia is a blood disorder caused by abnormal proteins in the blood called cryoglobulins. These proteins can be deposited in the small and medium sized blood vessels, which restrict blood flow and can lead to further problems. Cryoglobulinemia can affect the skin, kidneys, nerves and joints. Treating cryoglobulinemia consists of treatment of the underlying disease (hepatitis C) as well as the administration of medications to suppress the immune system, and plasmapheresis.[58]

Membranoproliferative Glomerulonephritis (MPGN) is a condition affecting the kidneys that is usually (but not always) associated with cryoglobulinemia. Symptoms include weakness, edema and arterial hypertension. Treatment consists of treating the underlying cause – hepatitis C. However, if there is severe kidney impairment, ribavirin should be avoided.[59]

Membranous Nephropathy is a disease of the kidneys that is associated with hepatitis C but not with cryoglobulinemia. It is believed that circulating HCV antibodies and viral particles are deposited in the kidneys causing damage. Treatment consists of treating hepatitis C. If there is severe kidney damage then the use of ribavirin should be avoided. [59]

Thyroid Disease including either hyperthyroidism or hypothyroidism. The direct link between hepatitis C and thyroid disease is unclear. HCV treatment can also induce thyroid disease, but thyroid function will return to normal for about 95% of people who develop treatment-related hypothyroidism when treatment is stopped. [56]

Other extra hepatic manifestations include insulin resistance and type 2 diabetes mellitus, Idiopathic Pulmonary Fibrosis and Chronic Obstructive Pulmonary Disease (COPD). [56]

Diagnosis

The optimal approach for detecting HCV infection is to screen persons for a history of risk of exposure to the virus, and to test selected individuals who have an identifiable risk factor. [60]

Center of disease control and prevention (CDC) outlined the list of persons who should be routinely screened for HCV infection. [61]

Persons for Whom HCV Screening is Recommended

- Persons who have injected illicit drugs in the recent and remote past, including those who injected only once and do not consider themselves to be drug users.

- Persons with conditions associated with a high prevalence of HCV infection including:

  - Persons with HIV infection

- Persons with hemophilia who received clotting factor concentrates prior to 1987

- Persons who have ever been on hemodialysis

- Persons with unexplained abnormal aminotransferase levels

• Prior recipients of transfusions or organ transplants prior to July 1992 including:

- Persons who were notified that they had received blood from a donor who later tested positive for HCV infection

- Persons who received a transfusion of blood or blood products

- Persons who received an organ transplant

• Children born to HCV-infected mothers

• Health care, emergency medical and public safety workers after a needle stick injury or mucosal exposure to HCV-positive blood

• Current sexual partners of HCV-infected persons.

Two classes of assays are used in the diagnosis and management of HCV infection: serologic assays that detect specific antibody to hepatitis C virus (anti-HCV) and molecular assays that detect viral nucleic acid. These assays have no role in the assessment of disease severity or prognosis.

Serologic Assays: Tests that detect anti-HCV are used both to screen for and to diagnose HCV infection. Anti-HCV can be detected in the serum or plasma using a number of immunoassays. Two enzyme immunoassays (EIAs), Abbott HCV EIA 2.0 and HCV Version 3.0 ELISA as well as one enhanced chemiluminescence immunoassay (CIA) Anti-HCV assay are approved by the U.S. Food

and Drug Administration (FDA) for clinical use, The specificity of current EIAs for anti-HCV is greater than 99%.[62] False positive results are more likely to occur when testing is performed among populations where the prevalence of hepatitis C is low. False negative results may occur in the setting of severe immunosuppression such as infection with HIV, solid organ transplant recipients, hypo- or aggammaglobulinemia or in patients on hemodialysis. [63, 64]

The recombinant immunoblot assay (RIBA) is also FDA approved. This assay was originally developed as a more specific, supplemental assay to confirm the results of EIA testing. [65] Given the widespread availability of nucleic acid testing, the role for RIBA testing in HCV diagnosis and management becomes limited.

Molecular Assays: The detection of HCV RNA in the serum by reverse transcriptase PCR (RT-PCR) is nowadays considered the gold standard for the diagnosis of HCV infection and for assessing the antiviral response to therapy.

Historically, qualitative assays have been more sensitive than quantitative assays. With the recent availability of real time polymerase chain reaction (PCR)- based assays and transcription-mediated amplification (TMA) assays, with sensitivities of 10-50 IU/mL, there is no longer need for qualitative assays.[66] A highly sensitive assay with this lower limit of detection is considered appropriate for monitoring during therapy. All currently available assays have excellent specificity, in the range of 98% to 99%. In 1997, the World Health Organization established the first International standard for HCV RNA nucleic acid technology, and

the IU rather than viral copies is now the preferred unit to report test results. [67]

Genotyping Assays: Genotyping is useful in epidemiological studies and in clinical management for predicting the likelihood of response and determining the optimal duration of therapy. Several commercial assays are available to determine HCV genotypes using direct sequence analysis of the 5´ non-coding region, Incorrect typing among the major genotypes is rare (less than 3%) and mixed genotypes occur but are uncommon. Occasionally less than 5% of tested samples cannot be genotyped, this usually results from low viral levels, issues with the PCR amplification step of the assay, or extreme nucleotide variability within the HCV genome. [68]

Diagnosis of Acute and Chronic HCV Infection and Interpretation of Assays

The differentiation of acute from chronic HCV infection depends on the clinical presentation and whether or not there was a prior history of ALT elevation and its duration because the serology of HCV infection does not follow the classical pattern of IgM response observed in other viral infections because it may be absent, late or persistent after HCV infection and does not correlate with the histologic activity. [69] After acute exposure, HCV RNA can be identified as early as 2 weeks following exposure whereas anti-HCV is generally not detectable before 8-12 weeks. [70]

Children should not be tested for anti-HCV before 12 months of age as anti-HCV from the mother may last until this age.

Diagnosis relies on presence of HCV RNA in baby blood after the second month of life.[71]

Diagnosis of liver fibrosis

The assessment of fibrosis in liver diseases provides much information not only for the diagnosis and prognosis of disease, but also for the therapeutic decision and for the monitoring and evaluation of treatment.

Methods to assess liver fibrosis include:

1. Invasive methods using liver biopsy

2. Noninvasive methods

 a. Fibrotic markers

 i. Direct serological markers

 ii. Indirect serological markers

 b. Imaging methods

 i. Ultrasonography (US)

 ii. Computed tomography (CT)

 iii. Magnetic resonance imaging (MRI)

3. Fibroscan

Liver Biopsy

Despite the development of potential diagnostic tests for fibrosis assessment, needle biopsy of the liver remains the gold standard that provides much useful information.[72] Liver biopsy remains the best way to assess the severity of chronic hepatitis C, to determine the prognosis and to evaluate the indication of therapy. The risk of developing cirrhosis depends on the stage (degree of fibrosis) and

the grade (degree of inflammation and necrosis) observed on the initial liver biopsy. Liver biopsy could be taken percutaneously, transvenously or directly during abdominal surgery.[72] Unfortunately, liver biopsy has some limitations and complications, including sample errors which is present in about 20% of liver biopsies, it may miss advanced fibrosis in 30% of patients, [73, 74] pain which is the most frequent risk of percutaneous liver biopsy,[75] bleeding which is encountered in one per 1000 liver biopsies, infection, trauma to internal organs and death .[76]

There are several methods to read a liver biopsy. The most common scoring methods are known as the Knodell, Ishak and Metavir Scores. It is important to remember that the size of the piece of liver taken at biopsy can affect the report. The knowledge of the doctor reading the biopsy is also important.

The Knodell score or histologic activity index (HAI) (table 1)

The Knodell score or HAI (histologic activity index) is a complex process composed of four individually assigned numbers that make up a single score. The first component (perioportal and/or bridging necrosis) is scored 0-10. The next two components (intralobular degeneration and portal inflammation) are scored 0-4. The combination of these three markers indicates the amount of inflammation in the liver. The fourth component indicates the amount of scarring in the liver and is scored from 0 to 4.[77]

Table (1): HAI for Numerical Scoring of Liver Biopsy Specimens[77]

I. Periportal ± bridging necrosis	S.	II. Intralobular degeneration and focal necrosis	S.	III. Portal inflammation	S.	IV. Fibrosis	S.
A. None	0	A. None	0	A. None	0	A. No fibrosis	0
B. Mild piecemeal necrosis	1	B. Mild (acidophilic bodies, ballooning degeneration and/or scattered foci of hepatocellular necrosis in < 1/3 of lobules or nodules)	1	B. Mild (sprinkling of inflammatory cells in <1/3 of portal tracts)	1	B. Fibrous portal expansion	1
C. Moderate piecemeal necrosis (involves less than 50 percent of the circumference of most portal tracts)	3	C. Moderate (involvement of 1/3 to 2/3 of lobules or nodules)	3	C. Moderate (increased inflammatory cells in 1/3 to 2/3 of portal tracts)	3	C. Bridging fiborisis (portal-portal or portal-central linkage)	3
D. Marked piecemeal necrosis (involves more than 50 percent of the percent of the	4	D. Marked (involvement of >2/3 of lobules or nodules)	4	D. Marked (dense packing of inflammatory cells in >2/3 of	4	D. Cirrhosis	4

			portal tracts)		
circumference of most portal tracts)					
E. Moderate piecemeal necrosis plus bridging necrosis	5				
F. Marked piecemeal necrosis plus bridging necrosis	6				
G. Multilobular necrosis	10				

The most frequently cited criticism of the Knodell HAI is that it is the sum of necroinflammatory and fibrosis scores and, therefore, does not distinguish ongoing hepatitis from parenchymal remodeling with fibrosis. Another criticism was that the categories had scores ranging from 0-10 for I, and 0-4 for II to IV but several numbers were omitted. In category I, there are 7 subgroups (A-G) but the scores 2, 7, 8 and 9 are missing. Similarly categories II to IV have 4 subgroups (A-D) with the score 2 missing in all of them. [78]

Ishak scoring system (Modified HAI)

The Ishak scoring system is a modification of Knodell (HAI) scoring system. It is reported to be more accurate and sensitive. The Ishak system assesses fibrosis in seven categories, ranging from normal to cirrhosis (table 2). Ishak scoring system also assesses grading as shown in table 3. [79]

Table (2): Liver fibrosis scoring according to Ishak classification [79]

Change	Score
No fibrosis	0
Fibrous expansion of some portal areas, with or without short fibrous septa	1
Fibrous expansion of most portal areas, with or without short fibrous septa	2
Fibrous expansion of most portal areas with occasional portal to portal (P-P) bridging	3
Fibrous expansion of portal areas with marked bridging [portal to portal (P-P) as well as portal to central (P-C).	4
Marked bridging (P-P and/or P-C) with occasional nodules	5

(incomplete cirrhosis)	
Cirrhosis, probable or definite	6

Table (3): Grading component of Ishak scoring system [79]

Periportal or Periseptal Interface Hepatitis (piecemeal necrosis) (A)	S.	Confluent Necrosis (B)	S.	Focal (spotty) Lytic Necrosis, Apoptosis, and Focal Inflammation* (C)	S.	Portal Inflammation (D)	S.
Absent	0	Absent	0	Absent	0	None	0
Mild (focal, few portal areas)	1	Focal confluent necrosis	1	One focus or less per 10x objective	1	Mild, some or all portal areas	1
Mild/moderate (focal, most portal areas)	2	Zone 3 necrosis in some areas	2	Two to four foci per 10x objective	2	Moderate, some or all portal areas	2
Moderate (continuous around <50% of tracts or septa)	3	Zone 3 necrosis in most areas	3	Five to ten foci per 10x objective	3	Moderate/marked, all portal areas	3
Severe (continuous around >50% of tracts or septa)	4	Zone 3 necrosis + occasional portal-central (P-C) bridging	4	More than ten foci per 10x objective	4	Marked, all portal areas	4

Zone 3 necrosis + multiple P-C bridging	5	
Panacinar or multiacinar necrosis	6	

Metavir Scoring system

The METAVIR system included both piecemeal necrosis and lobular necrosis in the definition of activity, but with different values. The rationale for overweighting the piecemeal necrosis item by comparison with lobular necrosis is that piecemeal necrosis is the major discriminating factor used to grade activity, as shown by stepwise discriminate analysis.

- The first criterion (piecemeal necrosis) scores are defined as follows: 0, absent; 1, focal alteration of the periportal plate in some portal tracts; 2, diffuse alteration of the periportal plate in some portal tracts or focal lesion around all portal tracts; 3, diffuse alteration of the periportal plate in all portal tracts.

- The second criterion (focal lobular necrosis) scores are defined as follows: 0, less than one necroinflammatory foci per lobule; 1, at least one necroinflammatory focus per lobule; 2, several necroinflammatory foci per lobule or confluent or bridging necrosis. The overall activity scores are defined as follows: 0, No activity; 1, mild; 2, moderate; 3, severe. [80]

The fibrosis scores are defined as follows: F0, no fibrosis; F1, portal fibrosis without septa; F2, portal fibrosis with rare septa; F3 numerous septa without cirrhosis; F4, cirrhosis. [81]

The METAVIR system has the advantage of simplicity, reproducibility and application to a large number of biopsies. This score is composed of a two-letter and two-number coding system: A= histological activity and F= fibrosis. [80] (Table 4, 5)

Table (4): The METAVIR System Algorithm for Evaluation of Histological Activity[80]

Piecemeal Necrosis +	Lobular Necrosis =	
Histological Activity score		
0 (none)	0 (none or mild)	0 (none)
0	1 (moderate)	1 (mild)
0	2 (severe)	2 (moderate)
1 (mild)	0, 1	1
1	2	2
2 (moderate)	0, 1	2
2	2	3 (severe)
3 (severe)	0, 1, 2	3

Table (5): The METAVIR System: Fibrosis Scoring [82]

Description	Score
No fibrosis	0
Stellate enlargement of portal tract but without septa formation	1
Enlargement of portal tract with rare septa formation	2
Numerous septa formation	3
Cirrhosis	4

Non Invasive Methods of Diagnosis of Liver Fibrosis

Fibrosis Markers:

In recent years, interest in identifying and describing liver fibrosis by using non invasive surrogate markers has been on the rise. Serum markers of liver fibrosis offer an attractive, cost effective alternative to liver biopsy for both patients and clinicians. In addition to being substantially less invasive, there are practically no complications, little or no sampling errors and small observer related variability. Moreover, measurements may be performed repeatedly, thus, allowing for a dynamic monitoring of fibrosis. [83]

The ideal marker for liver fibrosis should have the following characteristics: [84]

• Be highly sensitive and specific to identify different stages of fibrosis

• Be readily available, safe, inexpensive and reproducible

• Be applicable to the monitoring of disease progression or regression as apart of natural history of liver disease or treatment regimens.

Although no single ideal marker exists, several markers have been identified as possible useful indicators of fibrosis when used in conjunction with each other.

Biomarkers of fibrosis are commonly divided into direct and indirect markers. Direct markers are fragments of the liver matrix components produced by hepatic stellate cells (HSC) during the process of ECM remodeling. Indirect markers include molecules released into the blood due to liver inflammation, molecules synthesized /regulated or excreted by the liver, and markers of processes commonly disrupted due to liver function impairment. Direct and indirect markers may be used alone or more commonly - in combination with each other, to produce composite scores. The calculation of such scores can be relatively simple or can be based on complicated formulas. [85]

Direct serological markers of liver fibrosis

These markers are directly involved in fibrogenesis and fibrolysis. They include markers of matrix metabolism as well as cytokines. Serum levels of direct markers reflect simultaneously both processes as well as the total mass of ECM undergoing remodeling. [86] The assessment of direct markers could be useful for staging liver disease and for assessing the effect of treatment and predicting disease progression.

Serum markers of ECM synthesis: Liver fibrosis is associated with major alterations in both quantity and composition of ECM. In advanced stages, the liver contains approximately 6 times more ECM components than normal, including collagen type I, III, and

IV, fibronectin, undulin, elastin, laminin, hyaluronan, and proteoglycans. [87] Therefore, some parameters related to elevated ECM synthesis could be used as markers of liver fibrosis.

Collagens are synthesized by HSCs as precursor molecules with large pro-peptide extensions at both the N- and C-terminal ends. [88] The mature pro-peptide are cleaved from pro-collagen by N- and C-terminal proteinases, and the mature collagen is then integrated into the ECM. Both the pro-collagen and the pro-peptide reflect the synthesis of ECM. The N-terminal pro-peptide of collagen type III (PIIINP) is the most widely studied marker of liver fibrosis. [89] It is useful to detect cirrhosis with a sensitivity of about 94% and specificity of about 81%, which can be increased up to 93% if combined with additional serum markers. [90] In chronic hepatitis C (CHC) patients, PIIINP levels have not been shown to correlate with the degree of fibrosis but do correlate with scores for necrosis. [91] PIIINP levels are known to be elevated in acute and chronic active hepatitis and correlate with aminotransferase levels. However PIIINP is not specific for liver fibrosis as its levels are also elevated in lung fibrosis, chronic pancreatitis and rheumatoid arthritis (RA). [92, 93] Therefore PIIINP is more likely a marker of inflammation than of fibrosis.

Type IV collagen is regarded as a putative marker of basement membrane formation and sinusoids capillarization, which are important pathological processes in fibrosis disease. The serum levels of type IV collagen can be used for predicting the state of liver fibrosis. [94] P4NP 7S, the N-terminal pro-peptide of type IV collagen 7S domain, is significantly elevated in rats with liver fibrosis. [95] The N-terminal pro-peptide of collagen type I (PINP) has been shown to be associated with the development of liver

fibrosis. Thus, PINP may be useful in studying the pathogenesis of liver fibrosis. However, caution should be applied when interpreting PINP levels in other disease states such as inflammation. [96]

Hyaluronan (HA) is a glycosaminoglycan synthesized by HSCs and it is a component of the ECM. High levels of HA in serum may reflect increased synthesis of ECM by HSCs, and it appears to be the best individual test that reflects ECM concentration. [97]

Chondrex (YKL-40) is a 39-kilodalton glycoprotein that is involved in remodeling of the ECM. It is claimed that the serum level of YKL-40 is closely related to the degree of liver fibrosis. YKL-40 has been tested in HCV-patients showing a sensitivity and specificity of around 80%. [98]

Serum markers of fibrolytic processes: Products of matrix degradation result from the activity of a group of enzymes, matrix metalloproteinases (MMPs). MMPs are synthesized intracellularly and secreted as pro-enzymes. They are activated by a proteolytic cleavage by membrane type matrix metaloproteinase 1 (MT1-MMP) or plasmin and inhibited by binding to specific tissue inhibitors of metalloproteinase (TIMPs).

In the early stage of fibrosis, MMPs can degrade normal basal membranes and this may contribute to the pathogenesis of liver fibrosis. The two most relevant MMPs are gelatinase A (MMP-2) and gelatinase B (MMP-9). MMP-2 is secreted by activated HSCs, and MMP-9 is mainly secreted by activated Kupffer cells. In the progression of liver fibrosis, MMP-2 is also involved in the degradation of fibrotic matrix. Both MMP-2 and MMP-9 are

correlated with fibrosis. [99] The study conducted by Boeker and co-workers showed that TIMP-1 and MMP-2 levels are accurate in detecting cirrhosis in patients with HCV (sensitivity of TIMP-1 levels, 100%; specificity, 56%–75%; AUC for MMP-2 levels, 0.97). However they are not capable of differentiating between mild and moderate fibrosis, therefore their clinical utility has been demonstrated only in advanced stages of liver fibrosis. [100]

Serum markers of fibrogenesis-related cytokines: Unregulated cytokine synthesis and release contribute to the initiation, progression and maintenance of fibrosis. [101] Some cytokines thought to mediate hepatic fibrogenesis have been studied as potential markers of fibrosis.

Transforming Growth Factor β (TGF-β) is the major stimulus for HSCs to synthesize ECM. TGF-β concentrations in plasma are elevated in, and correlate with the severity of liver disease and were suggested as non-invasive biomarkers of fibrosis. TGF-β has been shown to correlate well with the presence of liver fibrosis in patients with alcoholic liver disease and HCV. [102]

Platelet derived growth factor (PDGF) is a potent fibrogenic growth factor known to synergize with TGF-β. [103] PDGF, mainly produced by Kupffer cells, is the predominant mitogen inducing migration and proliferation of mesenchymal cells including HSCs to the site of injury.[104] Serum levels of PDGF have shown high potential as markers for fibrosis progression.[105]

Connective tissue growth factor (CTGF) is another important fibrogenic factor which is synthesized by both HSCs and hepatocytes and is strongly dependent on TGF-β presence.[106] CTGF is a general mediator of fibre-fibre, fibre-matrix, as well as matrix-matrix interaction. It is proposed as a fibrogenic 'master switch' in the epithelial-to-mesenchymal transition which plays a key role in the increase of ECM-producing fibroblasts during liver fibrosis.[107] A recent preliminary study reported not only a

significant elevation in circulating CTGF in patients with fibrosis, but also a correlation with fibrogenesis. This study showed that CTGF levels decreased in fully developed, end-stage cirrhosis, in which the process of fibrogenesis is almost terminated. Thus, CTGF has been suggested as a valuable biomarker of active fibrogenesis. Serum CTGF is also suitable for determining hepatic fibrosis and it is a powerful marker in patients with chronic HCV infection.[108]

Indirect serological markers of liver fibrosis

AST/ALT

Aspartate aminotransferase (AST) and Alanine aminotransferase (ALT) are hepatic enzymes elevated in the blood before the clinical signs and symptoms of liver diseases appear. The AST/ALT ratio has been found useful in evaluation of chronic viral hepatitis.[109] The ratio of AST to ALT tends to increase with advancing stages of fibrosis from approximately 0.8 in healthy subjects. The greatest value of this ratio is that it is suitable for the non-invasive diagnosis of cirrhosis, where a ratio of more than 1 suggests the presence of cirrhosis.[110]

APRI

The AST-to-platelet ratio index (APRI) is calculated as (AST/upper limit of normal range)/platelet count (10^9/L) × 100. This index was first put forward by Wai and co-workers,[111] used to identify chronic hepatitis C (CHC) patients with significant fibrosis and cirrhosis with a high degree of accuracy. Several studies suggest that application of this index may decrease the need for staging liver biopsy specimens among CHC patients,[112] but a recent large meta-analysis suggested that APRI can identify hepatitis C-related fibrosis with a moderate degree of accuracy.[113]

Combination of serum markers

Fibrotest

The fibrotest is a composite of five serum biochemical markers (alpha-2-macroglobulin, apolipoprotein A1, haptoglobin, γ-glutamyl transpeptidase, and bilirubin) associated with hepatic fibrosis developed by Posynard and co-workers.[114] It generates a score that is correlated with the degree of liver damage in people with a variety of liver diseases. Due to the variability of components of assays and analyzers, fibrotest assays can only be performed in validated laboratories.[115] The test has been validated by multiple groups in several liver diseases, including chronic hepatitis. Although preliminary results are encouraging, frequently cited limitations include the assay cost, failed external validation, difficulty in differentiating intermediate fibrosis stages, and the inability to exclude other conditions such as steatosis.[116]

FibroSURE

HCV FibroSURE is a noninvasive blood test that combines the quantitative results of six serum biochemical markers, α2-macroglobulin, haptoglobin, apolipoprotein A1, bilirubin, γ-glutamyl transpeptidase (GGT), and ALT with a patient's age and gender in a patented artificial intelligence algorithm. HCV FibroSURE has been shown to lead to a reliable quantitative assessment of fibrogenic and inflammatory activity in the liver of HCV patients.[117] It provides an accurate measure of bridging fibrosis and/or moderate necroinflammatory activity with predictive values between 0.70 and 0.80 when compared with liver biopsy. [118]

Imaging Techniques

Ultrasonography, CT scan, or MRI have traditionally been used to explore the liver. These methods are able to detect changes in the

liver parenchyma when there is significant fibrosis (bridging fibrosis and mainly cirrhosis) and signs of portal hypertension (enlarged spleen, collateral venous circulation, enlarged portal vein). However, these methods are not useful for identifying patients with less advanced stages of fibrosis. Recently, optical analysis of CT images of the liver (Fibro-CT) has been used to assess fibrosis in patients with chronic hepatitis C virus (HCV) infection.[119] The use of fibro-CT, however, is time-consuming and more expensive than the noninvasive serum markers currently in use.

Fibroscan

Transient elastography (TE) is probably the most widely used noninvasive method in Europe for assessing the degree of liver fibrosis. The concept is simple: a vibration of mild amplitude and low frequency is transmitted to the tissue, which induces an elastic shear wave that propagates within the liver. Pulse-echo ultrasonic acquisitions follow the shear wave and measure its speed. The velocity of wave propagation relates directly to tissue stiffness (the harder the tissue is, the faster the shear propagates), which is measured in kilopascals. The advantages of this method are: (1) it is rapid, noninvasive, and reproducible; (2) it acquires information from a much larger portion of the tissue compared with liver biopsy, and therefore, the risk of sampling error is significantly lower; (3) it can be used in different liver diseases. TE has some limitations, it has proven unsuccessful in individuals with narrow intercostal spaces and morbid obesity, and increased liver stiffness is not always a surrogate of fibrosis (the presence of significant necroinflammation or extrahepatic cholestasis may increase liver stiffness values in the absence of fibrosis). Despite the forementioned limitations, several studies have already evaluated

the accuracy of TE in identifying patients with significant fibrosis or cirrhosis.[120] The diagnostic accuracy is sufficiently good in identifying significant fibrosis (particularly if the underlying disease is taken into account) and is excellent in identifying liver cirrhosis. [121]

Treatment

Treatment of chronic HCV infection has 2 goals. The first is to achieve sustained eradication of HCV (ie, sustained virologic response [SVR]), which is defined as the persistent absence of HCV RNA in serum 6 months or more after completing antiviral treatment. The second goal is to prevent progression to cirrhosis, hepatocellular carcinoma (HCC), and decompensated liver disease requiring liver transplantation. Because of the slow evolution of chronic HCV infection over several decades, it has been difficult to demonstrate that therapy prevents complications of liver disease Accordingly, treatment responses are defined by a surrogate virological parameter rather than a clinical endpoint. Short-term outcomes can be measured biochemically (normalization of serum ALT levels), virologically (absence of HCV RNA from serum by a sensitive PCR based assay), and histologically (> 2 point improvement in necroinflammatory score with no worsening in fibrosis score). [122]

Several types of virological responses may occur, labeled according to their timing relative to treatment (table 6). [70]

Table (6): Virological Responses During Therapy and Definitions (70)

Virological Response	Definition	Clinical utility
Rapid virological response (RVR)	HCV RNA negative at treatment week 4 by a sensitive PCR based quantitative assay	May allow shortening of course for genotypes 2&3 and possibly genotype with low viral load
Early virological response (EVR)	Equal or more than 2 log reduction in HCV RNA level compared to baseline HCV RNA level (partial EVR) or HCV RNA negative at treatment week 12 (complete EVR)	Predicts lack of SVR
End-of-treatment response (ETR)	HCV RNA negative by a sensitive test at the end of 24 or 48 weeks of treatment	
Sustained virological response (SVR)	HCV RNA negative 24 weeks after cessation of treatment	Best predictor of a long-term response to treatment

Breakthrough Relapse	-Reappearance of HCV RNA in serum while still on therapy -Reappearance of HCV RNA in serum after therapy is discontinued	
Non responder	Failure to clear HCV RNA from serum after 48 weeks of therapy	
Null responder	Failure to decrease HCV RNA by < 2 logs after 24 week of therapy	
Partial responder	Two log decrease in HCV RNA but still HCV RNA positive at week 24	

In 2014, the Infectious Diseases Society of America and the American Associations for the Study of Liver Diseases, in collaboration with the International Antiviral Society-USA, released a new section to their guidelines to cover information on when to begin therapy and in which patients with chronic hepatitis C virus (HCV) infection**.** The recommendations include the following: [123]

- Patients with advanced fibrosis, those with compensated cirrhosis, liver transplant recipients, and those with severe extraheptic manifestations are to be given the highest priority for treatment

- Treatment decisions should balance the anticipated reduction in transmission versus the likelihood of reinfection in patients whose risk of HCV transmission is high and in whom HCV treatment may result in a reduction in transmission (e.g., men who have high-risk sex with men, active injection drug users, incarcerated persons, and those on hemodialysis).

Antiviral therapy for chronic hepatitis C should be determined on a case-by-case basis. However, treatment is widely recommended for patients with elevated serum alanine aminotransferase (ALT) levels who meet the following criteria:- [124]

- Age greater than 18 years

- Positive HCV antibody and serum HCV RNA test results

- Compensated liver disease (eg, no hepatic encephalopathy or ascites)

- Acceptable hematologic and biochemical indices (hemoglobin at least 13 g/dL for men and 12 g/dL for women; neutrophil count >1500/mm^3, serum creatinine < 1.5 mg/dL)

- Willingness to be treated and to adhere to treatment requirements

- A further criterion is liver biopsy findings are consistent with a diagnosis of chronic hepatitis. However, a pretreatment liver biopsy is not mandatory. It may be helpful in certain situations, such as in patients with normal transaminase levels, particularly those with a history of alcohol dependence, in whom little correlation may exist between liver enzyme levels and histologic findings.

- No contraindications for treatment.

The treatment of hepatitis C has evolved over the years. Initial studies used interferon (IFN) monotherapy. Subsequently, combination of ribavirin and IFN or of IFN to which polyethylene glycol (PEG) molecules have been added (ie, PEG-IFN) were used.

Protease inhibitors have emerged as a third feature of combination therapy. The first protease inhibitor indicated for use in HCV infection, boceprevir, was approved by the FDA in May 2011 followed by approval of telaprevir. However these two protease inhibitors are not recommended due to the more recent availability of more effective options. A third protease inhibitor, simeprevir, was approved in November 2013 and is recommended as a part of combination therapy for chronic hepatitis C infection.

Most recently, HCV NS5B polymerase inhibitor sofosbuvir was shown to result in suppression of HCV replication and has emerged as an important component of currently recommended regimens. In November 2014, the FDA approved an all oral regimen of simeprevir plus sofosbuvir for treatment-naïve or treatment-experienced patients.The treatment duration is 12 weeks for patients without cirrhosis and 24 weeks for those with cirrhosis. [125]

Interferons and Pegylated Interferons

Interferons (IFNs) are proteins made and released by host cells in response to the presence of pathogens. IFNs belong to the large class of proteins known as cytokines, molecules used for communication between cells to trigger the protective defenses of the immune system that help to eradicate pathogens.

Based on the type of receptor, human interferons have been classified into three major types:-

- Interferon type I: All type I IFNs bind to a specific cell surface receptor complex known as the IFN-α/β receptor (IFNAR) that consists of IFNAR1 and IFNAR2 chains.[126] The type I interferons present in humans are IFN-α, IFN-β, IFN-ε, IFN-κ and IFN-ω.[127]

- Interferon type II: Binds to IFNGR that consists of IFNGR1 and IFNGR2 chains. In humans this is IFN-γ.

- Interferon type III: which was discovered more recently than type I and type II IFNs, recent information demonstrate the importance of Type III IFNs in some types of virus infections. [128]

The most 2 frequently used recombinant IFN preparations in clinical trials have been IFN alfa-2b and IFN alfa-2a, which differ from each other by only a single amino acid residue. Interferon-alfa is given subcutaneously at doses of 3 million units 3 times a week for 24 months. The addition of propylene glycol (PEG) molecules to IFN has led to the development of long-lasting IFNs that have better sustained absorption, a slower rate of clearance, and a longer half-life than unmodified IFN, which permits more convenient once-weekly dosing rather than administering two or three times per week. The FDA has approved PEG-IFNs for the

treatment of chronic hepatitis C in January 2001 for PEGylated interferon-alpha -2b followed by PEGylated interferon-alpha-2a in October 2002. PEGylated interferon is effective in treatment of hepatitis C; with response rate of 75% in hepatitis C genotypes 2 or 3 and 50% in genotype 1. [129]

Interferons and Ribavirin

A major advance in the treatment of chronic hepatitis C was the addition of the oral nucleoside analogue ribavirin to the IFN regimen. As with IFN alfa, the addition of ribavirin to PEG-IFN heralded a new era in the treatment of chronic HCV. The benefits of combination therapy were documented in 3 landmark trials: Manns et al from 2001,[130] Fried et al from 2002,[122] and Hadziyannis et al from 2004.[131]

Manns et al reported a significantly higher SVR rate in patients given higher-dose PEG-IFN alfa-2b plus ribavirin than in patients given lower-dose PEG-IFN alfa-2b plus ribavirin or given IFN alfa-2b plus ribavirin. Adverse-effect profiles in the 3 treatment groups were similar. Secondary analysis identified body weight and HCV RNA viral load less than 1 million copies per milliliter as important predictors of SVR. [130]

Fried et al found that patients who received PEG-IFN alfa-2a plus ribavirin had a significantly higher SVR rate than patients who received IFN alfa-2b plus ribavirin (56% vs 44%) or PEG-IFN alfa-2a alone (56% vs 29%). The SVR rates for patients with HCV genotype 1 were 46%, 36%, and 21%, respectively, for the 3 regimens.[122]

Hadziyannis et al reported that in patients infected with HCV genotype 1, 48 weeks of treatment was statistically superior to 24

weeks, and standard-dose ribavirin was statistically superior to low-dose ribavirin.[131]

In a study of ribavirin in combination with either PEG-IFN alfa-2b or PEG-IFN alfa-2a for the treatment of chronic HCV infection, Ascione et al reported a higher SVR rate with PEG-IFN alfa-2a than with PEG-IFN alfa-2b (68% versus 54.4%).[132]The duration of therapy depends on the genotype and level of viremia. In patients with genotype 2 or 3, the duration is 24 weeks, while patients with genotype 1 need 48 weeks of treatment.[133]

Hepatitis C virus genotype 4 (HCV-4) which is the most common variant of the hepatitis C virus (HCV) in the Middle East and Africa, particularly Egypt has been considered difficult to be treated because initial clinical trials using conventional inteferon (IFN) monotherapy produced a limited success. These studies found that conventional IFN_ monotherapy resulted in a sustained virologic response (SVR) in only 5%-25% of treated patients.[134]The subsequent inclusion of ribavirin in treatment regimens improved SVR, from rates of 8% reported for patients receiving IFN alone to 42% when used in combination with ribavirin (1000- 1200 mg/day).[135]Pegylated interferon markedly improved the rates of SVR in chronic HCV-4, investigations reported SVR rates of 50%-79% in patients receiving PEG-IFN alfa 2b plus ribavirin (800-1200 mg/ day) for 48 weeks and suggested that HCV-4 is easier to treat than previously believed. [136]

Adverse effects are common with IFN therapy. Approximately 75% of patients experience one or more of the following adverse effects: hematological complications (neutropenia, thrombocytopenia), neuropsychiatric complications (memory and

concentration disturbances, headaches, depression, irritability, psychosis, seizures ataxia), flu-like symptoms, immune disorders (autoimmune thyroid disease, autoimmune hepatitis, systemic lubus erythromatosis), gastrointestinal complications (nausea, vomiting, weight loss), dermatologic complications (psoriasis, erythema multiform), pulmonary complications (interstitial fibrosis), cardiac arrhythmias, dilated cardiomyopathy, retinopathy, hearing loss and acute renal failure. [137]

Relative contraindications to INF therapy include: Severe depression, decompensated cirrhosis (Child class B or C), autoimmune hepatitis, hyperthyroidism, coronary artery disease, renal transplant, pregnancy, seizures, retinopathy, leucopenia, thrombocytopenia and anaemia. [134]

Ribavirin can induce hemolytic anemia and can cause problems to patients with preexisting anemia, bone marrow suppression, or renal failure. In these patients, if combination therapy cannot be avoided, attempts should be made to correct the anemia. Hemolytic anemia caused by ribavirin can be life-threatening for patients with ischemic heart disease or cerebral vascular disease. Ribavirin should be contraindicated in end stage renal disease, heamoglobinopathies, ischemic heart disease, pregnancy, and uncontrolled hypertension.

Protease Inhibitors

This is a new class of direct-acting antiviral agents (DAAs) has revolutionized the treatment of HCV genotype 1 infection. These drugs target specific enzymes involved in viral replication. The addition of these new protease inhibitors to pegylated interferon and ribavirin is becoming the new standard of care for the treatment of chronic HCV infection. Boceprevir and telaprevir are HCV NS3/4A protease inhibitors and were approved by the

US Food and Drug Administration (FDA) in May 2011. However, treatment with either of these agents is no longer recommended because of higher efficacy with regimens that contain sofosbuvir or simeprevir which is a third HCV NS3/4A protease inhibitor approved in November 2013. Each of these is indicated for treatment of chronic HCV genotype 1 infection in combination with PEG-IFN alfa and ribavirin in adults with compensated liver disease, including cirrhosis, who are previously untreated or who have failed previous interferon and ribavirin therapy. In November 2014, the FDA approved an all-oral regimen of simeprevir plus sofosbuvir for treatment-naïve or treatment-experienced patients; the duration of treatment is 12 weeks for patients without cirrhosis and 24 weeks for those with cirrhosis.[125]

Polymerase inhibitors

HCV NS5B polymerase plays an essential role in HCV replication. Sofosbuvir is a NS5B polymerase inhibitor that results in suppression of HCV replication and life cycle. Sofosbuvir was approved by the FDA in December 2013 for treatment of CHC infection genotypes 1, 2, 3, and 4 as part of a combination antiviral regimen, including those with hepatocellular carcinoma meeting Milan criteria (awaiting liver transplantation) to prevent HCV recurrence and those with HCV/HIV-1 co-infection.

Sofosbuvir treatment regimens and duration are dependent on both viral genotype and patient population. Patients with genotype 1 or 4 are treated with sofosbuvir plus peginterferon alfa and ribavirin for 12 weeks. Those with genotype 2 or 3 are part of an all oral drug regimen consisting of sofosbuvir plus ribavirin for 12 or 24 weeks respectively. Based on the proportion of patients who had a sustained virologic response 12 weeks after completing therapy (SVR12), patients who achieve SVR12 are considered cured of HCV. Trial participants taking sofosbuvir-based therapy achieved SVR12 rates of 50-90%.[138, 139]

Harvoni is a combination oral product containing ledipasvir, an NS5A protease inhibitor, and sofosbuvir that was approved by the

FDA in October 2014 for HCV genotype 1. It is administered once daily and does not need to be administered with interferon or ribavirin. Studies showed a high, sustained virologic response (94-99%) in all treatment groups (i.e., treatment-naïve or experienced, and with or without cirrhosis).[140-142]

Current treatment recommendations for chronic hepatitis C infection (American Association for the Study of Liver Diseases [AASLD] and the Infectious Disease Society of America [ISDA]) [123]

Treatment-naïve patients:

- Genotype 1: sofosbuvir (SOF) 400mg daily with PEG IFN/RBV (weight-based, 1000 to 1200mg daily) for 12 weeks or simeprevir (SMV) 150mg with PEG IFN/RBV for 12 weeks followed by another 12 weeks of PEG IFN/RBV; an alternative for IFN-ineligible patients is combination SOF, SMV, and RBV for 12 weeks.

- Genotype 2: SOF 400mg daily with RBV (weight-based, 1000 to 1200mg daily) for 12 weeks.

- Genotype 3: SOF 400mg daily with RBV (weight-based, 1000 to 1200mg daily) for 24 weeks (an alternative is SOF with PEG IFN/RBV for 12 weeks).

- Genotypes 4: Daily sofosbuvir (400 mg) and weight-based RBV (1000 mg [< 75 kg] to 1200 mg [≥75 kg]) plus weekly PEG IFN for 12 weeks is recommended for IFN-eligible. Alternatively, Daily simeprevir (150 mg) for 12 weeks and weight-based RBV (1000 mg [< 75 kg] to 1200 mg [≥75 kg]) plus weekly PEG IFN for 24 to 48 weeks.

- For IFN-ineligible patients, daily sofosbuvir (400 mg) plus weight-based RBV (1000 mg [< 75 kg] to 1200 mg [≥75 kg]) for 24 weeks is recommended.

- Genotype 5 to 6: Daily sofosbuvir (400 mg) and weight-based RBV (1000 mg [< 75 kg] to 1200 mg [≥75 kg]) plus weekly PEG IFN for 12 weeks is recommended for IFN-eligible persons. Alternatively, Daily weight-based RBV (1000 mg [< 75 kg] to

1200 mg [≥75 kg]) plus weekly PEG IFN for 48 weeks is an acceptable regimen.

Patients with prior PEG IFN/RBV treatment failure:

- Genotype 1: SOF 400mg daily, SMV 150mg daily, and RBV (1000 to 1200mg daily) for 12 weeks (an alternative is SOF for 12 weeks with PEG IFN/RBV for 12 to 24 weeks or SMV 150mg for 12 weeks with PEG IFN/RBV for 48 weeks).

- Genotype 2: SOF 400mg daily with RBV (weight-based, 1000 to 1200mg daily) for 12 weeks (an alternative is SOF 400mg daily with PEG IFN/RBV [weight-based, 1000 to 1200mg daily] for 12 weeks).

- Genotype 3: SOF 400mg daily with RBV (weight-based, 1000 to 1200mg daily) for 24 weeks, an alternative is SOF 400mg daily with PEG IFN/RBV [weight-based, 1000 to 1200mg daily] for 12 weeks.

- Genotypes 4 to 6: SOF 400mg daily with PEG IFN/RBV (weight-based, 1000 to 1200 mg daily) for 12 weeks.

Treatment of hepatitis C in HIV-HCV Coinfection

The older therapy of HIV coinfected patients with PEG IFN/RBV and first-generation protease inhibitors has been replaced by SOF-based regimens. Current recommendations are very similar in HIV-HCV coinfected patients to those for HCV monoinfected patients. For treatment-naïve patients and prior relapsers: in genotype 1, first-line therapy is SOF with PEG IFN/RBV for 12 weeks (or SOF with RBV for 24 weeks in IFN-ineligible or unwilling patients); in genotype 2, SOF with RBV for 12 weeks (consider 16 weeks in cirrhotics); in genotype 3, SOF with RBV for 24 weeks; in genotype 4, SOF with PEG IFN/RBV for 12 weeks.

For treatment-experienced patients (partial and null responders): in genotype 1, prior PEG IFN/RBV nonresponders SOF, SMV (only with ART drugs with which SMV does not have drug-drug interactions), and RBV; in genotypes 2, 3, and 4, the same regimens as in treatment-naïve patients. [143]

Human Immunodeficiency Virus (HIV)

Human immunodeficiency virus (HIV) is a lentivirus (a member of the retrovirus family) that causes acquired immunodeficiency syndrome (AIDS) a condition in humans in which progressive failure of the immune system allows life threatening opportunistic infections.[144]

There are two recognized types of HIV, HIV1 and HIV2 which are similar to each other strikingly, however they differ with regard to the molecular weight of their proteins and their accessory genes.[145] Worldwide the predominant virus is HIV1, and generally when people refer to HIV without specializing the type of the virus they are referring to HIV1.[146]

HIV structure

HIV virus consists of a bar–shaped electron dense core containing the viral genome which is two short strands of ribonucleic acid (RNA) with 9200 nucleotide bases long– along with the enzymes reverse transcriptase, protease, ribonuclease, and integrase, all encased in an outer lipid envelope derived from a host cell. This envelope has 72 surface projections, or spikes, containing an antigen, gp120 that aids in the binding of the virus to the target cells with CD4 receptors. A second glycoprotein, gp 41, binds gp 120 to the lipid envelope.[147]
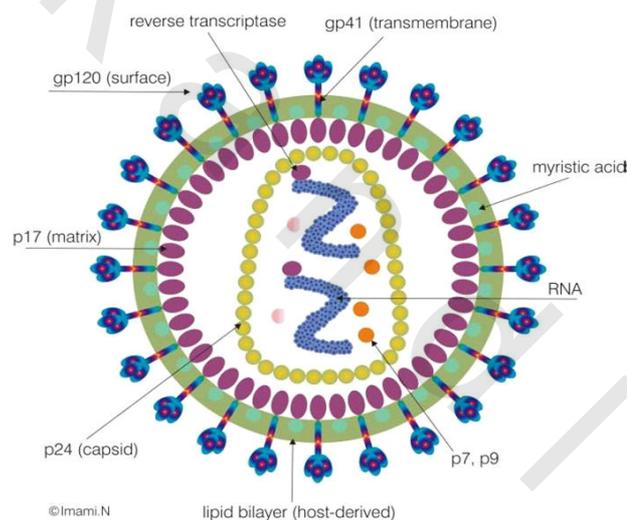
Figure (7): Structure of HIV viron particle. [147]

The genome of HIV contains three major genes, gag means group-antigen, pol represents polymerase, env is for envelope. These genes code for the major structural and functional components of HIV. The structural components encoded by env gene include the envelope glycoproteins: outer envelope glycoprotein (gp120) and transmembrane glycoprotein (gp41) derived from glycoprotein precursor gp160. Components encoded by the gag gene include core nucleocapsidproteins p55, p40, p24 (capsid, or core antigen), p17 (matrix), p7 (nucleocapsid). The

important proteins encoded by pol gene are the enzyme proteins p66 and p51 (reverse transcriptase), p11 (protease), and p32 (integrase).[148]Accessory genes carried by HIV include tat, rev, vif, vpr, and vpu (for HIV-1) or vpx (for HIV-2). The accessory genes play multiple roles in HIV pathogenesis, regulate the process of RNA transcription and modify the infected cell to make it more suitable for producing HIV virions.[148]
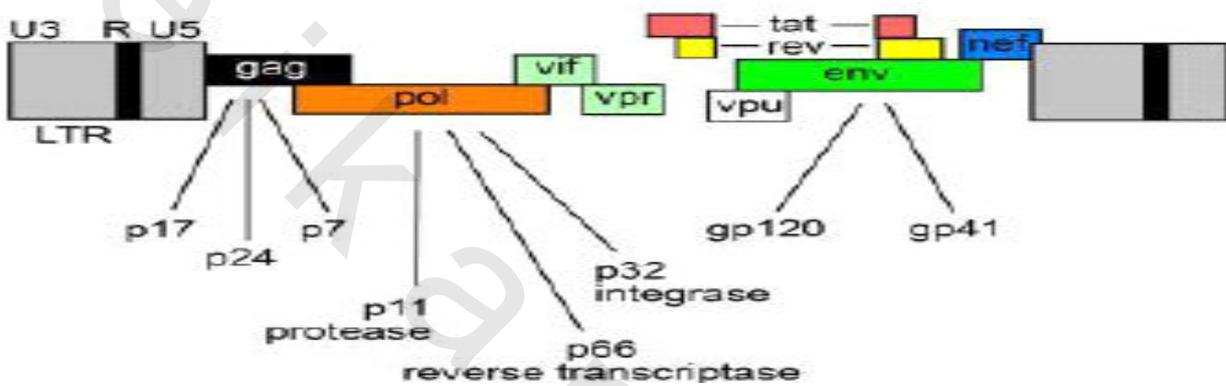

Figure (8): The viral genome for HIV-1[148]

HIV Replication cycle [149]

- **Binding and Fusion:** HIV begins its life cycle when it binds to a CD4 receptor on the surface of a CD4+ T- lymphocyte. The virus then fuses with the host cell. After fusion, the virus releases RNA, its genetic material, into the host cell.

- **Reverse Transcription**: A process in which the single-stranded HIV RNA is converted to double-stranded HIV DNA by reverse transcriptase enzyme.

- **Integration:** The newly formed HIV DNA enters the host cell's nucleus, where an HIV enzyme integrase integrates the HIV DNA within the host cell's own DNA. The integrated HIV DNA is called provirus. The provirus may remain inactive for several years, producing few or no new copies of HIV.

- **Replication:** The new DNA, formed by the integeration codes for the production of messenger RNA that initiates the synthesis of HIV proteins.

- **Budding:** The newly assembled virus buds from the host cell. During budding, the new virus steals part of the cell's outer envelope. This envelope, which acts as a covering, is studded with protein/sugar combinations called HIV glycoproteins.

- **Maturation:** The new virus has all the components necessary to infect other CD4 cells but cannot do so until it undergoes a maturation process. During this process, the HIV protease enzyme cuts the long HIV proteins of the virus into smaller functional units that then reassemble to form a mature virus. The virus is now ready to infect other cells.

Pathogenesis and Immune response

CD4 is a 58 kDa monomeric glycoprotein that can be detected on the cell surface of about 60% of T lymphocytes, on T cell precursors within the bone marrow and thymus, on monocytes, macrophages, eosinophils, dendritic cells and microglial cells of the central nervous system. [150]

In addition, besides CD4, galactosyl ceramide (Gal C) can serve as a major binding site for HIV in the bowel, vagina and brain. Moreover, HIV complexed with antibodies can gain entry into T cells and macrophages and other cells through Fc and complement receptors. [151]

After the virus enters the body there is a period of rapid viral replication, leading to an abundance of virus in the peripheral blood. This response is accompanied by a marked drop in the numbers of circulating CD4+ T cells. This acute viremia is associated with the activation of CD8+ T cells, which kill HIV-infected cells, and subsequently with antibody production, or seroconversion. The CD8+ T cell response is thought to be important in controlling virus levels, which peak and then decline, as the CD4+ T cell counts rebound. A good CD8+ T cell response has been linked to slower disease progression and a better prognosis, though it does not eliminate the virus. [152]

A major effect of acute HIV infection is the wide spread destruction of memory CD4 cells in the gastrointestinal tract. These T cells in the mucosal –associated lymphoid tissue (MALT) can induce the overall immune responses of the host. In addition, an interruption in the integrity of the gastrointestinal epithelium permitting bacteria to enter the blood can cause inflammation and activation that encourage further CD4 cell loss. [153]

Epidemiology

HIV/AIDS is a global pandemic, according to the latest estimates from UNAIDS, there were around 35.0 million people living with HIV in 2013. Of these, approximately 17.2 million are males, 16.8 million are females and 3.4 million are less than 15 years old. There were about 2.1 million new infections in 2013 and about 1.5 million people died of AIDS in the same year. Sub-Saharan Africa is the region most affected. In 2013, an estimated 24.9 million people living with HIV in sub-Saharan Africa, women accounts 58% of them.[154]

Egypt has low HIV prevalence among the general population (below 0.02 %) with a concentrated epidemic among men having sex with men (MSM) and injecting drug users (IDUs) as detected by the latest biological/behavioral surveillance survey completed in 2010 (BBSS 2010-second round BBSS). This is the second wave of biological/behavioral surveillance survey following the first round BBSS conducted in 2006. [155]

Data regarding detected cases indicate that the population group most affected is adults in the age group 25 – 40 years (60% of all detected cases).The male to female ratio of all detected cases is currently 4:1 (National AIDS Program data 2010).[155]

Since 1990 and to date, there has been a regular increase in HIV detected cases. Over the past ten years, the number of detected cases has increased from 1,040 HIV and AIDS cases (from 2001-2005) to 1,663 cases (from 2006-2009). The latest estimated number was 6,500 in 2013.[154]It is important to highlight that the prevalence of HIV in the country appears to remain below 0.02%.

However, a population based survey was never conducted in Egypt.[156]

Transmission

Although HIV is present in many body fluids, its presence doesn't necessarily constitute a risk for infection because it may be present only in very low concentrations. HIV has been found at infectious levels in five principal body fluids; blood, semen, cervicovaginal secretions, rectal secretions, and breast milk.[157]

The most important transmission route for HIV is sexual contact. Estimates of the probability of transmission per sex act vary according to male to female versus female to male transmission, stage of HIV infection in the positive partner, the effects of socio demographic characteristics, behavioral characteristics, as well as sexually transmitted infection cofactors.[158]

Regarding type of sexual act, anal sex is reported to be of a higher risk than vaginal sex, which in turn is a higher risk act than oral sex. There is also an increased risk associated with receptive intercourse (both vaginal and anal) compared with insertive intercourse. [159]

The risk estimates for the sexual transmission of HIV, per sex act, range widely, from 0.5% to 3.38% for receptive anal intercourse; 0.06% to 0.16% for insertive anal intercourse; 0.08% to 0.19% for receptive vaginal intercourse (i.e., male-to-female) ; and approximately 0.05% to 0.1% for insertive vaginal intercourse (i.e., female-to-male). The risk of transmission through oral sex was estimated to be approximately 0.04 % per contact.[159]

Injecting drug use (IDU) is a significant HIV transmission factor in many regions of the world. Current estimates put the number of injection drug users at 16 million. Of these, 10 million are HCV-seropositive and 3 million are HIV-seropositive.[160]

A significant mode of HIV acquisition is through mother to child transmission during pregnancy, childbirth, or breastfeeding.[161] In the absence of any preventive intervention, mother to child transmission ranges from about 15% to 45%. As with other modes of transmission, the most important risk factor is viral load. The probability of transmission of HIV through breastfeeding is in the range of 9% to 16%.Co-factors that are associated with risk of transmission from breastfeeding include duration and pattern of breastfeeding, maternal breast health, and high plasma or breast milk viral load. [162]

Beyond viral load, there are several factors associated with an increased risk of vertical transmission. Concurrent STIs and co-infection with either hepatitis C or active tuberculosis increase the risk of vertical transmission. As well as obstetric events, including prolonged rupture of membranes and intrapartum hemorrhage have been found to increase the risk of perinatal transmission of HIV.[159]

Blood transfusion with HIV-infected blood carries a risk of 95% HIV transmission.[163] The transmission of HIV via blood and blood products has been largely reduced on a global scale Since 1985 as all blood donations are tested for HIV via antibody tests.[163]

The main cause of infection in occupational settings is exposure to HIV infected blood via a percutaneous injury (i.e. from

needles, instruments, sharps which break the skin).The average risk for HIV transmission after such exposure to infected blood is as low as 3 per 1, 000 injuries (0.3%), while Research suggests that the risk of HIV infection after mucous membrane exposure is less than 1 in 1000 (0.09%), What renders the risk for HIV transmission after a percutaneous exposure less than that estimated for either HCV (approximately 1.8%) or HBV (approximately 6 to 30%).[164]

Clinical stages of HIV (Figure 9)

- **Stage 1: Primary HIV Infection**: Acute (primary) HIV infection is the phase of HIV disease immediately after infection during which the initial burst of viremia in newly infected patients occurs; anti-HIV antibodies are undetectable at this time while HIV RNA or p24 antigen are present. Early HIV infection phase is used to refer to either acute or recent HIV infection which lasts about 6 months after exposure, during which an estimated 40% to 90% of patients will experience symptoms of acute retroviral syndrome, characterized by fever, lymphadenopathy, pharyngitis, skin rash, myalgias/ arthralgias.[165]

Stage 2: Clinically Asymptomatic Stage: This stage occurs after - seroconversion associated with rising of CD4 cell count and falling of HIV viral load and appearance of anti- HIV antibodies. This asymptomatic phase may last up to 10 years or more. During this period there is a gradual fall in CD4 cells and a slow decline in the immune function. This stage could be characterized by Persistent generalized lymphadenopathy.[166]

Stage 3: Symptomatic HIV Infection: As the CD4 cell count - drops to below the lower limit of normal (500 cells/ml of blood), the person may experience some symptoms, such as weight loss (<10% of presumed or measured body weight) or severe forms of

infections, oral candidiasis, recurrent respiratory tract infections, herpes zoster (which may be bilateral), seborrhoeic dermatitis and Onychomycosis (fungal nail infections).[166]

Stage 4: Progression from HIV to AIDS: Serious complications with the occurrence of opportunistic infections or HIV related malignancies will occur as the CD4 cell count continue to decline overtime till it reaches below 200 cells/ml. Monitoring of HIV infection therefore involves routine checks of CD4 cell counts and VL. The amount of circulating virus reflects the activity of the virus and will generally correlate with the degree of damage that is occurring to the immune system. A VL of greater than 100 000 copies/mL of blood is considered high. In this stage diagnosis can be made also on the basis of clinical signs. [166, 167]
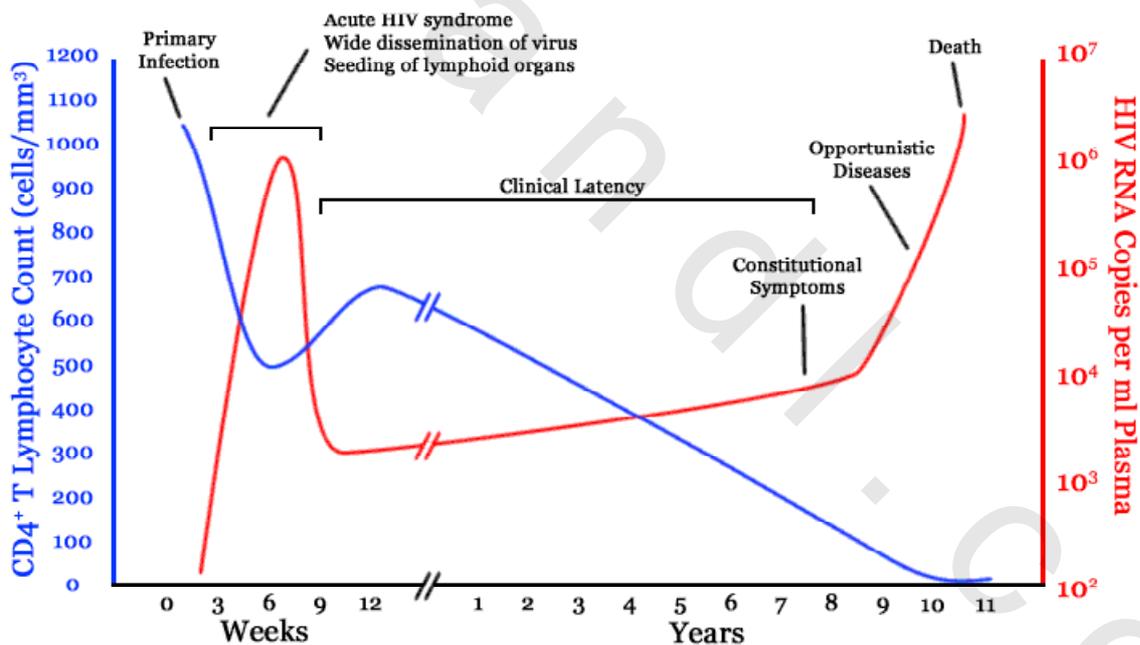
Figure (9): Progression of HIV-1 infection[166]

Diagnosis of HIV

HIV tests are able to detect antibodies, antigens or HIV-1 RNA in serum, plasma, whole saliva, dried blood spots, and urine. They are important for the primary diagnosis and monitoring of treatment. The diagnostic window for antibody tests is 22 days, for antigens 16 days and for molecular assays 7-14 days. [168]

The standard screening test for HIV is the ELISA, with a sensitivity over 99.5%. While the ELISA is extremely sensitive, it is not ideal with regard to specificity. False-positive ELISA tests may be associated with antibodies to class II antigens, autoantibodies (e. g. systemic lupus erythematosis), antibodies directed to other infectious agents, and the rheumatoid factor. A confirmatory test is thus needed by a positive or indeterminate ELISA test result. The most commonly used confirmatory test is the western blot. The western blot is considered to be positive if at least two bands (to p24, gp41, and gp120/160) are visible. [5]

RT-PCR can detect a target sequence of a highly conserved region of the HIV-1 genome. The lower limit of detection is 40copies/mL. Babies born to HIV positive mothers are usually tested using a PCR test because they retain their mother's antibodies for several months, making an antibody test inaccurate. Blood supplies in most developed countries are screened for HIV using PCR tests.[169]

The close relationship between clinical manifestations of HIV and CD4+ T cell count has made the determination of the CD4+ T cell level as a routine method for the evaluation of HIV infected individuals and gives information about the prognosis.[5]

Treatment of HIV

The cornerstone of medical management of HIV infection is antiretroviral therapy. Suppression of the HIV replication is an important component in prolonging as well as improving the quality of life of HIV infected patients. The agents have to comply with certain requirements: good activity against the virus, minimal toxicity (because they have to be applied lifelong), oral intake, good penetration into the central nervous system, and reduction of the viral load. Presently, six main drug groups are available: nucleoside reverse transcriptase inhibitors (NRTIs), non nucleoside reverse transcriptase inhibitors (NNRTIs), protease inhibitors (PIs), integrase inhibitors (IIs), fusion inhibitors (FIs) and chemokine receptor antagonists (CRAs). [170]

Agents of the NRTIs include zidovudine, stavudine, didanosine, abacavir, tenofovir, lamivudine and emtricitabine. NRTIs are nucleoside analogues that act as DNA chain terminators because of their inability to form a 3`-5` phosphodiester linkage with another nucleoside. The substances are applied orally. Major side effects include leucopenia, periphery neuropathy and pancreatitis.[171]

The group of the NNRTIs consists of nevirapine, efavirenz, and delavirdine. They are active inhibitors of reverse transcriptase. The effect of NNRTIs is very selective to HIV-1 and a monotherapy leads to a rapid emergence of drug-resistant-mutants. The main side effects are a maculopapular rash and an elevation of the hepatic enzymes.[171]

The PIs, saquinavir, ritonavir, indinavir, nelfinavir, amprenavir, fosamprenavir, lopinavir, ritonavir, tipranavir and atazanavir, are selective to the HIV-1 protease. They inhibit the split of the inactive precursor-proteins and consequently the maturation of the virus. The side effects are an elevation of the hepatic enzymes, redistribution of the body fat and metabolic disturbances. The PI-

monotherapy leads to a rapid development of drug-resistant isolates**.**[171]

Additionally, a fusion-inhibitor has recently been introduced. The drug is called Enfuvirtide (injection powder) binds to the viral fusion-proteins to inhibit the necessary conformation-change**,** integrase inhibitor Raltegravir oral tablets, Chemokine receptor antagonist (CCR5 antagonist) maraviroc oral tablets can also be used. [171]

The standard HIV-therapy is called highly active antiretroviral therapy (HAART). The basic combination consists of two NRTIs and one PI. Different combinations can be given and therapy has to be arranged individually with regard on side effects and compliance. HAART has proven to be the best combination to reduce the viral load, increase CD4+ T cell count, and prolong life. If an HIV infection becomes resistant to HAART, this may be overcomed by increasing the dosage or by switching to another antiviral drug. Even though HAART is a good therapy-combination, it is not possible to cure the disease and HIV may also be transmitted during therapy. This is another reason why it is very important to give the patients detailed education about their illness and how to cope with it. [172]

Certain factors can indicate the necessity to change HAART, drug regimen modifications can be based on the following: [173]

- Virologic failure shown by increases in HIV RNA viral load.

- Immunologic failure shown by decrease in CD4 cells count. Immunological response was defined as a rise in CD4Tcells >50 after 6 months or at least>100.

- Medication Toxicity

- Drug adherence or inability to comply with regimen.

- Drug resistance

HCV / HIV co infection

Several studies found that HIV/HCV-coinfected patients had a three fold greater risk of progression to cirrhosis or decompensated liver disease than HCV-monoinfected patients. This accelerated rate is magnified in HIV/HCV-coinfected patients with low CD4 T lymphocyte (CD4) cell counts.[174] Whether HCV infection accelerates HIV progression, as measured by AIDS-related opportunistic infections (OIs) or death is unclear.[175] ART may slow the progression of liver disease by preserving or restoring immune function and by reducing HIV-related immune activation and inflammation.[176] Therefore, ART should be initiated in most HIV/HCV-coinfected patients, regardless of CD4 count, although in HIV treatment-naive patients with CD4 counts >500 cells/mm^3, some clinicians may choose to defer ART until HCV treatment is completed to avoid drug-drug interactions. For patients with lower CD4 counts (e.g., <200 cells/mm^3), ART should be initiated expeditiously and HCV therapy may be delayed until the patient is stable on HIV treatment.[177]

Hyaluronic acid (HA)

Hyaluronic acid is a high molecular weight, nonsulfated, linear chain glycosaminoglycan. This molecule is present in extracellular, pericellular and intracellular spaces. It is composed of a repetitive sequence of hexuronic and amino sugar with acetyl groups (Figure 10).[178]

Glucuronic Acid (1→3) N-Acetyl-D-Glucosamine
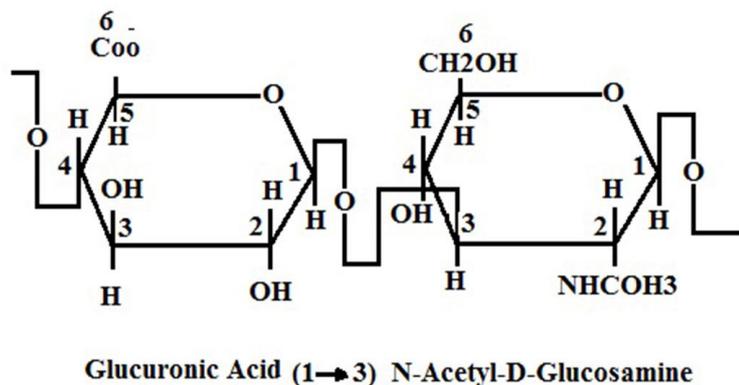
Figure (10): Structure of HA [179]

HA is synthesized by special enzymes that are located on the inner surfaces of plasma membranes in some tissue, such as the synovial lining cells or hepatic stellate cells. These enzymes are called hyaluronic acid synthases.[180] These enzymes are able to synthesize this molecule by adding the activated form of the sub-strates, i.e. UDP–glucuronic acid and UDP-N- acetylglucosamine, to the growing chain. The resulting molecules are passed through the plasma membrane and ultimately secreted into the extracellular space.[180] The liver is the major tissue for both removal and synthesis of the circulating form of this macromolecule. In the liver, HA is synthesized by HSC and finally degraded by sinusoidal endothelial cells. [181]

Clinical Significance of HA

High levels of serum HA have been detected in patients with liver diseases of different etiologies and particularly in those with cirrhosis.[182] Serum levels of HA have been shown to be related not only to the stage of fibrosis but also to the degree of necroinflammation.[183] It proved to be the best single biomarker of fibrosis with high sensitivities and specificities to discriminate cirrhosis from other fibrosis stages.[178]

Table (7): Summarizes the hyaluronic acid performance as a fibrosis biomarker in patients with different causes of liver disease

Aetiology	Diagnostic performance of HA
Hepatitis C virus	100% NPV (negative predicted value) for cirrhosis (cut off 50ng/ml) [178]
Hepatitis B virus	90.9 % sensitivity,98.1% specificity for extensive fibrosis (cut off 126.4 ng/ml)[183]
Alcoholic liver diseases	82.8% sensitivity,69% specificity for hepatic fibrosis (cut off 55.5 ng/ml)[184]
Non-Alcoholic Fatty liver disease	85% sensitivity,80% specificity for severe fibrosis (cut off 46.1 ng/ml) [185]
Haemochromatosis	100% sensitivity and specificity for cirrhosis (cut off 46.5 ng/ml) [186]