INTRODUCTION

In Egypt, an estimated 15% of the populations have chronic hepatitis C virus infection. Over 90% of chronically HCV infected patients have HCV genotype 4 infection **(Wantuck et al., 2014)**. Until recently, the standard of care for treatment of HCV genotype 4 has been pegylated interferon (Peg-IFNa) with ribavirin (RBV) for 24 to 48 weeks, depending on virological response **(Khattab et al., 2011).**

Despite advances in IFN-based therapy over the last 14 years, the ability to achieve a sustained virologic response (SVR) is limited to 43-70% in treatment naïve patients following old regimen **(Wantuck et al., 2014).**

The influence of HCV infection on the peripheral blood cell count has been well studied. A National Health and Nutrition Examination Survey of HCV-infected individuals in the United States showed that HCV antibody-positive subjects were more likely to have Anemia and low neutrophil counts than were HCV-negative individuals **(Dieterich and Spivak., 2003)**. Chronic HCV can also lead to thrombocytopenia with multiple mechanisms that has been proved to parallel the severity of the disease **(Mitchell et al., 2016)**.

It has been shown that HCV can replicate extrahepatically, specifically in the bone marrow which may contribute to the etiology of anemia, neutropenia and thrombocytopenia observed in HCV-infected patients (Dieterich and Spivak, 2003). Hypersplenism (secondary to portal hypertension and splenomegaly), immune mediated destruction of blood cells and impairment of production of important factors like thrombopoitin, are other possible causes of cytopenias caused by HCV **(Mousa., 2014).**

Not only the disease, but also therapy with Pegylated Interferon (PEGINF) or Ribavirin (RBV) results in hematologic abnormalities. Bone marrow suppression has always been the major complication of Interferon; Also Ribavirin-induced hemolytic anemia is a common cause of dose reduction or discontinuation of treatment **(Ong and Younossi., 2004)**.

An exciting new era in HCV therapy dawned in 2011 with the first two protease inhibitors, telaprevir and boceprevir approved to be utilized with PEGINF and RBV to treat chronic HCV genotype 1.The main limitation of these first-generation protease inhibitors was anemia as a side effect, which was more severe than observed with combined PEGINF and RBV regimen **(Shiffman., 2014)**.

In late 2013, another protease inhibitor simeprevir and the first polymerase inhibitor, sofosbuvir, were approved for HCV treatment. These two antiviral agents offer significant advantages compared with telaprevir and boceprevir when treating patients with HCV; the duration of therapy is shorter, the adverse effect profile is superior and the Sustained Virological Response (SVR) is higher. In addition, sofosbuvir is effective against all genotypes and when utilized with RBV represents the first interferon-free treatment for chronic HCV **(Lin and Chung., 2014)**.

During the past few years, several oral antiviral agents, which inhibit various HCV proteins, have been developed at a rapid pace. These include protease inhibitors, nucleotide and non-nucleotide polymerase inhibitors, NS5A inhibitors and cyclophilin inhibitors **(Shiffman., 2014)**.

There are limited data on the effect of direct-acting antiviral (DAA) therapies on blood indices for patients with HCV in routine medical practice. We aimed to evaluate real-world experience with DAA-based regimens.

AIM OF THE WORK

This study aims to screen patients treated with DAAs based, ribavirin free regimen to monitor the changes in blood indices during and after treatment to prove or disprove the direct effect of these new drugs on the hematological state.

Chapter I

HEPATITIS C VIRUS

HCV, a member of the Flaviviridae family, is a small, blood-borne enveloped virus with a 9.6 kb long ribonucleic acid (RNA) genome that serves as the blue print for 10 protein products: three structural (one core- and two envelope-associated proteins) and seven nonstructural proteins such as those involved in replication. Due to low fidelity during replication, the virus exists as 11 known genotypes (although the first six identified are the ones primarily addressed in reports and articles), and hundreds of subtypes and strains **(Thomson and Liang., 2000)**

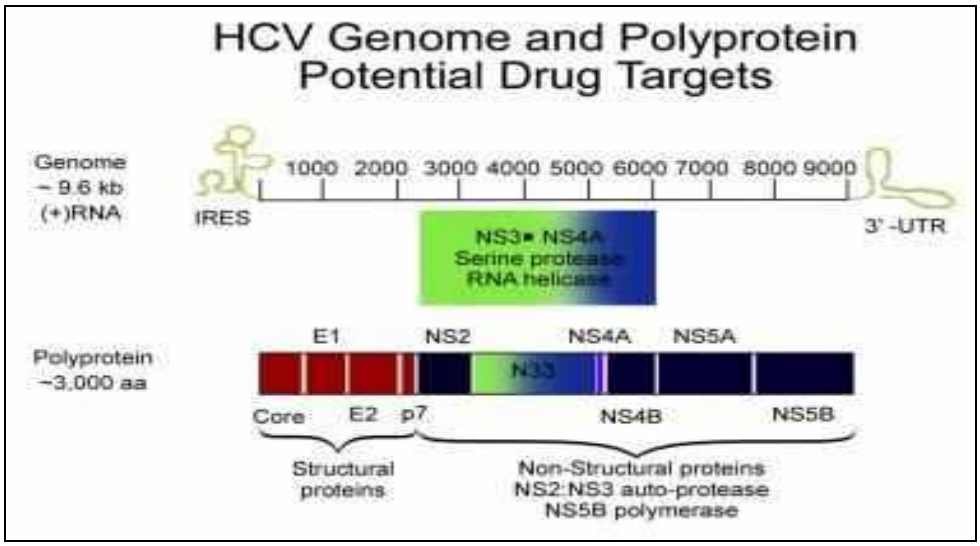

Fig. 1: HCV genome. Courtesy of Hepatitis Resource Network, 2016

Despite substantial progress in producing viral particles in cell culture and several biochemical and morphological studies, the structure of the HCV virion remains poorly characterized. This contrasts with the well-characterized flavivirus viral particles. A striking and unique feature of HCV biology is its association with lipoproteins, which exhibit an unusually low density **(Dubuisson and Cosset., 2014)**

HCV particles are 50–80 nm in diameter and contain the single-stranded RNA genome, core and the envelope glycoproteins, E1 and E2 **(Catanese et al., 2013)**.

Molecular differences between genotypes are relatively large, and they have a difference of at least 30% at the nucleotide level. The major HCV genotype worldwide is genotype 1, which accounts for 40-80% of all isolates. Genotype 1 also may be associated with more severe liver disease and a higher risk of hepatocellular carcinoma (HCC). Genotypes 1a and 1b are prevalent in the United States, whereas in other countries, genotype 1a is less frequent **(Zein.,2000)**

Genotype details are as follows:

- Genotype 1a occurs in 50-60% of patients in the United States
- Genotype 1b occurs in 15-20% of patients in the United States; this type is most prevalent in Europe, Turkey, and Japan
- Genotype 1c occurs in less than 1% of patients in the United States
- Genotypes 2a, 2b, and 2c occur in 10-15% of patients in the United States; these subtypes are widely distributed and are most responsive to medication
- Genotypes 3a and 3b occur in 4-6% of patients in the United States; these subtypes are most prevalent in India, Pakistan, Thailand, Australia, and Scotland
- Genotype 4 occurs in less than 5% of patients in the United States; it is most prevalent in the Middle East and Africa.
- Genotype 5 occurs in less than 5% of patients in the United States; it is most prevalent in South Africa
- Genotype 6 occurs in less than 5% of patients in the United States; it is most prevalent in Southeast Asia, particularly Hong Kong and Macao

Within a region, a specific genotype may also be associated with a specific mode of transmission, such as genotype 3 among persons in Scotland who abuse intravenous drugs**.** The most common HCV RNA genotype in Egypt is genotype 4, representing >85% of all HCV cases in Egypt **(Elgharably et al., 2017).**

Clinical course of HCV infection

Once infected, only 20 to 30% of individuals present with clinical symptoms in the acute phase. Once the virus enters the host, it travels to the liver where it begins replication. During the incubation period, the period between exposure to the virus and the onset of symptoms, HCV RNA can be detected in the blood 1 to 2 weeks after exposure. RNA levels continue to rise until the appearance of symptoms, which occurs between 2 to 12 weeks (with an average of 7 weeks) after exposure **(Chen and Morgan., 2006)**.

Currently, no vaccine is available for HCV. However, with the goal of developing a vaccine, ongoing research is looking at immune responses to the virus and the potential for acquiring immunity. A cellular immune response appears to be the primary path the body uses to combat HCV infection. A humoral response is evident by the presence of HCV antibody.

The effectiveness and role of antibodies is not fully understood, although neutralizing antibodies are thought to play a part in controlling the infection. The minority of infected individuals who spontaneously clear the virus in the acute phase demonstrate a relatively strong virus-specific T-cell response compared to those who progress to a chronic infection **(Law et al., 2013)**.

Most patients start to investigate HCV infection accidently when they notice elevation in the levels of serum alanine aminotransferase (ALT), a liver enzyme whose presence indicates hepatocyte necrosis, which start to become detectable around 2 to 8 weeks after exposure. At first, infected individuals experience nonspecific symptoms such as fatigue, anorexia, fever, nausea, and upper right quadrant discomfort. This period, known as the pre-icteric phase, lasts about 2 to 10 days. The icteric phase follows with the more characteristic symptoms of jaundice, lethargy, weakness, muscle fatigue and headaches, and upper right quadrant pain. During the icteric phase, usually 1 to 3 months after exposure, antibodies to HCV are detectable by immunoassay. Symptoms may worsen for 2 to 3 weeks after onset before subsiding. Mild symptoms may continue for several months. The disappearance of symptoms does not guarantee that the body has cleared the virus. In most

cases (55 to 90%), the virus persists and leads to a chronic infection, which is defined as the presence of HCV RNA in the blood more than 6 months after the acute infection phase **(Chen and Morgan., 2006)**.

According to the World Health Organization (WHO) (2012) HCV is most commonly transmitted through exposure to infected blood. This can occur through; contaminated blood transfusions, blood products and organ transplants, injections given with contaminated syringes and needle-stick injuries in health-care settings or through needle-sharing among drug-users. Sexual and perinatal transmission may also occur, although less frequently. Other modes of transmission such as social, cultural, and behavioral practices using percutaneous procedures (e.g. ear and body piercing, circumcision, tattooing) can occur if inadequately sterilized equipment is used **(Fikry et al., 2015)**.

According to the National Academy of Sciences (2010), the primary route of HCV transmission in developed world is intravenous drug use (IDU) while in developing world, the main methods for these widespread are due to unscreened blood transfusions and unsafe medical procedures **(Miller and Abu-Raddad., 2010)**.

Injection drug abuse, accounts for at least 60% of acute HCV infections in the United States. Healthcare exposures are important sources of transmission, including the receipt of blood products before 1992 (after which routine screening of blood supply was implemented), receipt of clotting factor concentrates before 1987, long-term hemodialysis, needle stick injuries among healthcare workers, and patient-to-patient transmission resulting from poor infection control practices. Other risk factors include having been born to an HCV-infected mother, having been incarcerated, and having received a tattoo in an unregulated setting. The importance of these risk factors might differ based on geographic location and population **(Fikry et al., 2015)**.

Although CDC and US Preventive Services Task Force hepatitis C testing guidelines do not specifically recommend testing immigrants from countries with a high prevalence (eg, Egypt or Pakistan) of HCV infection, such persons should be tested if they were born from 1945 through 1965 or if they have risk factors for infection **(Hosein and Wilson., 2013).**

CDC and the US Preventive Services Task Force both recommend a one-time HCV test in asymptomatic persons belonging to the 1945 to 1965 birth cohort and other persons

based on exposures, behaviors, and conditions that increase risk for HCV infection **(Witt et al., 2013)**.

All persons recommended for HCV testing should first be tested for anti-HCV using a Food and Drug Administration (FDA)-approved test including laboratory-based assays and a point-of-care assay. The latter is an indirect immunoassay with a sensitivity and specificity similar to those of FDA-approved laboratory-based anti-HCV assays **(Lee et al., 2011)**.

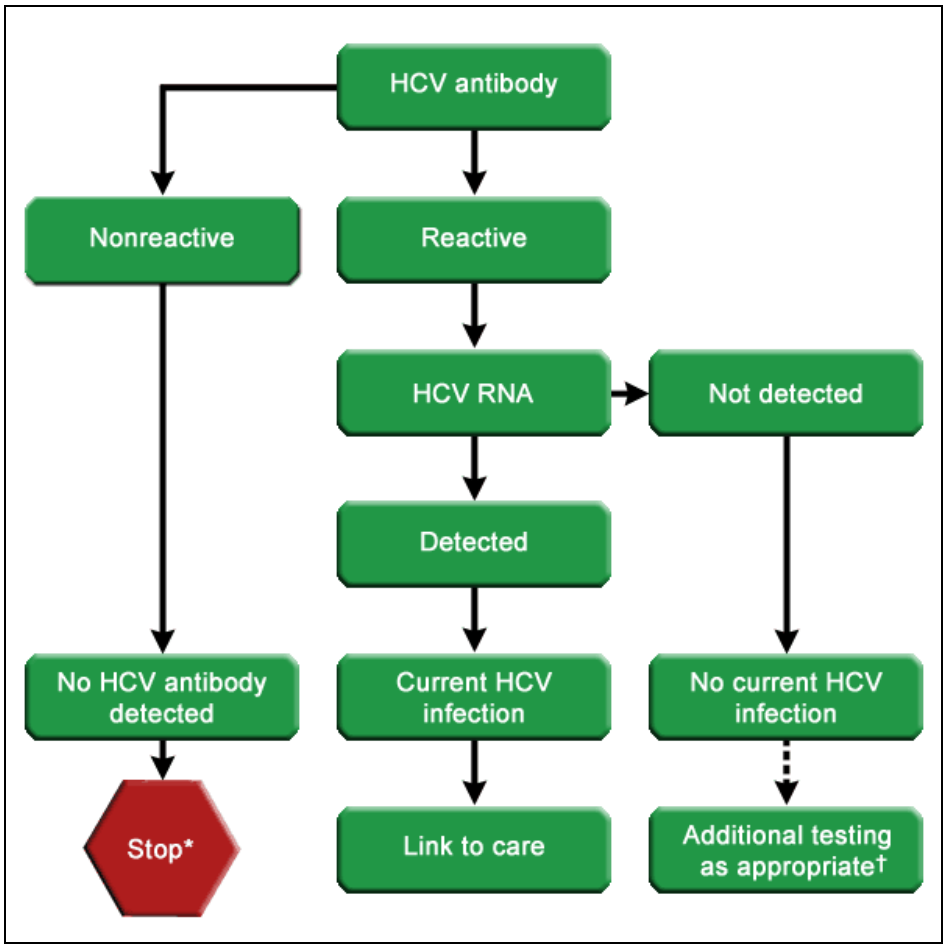

Fig.2: CDC Recommended Testing Sequence for Identifying Current HCV Infection **(CDC, 2013)**

Prior to the initiation of HCV therapy, quantitative HCV RNA testing may be used to determine the baseline level of viraemia (i.e., viral load) in order to define the duration of treatment for certain regimens. The degree of viral load decline after initiation of treatment is less predictive of sustained

virological response (SVR) in the era of direct-acting antiviral (DAA) therapy. Testing for HCV genotype helps to guide selection of the most appropriate treatment regimen **(Cavalcante and Lyra., 2015).**

The severity of liver disease associated with chronic HCV infection is a key factor in determining the initial and follow-up evaluation of patients. Although patients with more advanced disease may have a lower response to HCV therapy, they are also most likely to derive the greatest survival benefit **(Ghany et al., 2011).**

A liver biopsy can provide objective, semi-quantitative information regarding the amount and pattern of collagen or scar tissue in the liver that can assist with treatment and monitoring plans. The Metavir fibrosis score (F0-F4) and Ishak fibrosis score (0-6) are commonly used to score the amount of hepatic collagen. A liver biopsy can also help assess the severity of liver inflammation, or of hepatic steatosis, and help exclude competing causes of liver injury **(Kleiner., 2005)**. However, the procedure has a low but real risk of complications, and sampling artifact makes it's serial use in most patients less desirable **(Regev et al., 2002)**.

Noninvasive methods frequently used to estimate liver disease severity include a liver-directed physical exam (normal in most patients), routine blood tests (e.g. serum alanine aminotransferase (ALT), aspartate aminotransferase (AST), albumin, bilirubin, international normalized ratio levels, and complete blood cell counts with platelets), serum fibrosis marker panels, liver imaging (e.g. ultrasound, computed tomography scan) and transient elastography. Simple blood tests (eg, serum AST-to-platelet ratio index (APRI), Fibrosis-4 (FIB-4) **(Sterling et al., 2006)** and assessment of liver surface nodularity and spleen size by liver ultrasound or other cross-sectional imaging modalities can help determine if patients with HCV have occult portal hypertension, which is associated with a greater likelihood of developing future hepatic complications in untreated patients **(Chou and Wasson., 2013)**.

Successful HCV treatment results in SVR, which is tantamount to virologic cure and is defined as the continued absence of detectable HCV RNA at least 12 weeks after completion of therapy. SVR is a marker for cure of HCV infection and has been shown to be durable, in large prospective studies, in more than 99% of patients followed up for 5 years or more **(Manns et al., 2013)**. Patients in whom an SVR is achieved have HCV antibodies but no longer have