INTRODUCTION

Fetuin-A former name for the human α2 Heremans Schmid glycoprotein (AHSG), a 59 kDa glycoprotein, consisting of two cystatin-like domains and a smaller unrelated domain, is predominantly synthesized in liver. It is secreted into the blood stream and deposited as a noncollagenous protein in mineralized bones and teeth ***(Auberger et al., 1989).***

Fetuin-A occurs in high serum concentrations during foetal life, whereas its level declines following infection, inflamation and malignancy. Fetuin-A acts as an important circulating inhibitor of ectopic calcification, frequent complication of many degenerative diseases ***(Kalabay and Arnaud, 2002).***

Low serum level of Fetuin-A is associated with vascular and valvular calcification, atherosclerosis, malnutrition and higher cardiovascular mortality in chronic renal failure, liver cancer and liver cirrhosis patients on long-term dialysis ***(Kalabay and Arnaud, 2002).***

Since serum AHSG is produced exclusively by hepatocytes in adults and its concentration decreases during the acute phase reaction, study of its changes in patients with liver diseases as acute alcoholic hepatitis, acute drug-induced hepatitis, chronic autoimmune hepatitis, fatty liver, alcoholic and primary biliary cirrhosis, and hepatocellular cancer will be excellent indicator of liver function ***(Kalabay et al., 2005).***

Hepatitis C virus is one of the main causes of chronic liver disease worldwide. The long-term imapct of HCV infection is highly variable, from minimal changes to extensive fibrosis and cirrhosis with or without hepatocellular carcinoma (HCC) ***(European Association for the Study of the Liver, 2015).***

The asscoiation between a higher prevalence of Type 2 Diabetes Mellitus and HCV infection has been reported ***(Knobler et al., 2005).***

Type 2 diabetes represents a major global public health threat and, together with obesity, constitutes an important contributor to the predicted decline in life expectancy. The pathophysiology of type 2 diabetes is complex: In addition to impaired insulin secretion from cells, reduced insulin sensitivity was found to play a predominant role in the pathogenesis of the disease ***(Stumvoll et al., 2005).***

Fetuin-A is an endogenous inhibitor of the insulin-stimulated insulin receptor tyrosine kinase ***(Graham et al., 2006).***

Type 2 diabetes (T2D), has been recognized to modify the course of hepatitis C even at the stage of insulin resistance (IR), a condition that precedes the development of T2D. IR seems not only to accelerate the course of chronic hepatitis C, but also to influence the response to antiviral therapy ***(Negro and Alaei, 2009).***

Aim of the Work

This study will be conducted to find whether Fetuin-A secreted by the liver is affected by chronic liver disease caused by hepatitis C virus and whether the protein could be the link between hepatitis C and insulin resistance.

HEPATITIS C VIRUS (HCV)

Introduction:

Hepatitis C is an infection of the liver caused by HCV. The discovery of the HCV in 1989 was a major breakthrough. Before that point, it was clear that a major cause of acute hepatitis after a blood transfusion was neither related to hepatitis A nor to hepatitis B; hence, the early name for this disease, non-A non-B hepatitis. Although new infections resulting from blood transfusions are rare thanks to screening measures that began in 1990, the overall number of people facing death or serious liver disease from HCV is steadily rising because people often live decades with the virus before showing symptoms. HCV is a major cause of viral hepatitis. There are about 180 million people in the world who are chronically infected by this virus ***(Jay and Lefkowitch, 2009).***

Hepatitis C virus (HCV) is a globally prevalent pathogen and a leading cause of death and morbidity. The most recent estimates of disease burden show an increase in seroprevalence over the last 15 years to 2.8%, equating to >185 million infections worldwide. Persistent HCV infection is associated with the development of liver cirrhosis, hepatocellular cancer, liver failure, and death, and HCV is now the most common cause of death in HIV-positive patients on highly active antiretroviral therapy. While the incidence rate of HCV infection is apparently

decreasing in the developed world, deaths from liver disease secondary to HCV infection will continue to increase over the next 20 years ***(Messina et al., 2015).***

HCV genotype 1 is the most prevalent worldwide, comprising 83.4 million cases (46.2% of all HCV cases), approximately one-third of which are in East Asia. Genotype 3 is the next most prevalent globally (54.3 million, 30.1%); genotypes 2, 4, and 6 are responsible for a total 22.8% of all cases; genotype 5 comprises the remaining <1%. While genotypes 1 and 3 dominate in most countries irrespective of economic status, the largest proportions of genotypes 4 and 5 are in lower-income countries ***(Messina et al., 2015).***

It is difficult for the human immune system to eliminate the virus from the body, and infection with HCV usually becomes chronic. Long-term (chronic) HCV infection can lead to liver failure or liver cancer; prolonged inflammation may cause extensive scarring in the liver (cirrhosis). When the liver becomes cirrhotic, the liver fails to perform its normal functions (liver failure), and this leads to serious complications and even death. Cirrhotic livers also are more prone to become cancerous ***(Ge et al., 2009).***

(1) Structure:

HCV is a small (55-65 nm in size), enveloped, positive sense single strand RNA virus in the family Flaviviridae and genus hepacivirus. Although Hepatitis A virus, Hepatitis B

virus, and Hepatitis C virus have similar names (because they all cause liver inflammation), these are distinctly different viruses both genetically and clinically. The HCV particle consists of a core of genetic material (RNA), surrounded by a protective shell of protein, and further encased in a lipid (fatty) envelope of cellular origin. Two viral envelope glycoproteins, E1 and E2, are embedded in the lipid envelope ***(Marcelo et al., 2007).***

(2) Genotypes:

Because the virus mutates rapidly, changes in the envelope proteins may help it evade the immune system. There are at least six major genotypes and more than 50 subtypes of HCV. The different genotypes have different geographic distributions. Structural proteins made by the HCV include E1 and E2; nonstructural proteins (figure 1) include NS2, NS3, NS4, NS4A, NS4B, NS5, NS5A, and NS5B ***(Jay and Lefkowitch, 2009).***

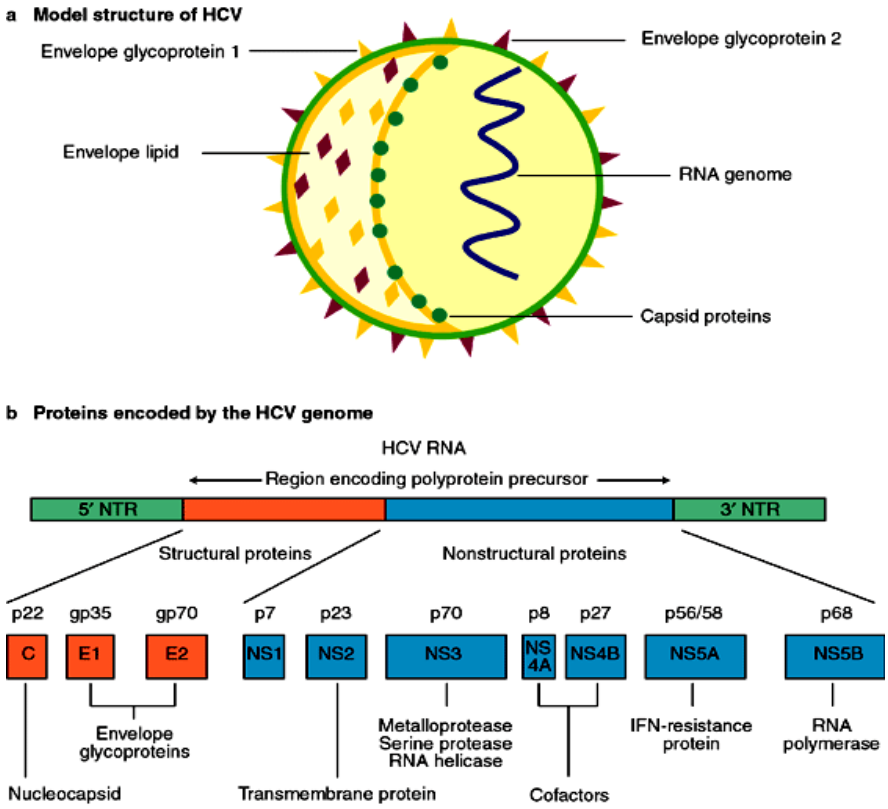

Figure (1): Simplified diagram of the structure and genome of the Hepatitis C virus particle ***(Dubuisson, 2007).***

(3) Replication:

Replication of HCV involves several steps (figure 2). The viruses need a certain environment to be able to replicate, and must therefore first move to such areas. HCV has a high rate of replication with approximately one trillion particles produced each day in an infected individual. Due to lack of proofreading by the HCV RNA polymerase, 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, the viruses reach these proper locations. Circulating HCV particles bind to receptors on the surfaces of hepatocytes and subsequently enter the cells ***(Ge et al., 2009).***

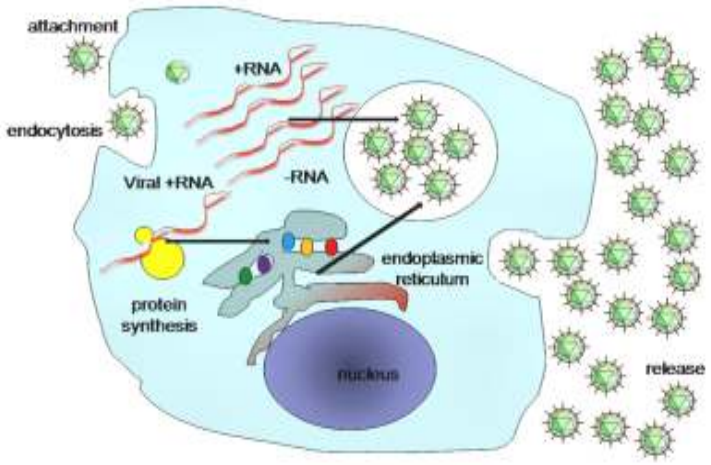

Figure (2): A simplified diagram of the HCV replication cycle ***(Ge et al., 2009).***

(4) Types of Hepatitis C Infection:

a) Acute Hepatitis C:

Acute hepatitis C is diagnosed in persons who have symptoms such as jaundice, fatigue, and nausea, together with marked increases in serum ALT (usually greater than 10-fold

elevation), and the presence of anti-HCV. Diagnosis of acute disease can be problematic because anti-HCV is not always present when the patient develops symptoms and sees the physician. In 30 to 40 percent of patients, anti-HCV is not detected until 2 to 8 weeks after onset of symptoms. In this situation, testing for HCV RNA is helpful, as this marker is present even before the onset of symptoms and lasts through the acute illness. Another approach to diagnosis of acute hepatitis C is to repeat the anti-HCV testing a month after onset of illness. Of course, a history of an acute exposure is also helpful in suggesting the diagnosis ***(Armstrong et al., 2006).***

b) Chronic Hepatitis C:

Chronic hepatitis C is diagnosed when anti-HCV is present and serum aminotransferase levels remain elevated for more than 6 months. Testing for HCV RNA by polymerase chain reaction (PCR) confirms the diagnosis and documents that viremia is present; almost all patients with chronic infection will have the viral genome detectable in serum by PCR. Diagnosis is problematic in patients who cannot produce anti-HCV because they are immunosuppressed or immunoincompetent. Thus, HCV RNA testing may be required for patients who have a solid-organ transplant, are on dialysis, are taking corticosteroids, or have agammaglobulinemia. Diagnosis is also difficult in patients with anti-HCV who have another form of liver disease that might be responsible for the liver injury, such as alcoholism, iron overload, or autoimmunity.

The anti-HCV may represent a false-positive reaction, previous HCV infection, or mild hepatitis C occurring on top of another liver condition. HCV RNA testing in these situations helps confirm that hepatitis C is contributing to the liver problem ***(Conjeevaram et al., 2006).***

(5) Pathogenesis:

Infection course and pathogenic mechanisms: The course of infection and the eventual clinical sequelae are very variable, the clinical sequelae being - surplus liver cell replacement / turnover: liver inflammation, liver fibrosis, liver cirrhosis, hepatocellular carcinoma (HCC) and extra hepatic manifestations: cryoglobulinopathy/ vasculitis; these events do often take an independent course. The individual course of infection is not predictable. Also the crucial events of infection must have complex self-tuning and interaction mechanisms, the crucial events being: viral replication, viral clearance. HCV is not essentially cytopathogenic; Immune reactions are thought to be essential for viral elimination and pathogenic events leading to the clinical sequelae. The essential immune reactions are **HCV-specific reactions**: cytotoxic T lymphocytes, T1 and T2 helper lymphocytes (cytokine release), and B lymphocytes/plasma cells producing anti-HCV. **Non-specific reactions**: (co-activated lymphocytes, macrophages, other Inflammatory cells) leading to a surplus production of cytokines ***(Jay and Lefkowitch, 2009).***

(6) Prevention of HCV infection:

At present, the only means of preventing new cases of hepatitis C are to screen the blood supply, encourage health professionals to take precautions when handling blood and body fluids, and inform people about high-risk behaviors. Programs to promote needle exchange offer some hope of decreasing the spread of hepatitis C among injection drug users. Furthermore, all drug users should receive instruction in safer injection techniques and simple interventions that can be life-saving. Vaccines and immunoglobulin products do not exist for hepatitis C, and development seems unlikely in the near future because these products would require antibodies to all the genotypes and variants of hepatitis C. Nevertheless, advances in immunology and innovative approaches to immunization make it likely that some form of vaccine for hepatitis C will eventually be developed ***(Alavian, 2006).***

(7) HCV infection and iron metabolism:

Iron and the liver: A greater understanding of iron metabolism with the discovery of new iron-related genes including the hepcidin gene which plays a critical role in regulating systemic iron homeostasis. Consequently, the distinction between (a) genetic iron-overload disorders including haemochromatosis related to mutations in the haemochromotosis ptn (HFE), haemojuvelin (HJV), transferrin receptor 2 (TfR2) and hepcidin genes and (b) non-haemochromatotic conditions related

to mutations in the ferroportin (FPN), ceruloplasmin, transferrin (Tf) and divalent metal transporter 1 (DMT1) genes, and (c) acquired iron-overload syndromes has become easier. However, major challenges still remain which include understanding of the regulation of hepcidin production, the identification of environmental and genetic modifiers of iron burden and organ damage in iron-overload syndromes, especially HFE haemochromatosis, indications regarding the new oral chelator, deferasirox, and the development of new therapeutic tools interacting with the regulation of iron metabolism ***(Deugnier et al., 2008).***

Fundamental cellular operations, including DNA synthesis and the generation of ATP, require iron. Viruses hijack cells in order to replicate, and efficient replication needs an iron-replete host. Some viruses selectively infect iron-acquiring cells by binding to TfR1 during cell entry. Other viruses alter the expression of proteins involved in iron homeostasis. HCV infection, iron overload is associated with poor prognosis and could be partly caused by the viruses themselves. Understanding how iron metabolism and viral infection interact might suggest new methods to control disease ***(Darke and prentice, 2008).***

(8) Hepatitis C in Egypt:

Egypt has the largest epidemic of HCV in the world. The recently released **Egyptian Demographic Health Survey [EDHS]** tested a representative sample of the entire country for

HCV antibody**.** EDHS data estimate that 14.7% of the people in Egypt have been infected; this is more than 10 times greater than in any other country in the world. The prevalence of HCV in Western countries is less than 2% ***(Saleh et al., 2008).*** Egypt has a very high prevalence of HCV and a high morbidity and mortality from chronic liver disease (CLD), cirrhosis, and HCC. Approximately 20% of Egyptian blood donors are anti-HCV positive. Egypt has higher rates of HCV than neighboring countries as well as other countries in the world with comparable socioeconomic conditions and hygienic standards for invasive medical, dental, or paramedical procedures. The strong homogeneity of HCV subtypes found in Egypt (mostly 4a) suggests an epidemic spread of HCV ***(Saleh et al., 2008).***

Since a history of injection treatment has been implicated as a risk factor for HCV, a prime candidate to explain the high prevalence of HCV in Egypt is the past practice of parenteral therapy for schistosomiasis. The large reservoir of chronic HCV infection established in the course of these campaigns remains likely to be responsible for the high prevalence of HCV morbidity and may be largely responsible for the continued endemic transmission of HCV in Egypt today ***(Mostafa et al., 2010).***

The current population in Egypt is about 78 to 80 million. 14.7% of this population (0.147 X 78 million) is 11,466,000 persons who have been infected with this virus. Not

everyone remains infected but EDHS reported that 9.8% continue to have HCV RNA ***(El-Zanaty and Way, 2009).*** That means almost 10% of the total population are infected and are infectious to other people. That is 7.8 million people with chronic active HCV infection. The issue of treatment for those that develop HCV related liver disease is essentially a medical care crisis for the country. Transmission of HCV from person to person in Egypt is of course continuing, using computer models to estimate how many people in Egypt are becoming infected each year based on the data from **EDHS**. The percentage (prevalence) of those with antibodies to HCV increase with age. Using this information, models estimate an overall 6/1000 new infections each year. In terms of absolute numbers of people in Egypt getting infected this over 500,000 individuals. 70,000 of them are children ***(Mostafa et al., 2010).*** This is a public health emergency ***(El-Zanaty and Way 2009).***

Preventing HCV Infection The most important public health issue now in Egypt is to prevent people from getting infected with HCV. This means stopping or reducing the transmission of HCV from an infected person to a person who is not infected ***(Mostafa et al., 2010).***

(9) Current treatment of chronic HCV infection:

Therapy for chronic HCV infection has evolved substantially during the past decade. The goal of treatment is to prevent complications of HCV infection; which is mainly

achieved by elimination of the virus. Accordingly, treatment responses are dependent on the results of HCV RNA testing and infection is considered eradicated when there is a sustained virological response (SVR) i.e. HCV RNA negative 4 weeks after cessation of treatment ***(Strader et al., 2004).***

The current standard of care is pegylated IFN (PEG-IFN) with ribavirin given for 48 weeks ***(Bruno et al., 2000).*** PEG-IFN is synthesized by the chemical conjugation of a branched methoxy polyethylene glycol molecule with Interferon (either ∝ 2a 180µg/week or ∝ 2b1.5 µg/kg per week) that had resulted in decreased clearance, increased serum half-life and reduced immunogenicity for a number of proteins ***(Zeuzem et al., 2001).*** Ribavirin should be orally administered according to the body weight of the patient, the optimal ribavirin dose is at least 11 mg/kg ***(Manns et al., 2001).*** A ribavirin dose of 15 mg/kg would be ideal, although higher doses are associated with higher rates of anemia ***(Snoeck et al., 2006).***

In 2011 the first direct acting antivirals, the drug class of protease inhibitors (PI) represented by boceprevir and telaprevir, were approved by the authorities for treatment of chronic Hepatitis C virus genotype 1 infections. Due to superior antiviral efficacy in combination with pegylated interferon (PEG-IFN) and ribavirin (RBV) their use was quickly adopted in the clinic ***(Sarrazin et al., 2012).***

Sofosbuvir is the first all-oral, Interferon-free regimen approved for treating chronic Hepatitis C ***(Tucker, 2013).***