Introduction

The highest hepatitis C virus (HCV) prevalence in the world occurs in Egypt at an estimated ١٢٪ ***(Marzouk et al., ٢٠٠٧).*** The bulk of chronic infection is age-related ***(Alter, ٢٠٠٧)*** and occurs among persons of rural origin. Cohort studies have estimated a ٩٪ prevalence and ٠,٨/١٠٠٠ person-years incidence in Upper Egypt, and a ٢٤٪ prevalence and ٦,٨/١٠٠٠ incidence in the Nile Delta ***(Mohamed et al., 2005).***

HCV is the cause of many different forms of heart disease worldwide, and few cardiologists are aware of it as an etiology of heart disease, or its treatment ***(Crabb, 2001).***

Myocarditis is believed to be the major cause of dilated cardiomyopathy, myocarditis is often associated with hypertrophic cardiomyopathy which is caused by a viral infection such as HCV ***(Matsumori, 2005).***

A combination of factors including cytokine levels, increased levels of markers of inflammation, thrombosis, endothelial dysfunction, behavioural and social risk factors, malnutrition and liver problems are the likely to be the reason why patients with HCV have an increased risk of cardiovascular disease ***(Blutt et al., 2009).***

It is biologically plausible that HCV may increase the risk of disease such as heart attack and stroke as non alcoholic fatty liver disease, a common complication of HCV infection, has been associated with increased levels of inflammation and metabolic syndrome ***(Blutt et al., 2009).***

Aim of this Study

To evaluate the effect of chronic HCV infection on myocardium and cardiac function in Egyptian patients with chronic HCV either chronic hepatitis or compensated HCV related chronic liver disease.

Chapter (1)

Hepatitis C Virus

Introduction

Hepatitis C virus (HCV) is an RNA virus that is recognized as a major threat to global public health. It is the most common chronic blood-borne infection, with approximately ٣٪ of the world's population (roughly ١٧٠-٢٠٠ million people) infected with HCV. It is contracted chiefly through parentral exposure to infected material such as blood transfusions or injections with dirty needles. Those at highest risk for development of hepatitis C infection are injection-drug users, people who snort cocaine with shared straws, and health care workers who are at risk for needle-stick exposure. Although the incidence of acute hepatitis C infection has fallen dramatically in the United States during the past decade, the prevalence of infection remains high (approximately ٢,٧ million Americans) because chronic HCV develops in about ٧٥٪ of those infected ***(Bonkovsky and Mehta, ٢٠٠١)***.

In Egypt HCV has become a major public health problem. There are some claims that HCV infection is hyperendemic, especially in the Nile Delta governorates. It is the most common etiology of chronic liver disease (CLD). HCV infection becomes chronic in a considerable proportion of cases. In these cases cirrhosis of the liver is the final outcome

after many years. HCV genotype ٤ seems to be fairly unique to Egypt ***(Medhat et al., 2002)***.

HCV may increase the risk of disease such as heart attack and stroke as hepatitis steatosis (fatty liver), a common complication of HCV infection, has been associated with increased levels of inflammation and metabolic syndrome (***Blutt et al., 2009***).

Virology of HCV

The HCV is a small, enveloped, RNA virus. Although humans are the only known reservoir of HCV, the virus has been successfully transmitted to chimpanzees in experimental settings. Given its high rate of mutation, at least ٦ distinct classes of HCV, and more than ١٠٠ subtypes, have been identified by nucleotide sequencing. classes ١-٣ have a worldwide distribution; classes ٤ and ٥ are found largely in Africa and class ٦ is confined largely to Asia (***Forns and Bukh, 1999.***).

The HCV particle consists of a core of genetic material (RNA), surrounded by an icosahedral protective shell of protein, and further encased in a lipid envelope of cellular origin. Two viral envelope glycoproteins, E١ and E٢ are embedded in the lipid envelope ***(Beek and Dubuisson, 2003)***.

Life cycle of HCV:

The polyprotein cleaves at the N-terminal side of three structural proteins, the core, envelop ١ (E١) and envelope ٢ (E٢), all of which involved in the architectural organization of HCV ***(Bartosch et al., 2003)***.

At the carboxyl-terminal side, the polyprotein cleaves to non structural proteins NS٢, NS٣, NS٤A, NS٤B, NS٥A, NS٥B and NS٦ which are responsible for the life cycle of the virus. Based on density gradient analysis, two populations of virus have been described in sera of infected hosts. A high density fraction is believed to be composed of free or immunoglobulin-bound viral particles, whereas a low-density fraction appears to be bound to low density lipoproteins ***(Penin et al., 2004)***.

Circulating HCV interact with the host cell membrane, enters the cell through endocytosis. HCV E٢ and E١ proteins recognize and bound with the CD٨ receptors present on surface of hepatocytes and lymphocytes. In the cytoplasm, the mRNA undergoes translation, and polyproteins are processed; the HCV RNA then replicates after which the new viral RNAs are packaged and transported to the surface of the host cell, so that they can disseminate and complete a new cycle ***(Cormier et al., ٢٠٠٤).***

After entering a susceptible host, HCV invades, infects and replicates within the blood stream, repeating the process in

various tissues, as well as in peripheral B and T lymphocytes. It proceeds to the liver by tropism, passing through various tissues such as those of the pancreas, thyroid, adrenal glands, spleen and bone marrow. Since HCV can also directly infect the lymphatic tissue, its stimulation can lead to development of B-cell lymphomas ***(Kato et al., 2003)***.

The HCV replication rate is high, approximately ١x١٠^١٢ virions per day, thus leads to great heterogeneity in its presentations, which are known as quasispecies. The selection of and host adaptation to HCV quasispecies have given rise to distinct genotypes whose classification is based on the similarity of the sequence of nucleotides ***(Bartosch et al., ٢٠٠٥)***. Study suggests that specific genotypes, such as genotype ١, can be more cytopathic or can induce more rapid progression of the disease than do other genotypes. The risk of cirrhosis and hepato-cellular carcinoma (HCC) has been shown to be greater in individuals presenting genotype ١b than in those presenting genotype ٢ and ٣ ***(Seeff and Everthart, 2005)***.

However, over time, other mutations can result in changes that allow the virus to replicate and to escape immune surveillance. The net result is that HCV that infect humans are remarkably heterogenous, with only about ٧٠٪ similarity among all known isolates ***(Thomas et al., 2000)***.

Genotypes of HCV:

Based on genetic differences between HCV isolate, the HCV species is classified into ٦ genotypes (١-٦) with several subtypes within each genotype. The preponderance and distribution of HCV genotypes varies globally. For example, in North America, genotype ١a predominates followed by ١b, ٢a, ٢b. In Europe, genotype ١b is predominant followed by ٢a, ٢b, ٢c and ٣a. Genotype ٤ and ٥ are found almost exclusively in Africa. Genotype is clinically important in determining potential response to interferon-based therapy and the required duration of such therapy. Genotype ١ and ٤ are less responsive to interferon-based treatment than are the other genotypes ***(Simmonds et al., 2005)***. Infection with one genotype does not confer immunity against others, concurrent infection with two strains is possible ***(Laskus et al., 2006).***

HCV in Egypt

Chronic hepatitis C is the most common cause of chronic liver disease and cirrhosis ***(Wasley and Alter, 2000; Charlton, ٢٠٠١).*** Approximately ١٧٠ million people are affected with HCV worldwide, comprising ~٣٪ of the global population ***(NIHCD, 2002).*** HCV is the most common chronic blood-borne infection in the U.S., and is involved in ٤٠٪ of chronic liver disease ***(CDCP, 1998).***

Egypt has the highest seroprevalence for HCV. There is a hypothesis that the prevalence is linked to a now discontinued mass treatment campaign for schistosomiasis, which is endemic in Egypt ***(Mohamed et al., 2005b)***.

The highest HCV prevalence in the world occurs in Egypt at an estimated ١٢٪ ***(Marzouk et al., 2007).*** The bulk of chronic infection is age-related ***(Alter, 1997)*** and occurs among persons of rural origin. Cohort studies have estimated a ٩٪ prevalence and ٠,٨/١٠٠٠ person-years incidence in Upper Egypt, and a ٢٤٪ prevalence and ٦,٨/١٠٠٠ incidence in the Nile Delta ***(Mohamed et al., 2005a).***

In Egypt, HCV is considered the most common cause of chronic liver disease (CLD), where prevalence of antibodies to HCV is approximately ١٠ fold greater than in US and Europe. Ten to twenty percent of the general population was infected and HCV is the leading cause of HCC and cirrhosis in the country. Approximately ٩٠٪ of Egyptian HCV isolates belong to single subtype ٤a ***(Abdel-Aziz et al., 2000).***

Modes of transmission of HCV

The HCV is transmitted by blood-to-blood contact. In developed countries, it is estimated that ٩٠٪ of persons with chronic HCV infection were infected through transfusion of unscreened blood or blood products or via injection drug use or by inhalational drug use ***(Karmochkine et al., 2006).***

In Egypt, the major route of exposure appears to be due to medical therapy and inadequate sterilization techniques and supplies. In addition, farmers and rural populations are at greater risk, and this is supported by the higher prevalence rate of HCV in the Nile Delta and rural areas. Further, transmission of HCV through unusual routes has become significant as tattooing, circumcision or other medical procedures performed by non medical personnel. In addition house hold transmission, vertical and sexual transmission routes are also under investigation ***(Strader et al., 2004)***.

The modes of transmition

١. Injection drug use: Individuals most affected by HCV infection are present and former injection drug users; estimates of the percentage of these individuals who are seropositive for HCV infection range from ٦٠٪ to ٨٠٪ ***(NIH, 2002).*** It considered a risk factor for HCV infection in Egypt ***(Mohamed et al., 2009).***

٢. Invasive health care procedures (Haemodialysis, multiple surgeries, endscopy and contaminated syringes): These procedures are associated with risk of developing HCV infection through increased risk of contact with infected blood ***(Mohamed et al., 2009)***.

٣. Interfamilial viral transmission: HCV infection has a strong familial component explained by genetic predisposition to infection ***(Mohamed et al., 2008).***

٤. Sexual transmission is relatively infrequent because of the low levels of virus in semen, saliva, and vaginal secretions. The rate of sexual transmission of HCV is generally accepted to be low, with an overall risk of <٥٪, and studies of long-term monogamous couples suggest a low risk of transmission from an infected individual to his/her spouse or partner ***(Sarbah and Younossi, 2000; Ortiz and Lane, 2004)***.

٥. Needle-involved procedures such as body piercing, tattooing, or acupuncture ***(Haley and Fischer, 2003; Leung, 2003)***. Shaving by a community barber is a risk factor for HCV infection ***(El Sadway et al., 2002)***.

٦. Parentral antishistosomal therapy: It had a major role in the spread of HCV infection throughout Egypt. This intense transmission established a large reservoir of chronic HCV, responsible for the high prevalence of HCV infection and high rate of transmission ***(Mohamed et al., 2005b)***.

٧. Blood transfusion: Between January ١٩٨٨ and June ١٩٩٢, an estimated ٣٠٠,٠٠٠ Americans received blood transfusions contaminated with HCV, although blood screening instituted since mid-١٩٩٢ has reduced the risk to less than ١ in ١٠٠,٠٠٠ units ***(Ortiz and Lane, 2004).*** Blood-derived products manufactured prior to ١٩٨٧, when viral inactivation practices were initiated and HCV-contaminated immune globulin, were also sources of infection ***(Kenny-Walsh, 1999; Adams et al., ٢٠٠٤).***

٨. Breastfeeding need not be restricted in infected mothers, since the transmission of virus through breast milk has not been demonstrated ***(Stephenne and Sokal, 2002)***.

The Immunopathogenesis of HCV infection

Despite advances in the knowledge of the epidemiology and molecular virology of HCV, the mechanism of hepatocellular injury in HCV infection is not completely understood. After infection with HCV, multiple factors influence the host-virus interaction, resulting in a unique and individual disease pattern. Viral factors include direct cytopathic effects, replication efficiency, nucleotide substitution rates and viral heterogeneity (quasispecies). Host factors may include the competence of the innate immune system, local and systemic cytokine production and the humoral and adaptive cellular immune responses ***(Rothman et al., 2005).***

Other environmental factors, especially alcohol abuse and immunosuppression may alter the progression of liver disease. The host immune response plays the major role in controlling HCV and causing hepatocellular damage. It is a non-specific immune response, including interferon (IFN) production and natural killer (NK) cell activity, and also an HCV-specific immune response, including humoral and cellular components ***(Chang, 2003)***.

Antibodies to HCV structural and non structural proteins develop during infection and form the basis for the detection

assay for the host's exposure to this viral infection. The cellular immune response is also activated in these patients, with the presence of $CD_{٤+}$ and $CD_{٨+}$ cells that recognize and respond to various processed HCV antigens. Neither the humoral nor the cellular immune response, however, seems sufficient to eradicate infection in most patients ***(Accapezzato et al., 2004)***.

Immune-mediated mechanisms of HCV infection:

١- Humoral immune response to HCV infection:

Following exposure to HCV, viremia develops within one week. In immunocompetent host, the viremia is followed in several weeks by elevated transaminase levels and then by a delayed antibody seroconversion. Intrahepatic lymphoid aggregates, composed of a germinal center of activated B cells, are commonly observed in patients with chronic HCV infection and represent the host B-cell response ***(Logvinoff et al., 2004)*.**

A wide variety of antibodies are generated to both structural and non-structural regions of the virus. Antibodies against conserved epitopes of the envelope glycoproteins ($E_{١}$, $E_{٢}$) are found in more than ٩٠٪ of patients with chronic HCV infection. This specific antibodies response gradually decreases after recovery from HCV infection and can eventually disappear. The clinical significance of antibody to HCV polypeptides with respect to control of infection and the pathogenesis of liver disease is still largely unknown. Antibodies against envelope proteins often have neutralizing

ability and may prevent viral entry into cells or target the virus for elimination by scavenger cells ***(Strader et al., 2004)***.

The neutralizing responses are highly strain-specific and the quasispecies nature of the virus allows other strains to emerge when the predominant strain is under immune pressure. This variability represents a major obstacle in the development of an effective vaccine, because vaccine development depends on induction of neutralizing antibodies to conserved epitopes of the envelope proteins ***(Zeisel et al., 2007)***.

٢- Cellular immune response to HCV infection :

It involves both an innate response and an adaptive antigen-specific phase. Eradication of the virus depends on classical CD٤+ and CD٨+ cytotoxic T- lymphocytes responses. Intracellular and extracellular viral proteins represent quite different challenges to the immune system, both in terms of recognition and of appropriate response. Unlike Ag recognition by B-cell immune globulin receptors, the two general classes of T cells don't recognize native antigen; rather they recognize antigenic peptide that have been processed and presented on the cell surface ***(Quiroga et al., 2006)***.

Natural history of HCV infection

Acute hepatitis C:

Acute hepatitis C infection is infrequently diagnosed because the majority of acutely infected individuals are asymptomatic. In the transfusion setting, where acute onset of HCV infection has been best documented, ٧٠٪ to ٨٠٪ of cases are asymptomatic. The symptomatic onset ranges from ٣ to ١٢ weeks after exposure. Symptoms may include malaise, weakness, anorexia, and jaundice. Serum alanine transferase (ALT) levels, signifying hepatocyte necrosis, begin rising ٢ to ٨ weeks after exposure and often reach levels of greater than ١٠ times the upper limits of normal. HCV RNA can be detected in the serum within ١ to ٢ weeks after exposure. The level of HCV RNA rises rapidly during the first few weeks, and then peaks between $١٠^{٥}$ to $١٠^{٧}$ IU/ml, shortly before the peak of ALT level and onset of symptoms. In self-limited acute hepatitis C, symptoms can last several weeks and subside as ALT and HCV RNA levels decline. Acute HCV infection can be severe, but fulminant liver failure is rare. The antibody to HCV, as detected by enzyme immunoassay, becomes positive near the onset of symptoms, approximately ١ to ٣ months after exposure. Up to ٣٠٪ of patients will test negative for anti-HCV at onset of their symptoms, making anti-HCV testing unreliable in diagnosis of acute infection. Almost all patients eventually develop the antibody to HCV; however, titers can be low or undetectable in immunodeficient patients. The anti-HCV assay detects greater