Introduction

Over 75% of patients with acute hepatitis C (HCV) will develop into chronic infection associated with chronic hepatitis, end stage liver disease or hepatocellular carcinoma. HCV infection is usually ignored because the level of HCV in serum of some patients is very low and most patients with chronic HCV infection are asymptomatic. Some patients with persistent abnormal liver function may be infected with HCV although HCV RNA and HCV antibodies in serum are negative because their HCV load is under the current detectable limit or sometimes HCV is absent in serum but present in the liver or peripheral blood mononuclear cells (PBMCs) and other tissues .Replication of HCV in PBMCs causes relapse and chronic infection. Therefore PBMCs play an important role in replication, relapse and chronic infection of HCV ***(Yan-Feng et al., 2007).***

It is generally accepted that HCV replicates by making a cRNA strand known as the negative or replicative strand. Although the liver is the main site of virus replication, there is an increasing body of evidence for virus propagation in extrahepatic locations, including cells of the lymphatic and the central nervous systems. In regard to infection of lymphoid cells, HCV positive and negative strands were detected in the peripheral blood mononuclear cells (PBMC) and the bone marrow from chronically infected patients. It was also shown that HCV can propagate in lymphoid cell cultures and that the virus derived is infectious ***(Pham et al., 2004).***

Treatment for hepatitis C consists of the administration of pegylated interferon-alpha (IFN-alpha) in combination with ribavirin; the duration of treatment adequate for each patient is determined according to the genotype of virus. At the end of treatment, patients are classified as responders if no HCV RNA is detected by PCR or as nonresponders if HCV-PCR is positive. The virological response is evaluated again after six months, and a sustained virological response (SVR) is defined as negative HCV-PCR and a nonsustained virological response is defined as positive HCV-PCR ***(Cavalheiro et al., 2007).***

Aim of the Work

The aim of this study was to evaluate the persistence of hepatitis C in peripheral blood mononuclear cells of sustained viral responders to pegylated interferon and ribavirin therapy.

Chapter (1): Epidemiology of Hepatitis C

Hepatitis C virus continues to be a major disease burden on the world. The WHO estimated a worldwide prevalence of about 3% with the virus affecting 170 million people worldwide. Generally, most studies of prevalence use blood donors to report the frequency of HCV usually by anti-HCV antibodies and do not report follow-up HCV testing. Using blood donors as a prevalence source may underestimate the real prevalence of the virus because donors are generally a highly selected population ***(Theodore & Jamal, 2006).***

There are both geographic and temporal differences in the patterns of HCV infection. For example, vastly different countries, including the United States, Australia, Turkey, Spain, Italy, and Japan, belong to regions of the world with similar overall average prevalences of HCV infection (1.0%-1.9%), but have different patterns of age-specific prevalence. In the United States, prevalence is highest among persons 30-49 years old, who account for two-thirds of all infections, and lower than average among persons less than 20 and greater than 50 years old. This pattern indicates that most HCV transmission occurred in the last 20-40 years, and primarily among young adults ***(Alter, 2007).*** In contrast, the age-specific prevalences of HCV infection increase steadily with age in Turkey, Spain, Italy, Japan, and China. In these countries, persons > 50 years old account for most infections, which suggests a cohort effect in which the risk for HCV infection was higher in the distant past, i.e., 40-60 years previously.

Region-specific estimates range from <1% in Northern Europe to >2.9% in Northern Africa. The lowest prevalence (0.01%-0.1%) has been reported from countries in the UK and Scandinavia; the highest prevalence (15%-20%) has been reported from Egypt ***(Shepard et al., 2005)***. HCV is less prevalent in countries neighboring Egypt that have similar sociomedical conditions and similar HCV strains ***(Shobokshi et al., 1999)***.

HCV infection is a major public health problem in Egypt. Blood bank and community-based surveys conducted in Egypt have reported sero-prevalence rates of HCV to be as high as 40% in some parts of the country. These rates are substantially higher in the Nile Delta region compared with the rest of the country ***(Rao et al, 2002).***

The highest HCV prevalence in the world occurs in Egypt, where the prevalence of infection increases steadily with age, and high rates of infection are observed among persons in all age groups. This pattern indicates an increased risk in the distant past followed by an ongoing high risk for acquiring HCV infection, although there are regional differences in average overall prevalence ***(Alter, 2007)***.

The risk factor for HCV transmission that specifically sets Egypt apart from other countries is a personal history of parenteral antischistosomal therapy (PAT). A review of the Egyptian PAT mass-treatment campaigns, discontinued only in the 1980s, show a very high potential for transmission of blood-borne pathogens ***(Frank et al., 2000).***

Studies of the epidemiology of HCV infections have suggested that the Nile delta region of Egypt has a prevalence rate among the highest prevalence rates of HCV in the world, with seroprevalence rates of 30-40% in villagers over the age of 30 years ***(Darwish et al., 2001).***

In the 1960s, 1970s and early 1980s, mass campaigns were conducted to treat schistosomiasis infections in these areas, during which individuals older than 5 years of age were treated with tartar emetic injections. Sero-surveys conducted in the 1990's in Egypt have reported positive associations between HCV infections and a history of schistosomiasis or a history of having received injections for the treatment of schistosomiasis. Based on this evidence, the studies suggest that inadequately sterilized needles and syringes used during the campaign were probable causes for transmission of HCV in the region ***(Rao et al., 2002).***

Prevalence of HCV in African Americans is about 3 times greater than Caucasians. The problems of HCV infected African Americans are that they are more likely to be infected with genotype 1, more likely to have HCV complications, have higher rates of cirrhosis, HCC and death due to HCV ***(Hoefs & Aulakh, 2006).***

There is no evidence that casual contact, such as kissing, hugging, or sharing eating utensils, is associated HCV transmission ***(Booth et al., 2001)***. The intrafamilial transmission rate of HCV is similar to the rate of transmission in healthy subjects. However, the duration of exposure with anti-HCV positive index cases is a significant risk factor particularly for spouses in the intrafamilial transmission ***(Celen et al., 2007).***

Risk Factors:

The transmission of HCV is primarily through exposure to infected blood. Risks for transmission include blood transfusion before 1992, intravenous drug use, high-risk sexual activity, solid organ transplantation from an infected donor, occupational exposure, hemodialysis, household exposure, birth to an infected mother, and intranasal cocaine use. A potential risk factor can be identified in approximately 90% of persons with HCV infection. In the remaining 10%, no recognized source of infection can be identified, although most persons in this category are associated with low socioeconomic level ***(Chen & Morgan, 2006).***

The most efficient transmission of HCV is through large or repeated direct percutaneous exposures to blood (e.g., transfusion or transplantation from infectious donors, injecting drug use). HCV is less efficiently transmitted by single small dose percutaneous exposures (e.g., accidental needlesticks) or by mucosal exposures to blood or serum-derived fluids (e.g., birth to an infected mother, sex with an infected partner) ***(Alter, 2007).***

In developing countries, transmission of HCV typically results primarily from iatrogenic factors, such as blood transfusion and inadequate sterilization or reuse of medical equipment, but in industrialized countries, risk resulting from these factors has been greatly reduced ***(Liu et al., 2009).*** Esophageal balloon examination, a less commonly identified route of HCV infection, also increased

the risk for HCV infection. In the recent past (1980–2000), esophageal balloon examination, which was designed for early cytologic detection of esophageal lesions, was relatively common in China. In this technique, the patient swallows a balloon covered with a cotton net. The balloon is inflated within the patient's stomach. Exfoliated esophageal cells are then scraped off the mucosa by pulling out the balloon. Bleeding of esophageal mucosa can occur. The balloon and cotton net were designed to be nonreusable. Nonetheless, on some occasions, balloons were reused after manual cleaning.

Intravenous Drug Use

Transmission of Hepatitis C virus has been strongly associated with intravenous and percutaneous drug and needle use ***(Theodore & Jamal, 2006)***.

It has been estimated that approximately 2 million HCV infections are acquired annually from contaminated health care injections, and may account for up to 40% of all HCV infections worldwide. In many developing countries, supplies of sterile syringes may be inadequate or nonexistent, non-professionals often administer injections outside the medical setting, and injections are often given to deliver medications that could otherwise be delivered by the oral route. In addition to unsafe injection practices, lack of attention to appropriate cleaning and disinfection of equipment used in hospital and dental settings also may be a source for HCV transmission ***(Alter, 2007).***

Reuse of glass syringes during the early campaign to treat schistosomiasis in Egypt appeared to be responsible for the largest outbreak of iatrogenic transmission of a blood born pathogen ever recorded ***(Frank et al., 2000).***

Intravenous drug use is the major risk factor for the acquisition of HCV and intravenous drug users (IDUs) constitute by far the largest pool of HCV infection in the UK and other industrialized countries. Prevalence varies between 27% and 74% in different cohorts. Despite these very high rates of infection, antiviral therapy has not been consistently offered to this population. IDUs often do not attend hospital clinics regularly, and are perceived to be at greater risk from illicit drug use than HCV infection. Active IDUs are also at theoretical risk of re-infection and may not therefore represent a cost-effective use of high cost combination therapy ***(Thomson, 2009).***

Blood Product Transfusion

Many cases of post transfusion hepatitis that occurred before the identification of HCV were retrospectively analyzed and identified as HCV infection. The infection rates range from 19% seroprevalence in patients receiving packed red cells or plasma during open heart surgery to more than 95% in hemophiliac patients treated with unheated or dry heat-treated factor VIII or IX concentrates ***(Bresee et al., 1999).***

At present, Transfusion-associated Hepatitis incidence is so low that it has to be mathematically modeled, and the risk of hepatitis C is calculated to be 1

case in every 1.5 million to 2 million exposures, a remarkable incidence compared with the 30% rate that prevailed in 1970 and the 10% rate in 1980 ***(Alter & Klien, 2008).***

Organ Transplantation

Among recipients of organs from anti-HCV positive donors, 35% developed posttransplant liver disease, 50% tested positive for anti-HCV posttransplant, and 74% tested positive for HCV RNA ***(Lunel et al., 2000).***

Hepatitis C (HCV) universally recurs following orthotopic liver transplantation (OLT), representing an important cause for retransplantation ***(Mukherjee et al., 2003).***

Health Care Workers

The National Institute of Health (NIH) has recently revised its recommendations regarding follow-up of needle-stick injuries in the health care sitting. Given the ability of PCR to detect HCV RNA two weeks after transmission, the NIH recommends exposed healthcare workers should be checked at 2 and 8 weeks after injury. A similar approach to needle-stick injuries in the non-health care setting may facilitate the earliest possible diagnosis for risk management purposes, but to identify persisting infection that requires antiviral treatment, testing at 12 weeks may be more appropriate ***(Haber et al., 2006).***

Occupational transmission of HCV infection is largely confined to health care workers who have sustained contaminated needle stick injuries, average incidence of anti-HCV seroconversion from an HCV-positive source is 1.8% ***(Alter et al., 2007).***

Transmission has been associated with hollow-bore needles and deep injuries. Transmission rarely occurs from mucous membrane or non-intact skin exposure to blood and no transmission to health care workers has been documented from intact skin exposure to blood ***(Yazdanpanah et al., 2005).***

The risk of needle-stick transmission is related to the presence and concentration of virus, the inoculum volume, the nature of injury to the skin, and ambient environmental factors. The level of HCV RNA in blood generally declines hours after removal from the host, suggesting that infectivity of a needle is dependent on the time between the needle being discarded and the subsequent injury ***(Haber et al., 2006).***

Vertical Transmission

The rate of perinatal transmission of HCV is 4% to 7% per pregnancy and occurs only when HCV RNA is detectable in maternal serum at delivery. Transmission may be related to higher levels (above 106 copies per mL), although data on the effect of virus concentration have been inconsistent. Prolonged labor after membrane rupture and internal fetal monitoring have been associated with perinatal infection ***(Alter, 2007).***

The European Paediatric HCV Network recommends that, on the basis of current evidence, neither elective Caesarean section nor avoidance of breast feeding is of benefit in preventing HCV transmission from mother to child. Mothers co-infected with HCV and HIV should follow the existing HIV guidelines, which emphasize maternal choice but recommend elective Caesarian section at 38 weeks ***(Thomson, 2009).***

Sexual Transmission

HCV is more likely to be transmitted by sexual intercourse when the infected partner is in the early phase of acute infection; virus concentration is high and there is no antibody to complex with antigen ***(Gambotti et al., 2005).***

Monogamous couples do not need to use barrier protection but should be advised that condoms may reduce the already low risk of HCV transmission ***(Terrault, 2002).***

In contrast to hepatitis B virus, sexual transmission of HCV is rare. A long-term prospective study of HCV among 895 monogamous heterosexual partners of individuals chronically infected with HCV, with a total follow-up period of more than 8000 person-years, found an extremely low or null risk of sexual transmission ***(Thomson, 2009).***

Haemodialysis

The prevalence of HCV antibodies among haemodialysis patients is about 8%, and the infection is presumed to have been transmitted by inadequate infection control practices ***(Tokars et al., 2002).***

Despite screening of blood products for anti-HCV and implementation of precaution measures, HCV infection is still a major problem in haemodialysis units. Routine serologic controls performed biannually (including qualitative PCR technology) and testing at least every 2 months for alanine aminotransferase and gamma glutamyl transpeptidase levels are important for monitoring viral hepatitis transmission in haemodialysis units ***(Barril et al., 2008).***

Because of the wide variety of human activities that involve the potential for percutaneous exposure to blood or blood-derived body fluids, there are numerous other biologically-plausible modes of transmission besides those with clearly-demonstrated epidemiologic associations with infection. These include cosmetic procedures (tattooing, body-piercing), intranasal drug use, and religious or cultural practices such as ritual scarification, circumcision, acupuncture, and cupping ***(Alter, 2007).***

CHAPTER (2): HCV STRUCTURE

HCV is a small enveloped RNA virus which has been allocated to an unique genus, designated *Hepacivirus*, within the family *Flaviviridae* ***(Thomson & Finch, 2005).***

Hepatitis C is an infectious disease affecting the liver, caused by the hepatitis C virus. The hepatitis C virus is a small, enveloped, single-stranded, positive sense RNA virus with a large genetic heterogeneity. Isolates have been classified into at least eleven major genotypes, based on a nucleotide sequence divergence of 30-35% ***(Zarkesh-Esfahani, 2010).***

The major features of HCV structure, replication, transmission and ability to establish persistent infection are shared between all known variants. Indeed, viewed purely as a survival machine, the widespread distribution of genotypes 1–6 in human populations indicates that that each is equally successful in maintaining infections in human populations. The clearest difference between genotypes is in their susceptibility to treatment with IFN monotherapy or IFN/ribavirin (RBV) combination therapy ***(Simmonds, 2004).***

HCV demonstrates a high degree of sequence variation throughout its genome and exists in vivo as a group of heterogeneous but closely related quasispecies. However, the levels of heterogeneity differ considerably among the various regions of the virus, ranging from as little as 10% in the 5' untranslated region (5' UTR) to 50% or more within the E1 region ***(Chen & Morgan, 2006).***

HCV is classified in the family Flaviviridae, although it differs in many details of its genome organization from the original (vector-borne) members of the family. HCV is additionally distinct and somewhat unusual for an RNA virus in being able to establish persistent infections in the majority of exposed individuals. This phenomenon has attracted the greatest interest in HCV research, not at least because long-term, chronic infections underlie its disease manifestations and effective therapy must break this ongoing cycle of replication in the liver ***(Simmonds, 2004).***

The HCV RNA genome is approximately 9600 nucleotides in length. In common with other members of the flavivirus family, the viral genome is composed of a 5' non-coding region (NCR), a single, long open-reading frame with the potential to encode a polyprotein precursor of about 3000 amino acids and a 3' NCR. The HCV polyprotein is co- and post-translationally processed by cellular and viral proteases to yield the mature structural and non-structural proteins ***(Thomson, 2009).***

Most of the genome forms a single open-reading frame that encodes three structural (core, E1, E2) and non-structural (p7, NS2-NS5) proteins ***(Cavalheiro et al., 2007).***

There are six known genotypes (numbered 1 through 6) and more than 50 subtypes (e.g., 1a, 1b, 2a...). Frequent HCV mutations and numerous subtypes have made the search for an HCV vaccine challenging ***(Chen & Morgan, 2006)***. HCV genotype 1 is more common in Western countries, genotype 2 is more common in Western Far East and Mediterranean countries, genotype 3