Prevalence of Occult Hepatitis C Virus Infection among Egyptian Haemodialysis Patients

Thesis

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List of Abbreviations

Abb.	Full term
ALT	*Alanine aminotransferase*
Anti-HCV	*Antibody to HCV*
CAPMAS	*Central Agency for Public Mobilisation and Statistics*
CHD	*Chronic hemodialysis*
ELISA	*Enzyme-linked immunosorbent assay*
FDA	*Food and Drug Administration*
HBV	*Hepatitis B virus*
HCV	*Hepatitis C Virus*
HIV	*Human immunodeficiency virus*
OCI	*Occult HCV infection*
PBMCs	*Peripheral blood mononuclear cells*
PEG-IFN	*Pegylated-interferon*
PMNCs	*Polymorphonuclear Cells*
RIBA	*Recombinant immunoblot assay*
RT-PCR	*Reverse-transcription polymerase chain reaction*
TBRI	*Theodor Bilharz Research Institute*

INTRODUCTION

Hepatitis C Virus (HCV) infection is a global health problem. Upon exposure to HCV, the immune system responds by producing antibodies against the virus. A new entity of HCV infection was first described in 2004 in patients with persistently elevated liver function tests and who were anti-HCV and serum HCV RNA negative. Despite the absence of conventional HCV markers, 57% of these patients had HCV RNA in the liver and so this clinical situation was termed "occult HCV infection" (OCI) ***(El-Sayed, 2014).***

Chronic HCV infection is a significant reason for morbidity and mortality in patients who have End Stage Renal Disease (ESRD), where the prevalence of HCV infection in patients on hemodialysis is higher than in the general population ***(Kosaraju et al., 2013).***

Fabrizi et al. (1997) revealed that in HD patients, HCV carriage is associated with 1.57 times increased risk of death. Liver cirrhosis and hepatoma contribute to the increased mortality.

The proven ability of HCV replication in PBMCs raises the question of potential transmission of infection between OCI carriers and people undergoing hemodialysis. Patients with OCI can be potentially infectious, they have a better immune response that could be the cause of milder disease compared with chronic HCV patients, and anti-HCV treatment is advisable in this situation ***(Abdel Rahim et al., 2016).***

AIM OF THE WORK

The aim of this study is to evaluate the hidden infection of hepatitis C virus among patients on maintenance hemodialysis with negative HCV Ab by ELISA and negative HCV(RNA) PCR in plasma by using PCR in peripheral mononuclear cells as a marker in these patients.

Chapter 1

HEPATITIS C VIRUS IN PERIPHERAL MONONUCLEAR CELLS (OCCULT HCV)

Egypt has the highest prevalence rate of hepatitis C virus (HCV) in the world, making it the most challenging public health problem facing the country. It was found that about 20% of Egypt's population had HCV infections, the highest prevalence anywhere in the world ***(Reker and Islam, 2014).***

Upon exposure to HCV, the immune system responds by producing antibodies against the virus. These antibodies remain and could be detected even after HCV is cleared from the body. An active infection develops when the virus starts to increase in count and cause damage to the liver. This is when patients develop symptoms of hepatitis, seek medical help and get diagnosed. Some age groups suffer prevalence rates of up to 50%. As for the geographical distribution of anti-HCV in persons aged 10-50 years: the Nile Delta and Upper Egypt have rates of 28% and 26% respectively ***(Egyptian National Control Strategy for Viral Hepatitis, 2012).***

Incidence rates are estimated at 2-6 per 1000 per year, that is, at least 170,000 new cases every year, which means maintaining a prevalence rate of 5-15% in the foreseeable

future ***(Egyptian National Control Strategy for Viral Hepatitis, 2012).*** Liver mortality in Egypt reaches 40,000 per year, making 10% of total mortality, and comes second after heart diseases ***(El-Sayed, 2014).***

A new entity of HCV infection was first described in 2004 in patients with persistently elevated liver function tests and who were anti-HCV and serum HCV RNA negative. Despite the absence of conventional HCV markers, 57% of these patients had HCV RNA in the liver and so this clinical situation was termed "occult HCV infection". Moreover, it was proven that the antigenomic HCV RNA strand could be detected also in the hepatocytes of a high proportion of those patients with occult HCV infection, this indicating an active viral replication. Occult HCV infection has also been described in two other different clinical settings. One of these is in anti-HCV positive, serum HCV-RNA negative subjects with persistent normal values of liver enzymes (asymptomatic HCV carriers), of whom nearly 90% have detectable viral RNA in liver and in peripheral blood mononuclear cells (PBMCs). The second one is in anti-HCV positive individuals who resolved HCV infection either spontaneously or after antiviral treatment ***(Carreño et al., 2006).***

In these patients, HCV RNA is detected in liver and in PBMCs years after apparent recovery from the disease (normalization of liver enzyme values and loss of serum HCV RNA). This occult HCV infection is related to the persistence

of necroinflammation activity in the liver of the sustained responders. Thus, there are two types of occult HCV infection: one can be found among anti-HCV seropositive individuals with normal values of liver enzymes and the other is found among anti-HCV seronegative patients with abnormal levels of liver enzymes ***(Pham et al., 2012).***

Socioeconomic nature of HCV in Egypt:

The HCV epidemic in Egypt is of a socioeconomic nature. It is mostly prevalent among lower social and economic segments of the population. Historically, it started by the parentral anti-schistosomal (bilharzia) treatment campaigns undertaken in the 1960s and 1970s in rural areas using improperly sterilised glass syringes (tartar emetic injections) ***(Esmat, 2013).*** This is behind the high HCV prevalence rate in rural compared to urban areas; 12% and 7% respectively ***(El-Zanaty and Way, 2009).***

HCV prevalence also varies with wealth: 12% in the lowest wealth quintile compared to7% in the highest quintile. In Egypt, 26% of the population live with less than USD 1.6 per day (the national poverty line) ***(El-Zanaty and Way, 2009).*** With this level of poverty in the country, it could be concluded that hepatitis C is a socioeconomic condition, hitting the poorest segments of the population ***(Central Agency for Public Mobilisation and Statistics (CAPMAS), 2013).***

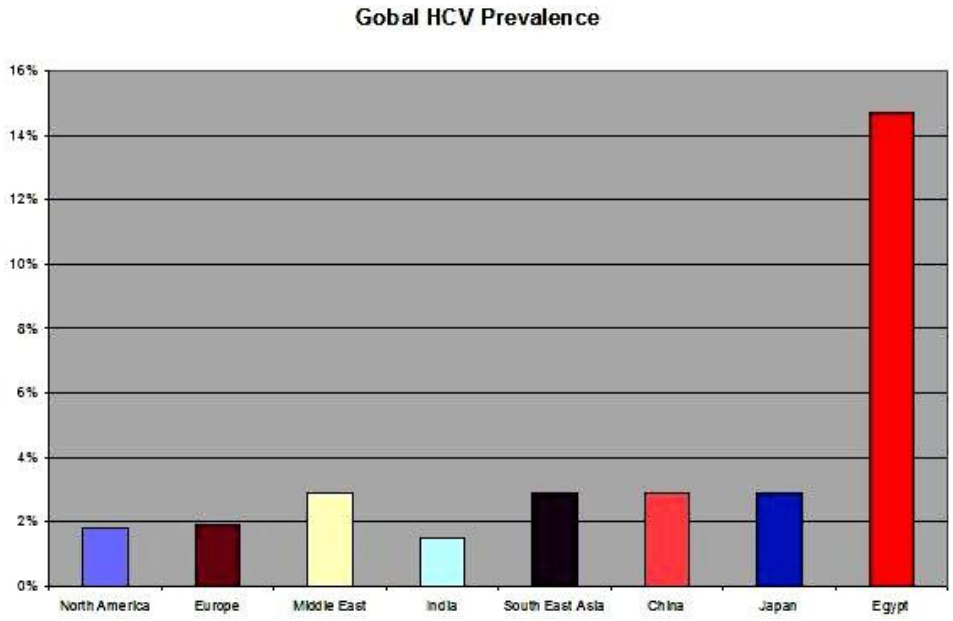

Figure 1: Hepatitis C virus in Egypt compared to other countries in the world ***(El- Zanaty et al., 2009).***

Mode of HCV transmission:

HCV is transmitted by blood from the infected person. The most common exposure to HCV infection in Egypt is from formal and informal medical and dental care. There are many procedures and treatments that are percutaneous or cause bleeding. If the person doing this is not using sterile equipment or materials or may be has not changed their latex gloves, he could be exposed to HCV infection ***(El- Zanaty et al., 2009).***

Methods of detection of occult HCV:

Occult HCV infection was first identified in liver of anti-HCV and serum HCV RNA negative patients with abnormal liver function tests and it was also found that viral RNA could

be present in the PBMCs of nearly 70% of these patients. Furthermore, it was demonstrated that occult HCV replicates in these cells ***(Castillo et al., 2005).***

By detecting HCV RNA in liver biopsies or in PBMCs, other groups in Japan, Italy, Egypt, Colombia, Pakistan and Iran have confirmed the existence of occult HCV infection in patients with elevated liver enzymes and without conventional HCV markers. Occult HCV infection has also been found in hemodialysis patients who were persistently anti-HCV and serum HCV RNA negative but with abnormal values of liver enzymes, in the family setting of patients with occult hepatitis C and even in healthy subjects with normal alanine aminotransferase (ALT) levels and no clinical evidence of liver disease ***(Idrees et al., 2011).***

Since HCV was replicating in the liver and PBMCs of patients with occult HCV infection, it was speculated that it should exist as circulating viral particles but at such low levels that the virions could not be detected even using the most sensitive reverse-transcription polymerase chain reaction (RT-PCR) techniques. This hypothesis was tested by concentrating HCV virions by ultracentrifugation of 2 mL of serum from patients with occult HCV prior to HCV RNA detection by RT-PCR ***(Bartolomé et al., 2007).***

In this way, serum viral RNA was found in nearly 60% of the patients. In addition, it was found that the density of the

viral particles isolated from patients with occult HCV infection was similar to the highly-infectious lipoviral particles present in the serum of patients with classical chronic hepatitis C, suggesting that serum from patients with occult HCV is potentially infectious ***(Nielsen et al., 2006).***

HCV-specific T-cell responses and the occult infection:

Functional virus-specific memory CD4+ and CD8+ T-cells have been documented in the circulation of patients with HCV RNA persistence in the liver and so assaying cellular immunity has been proposed as a surrogate marker of occult HCV infection. T-cell responses found in occult HCV infection are similar to those described in anti-HCV-positive patients following spontaneous or treatment- induced recovery ***(Pham et al., 2010).***

HCV-specific T-cell responses have been detected often among occult HCV-infected hemodialysis patients, family members of patients with occult or overt HCV infection and among HCV- seronegative sexual partners of patients with chronic hepatitis C, supporting exposure to trace amounts of HCV RNA. The maintenance of such immune responses may require only a low level productive infection. In fact, sporadic reappearance of minute amounts of HCV RNA stimulates cellular immunity ***(Veerapu et al., 2011).***