Detection of Occult Hepatitis C Virus Infection in Patients Who Achieved a Sustained Virologic Response to Direct-Acting Antiviral Agents

Thesis

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Amr Adel Elzohary Mohamed

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List of Abbreviations

Abb.	Full term
AEs	*Adverse events*
AFP	*Alpha feto protien*
ALT	*Alanine aminotransferase*
ARFI	*Acoustic radiation force impulse*
AST	*Aspartate aminotransferase*
CLD	*Chronic liver disease*
CTP	*Child-Turcotte-Pugh*
DAA	*Direct acting antivirals*
DCV	*Daclatasvir*
ECM	*Extracellular matrix*
ELISA	*Enzyme-linked immunosorbent assay*
GGT	*γ glutamyl transferase*
HA	*Hyaluronic acid*
HBV	*Hepatitis B virus*
HCC	*Hepatocellular carcinoma*
HCV	*Hepatitis C virus*
HIV	*Human immunodeficiency virus*
HSCs	*Hepatic stellate cells*
INR	*International normalised ratio*
LPDs	*Lymphoproliferative disorders*
MMPs	*Matrix metalloproteinases*
MRE	*Magnetic Resonance Elastography*
NAFLD	*Non-alcoholic fatty liver disease*
NAT	*Nucleic acid test*
NHL	*Non-Hodgkin lymphoma*
NNIs	*Non-nucleoside polymerase inhibitors*
OCI	*Occult HCV infection*
PBMCs	*Peripheral blood mononuclear cells*

List of Abbreviations cont...

Abb.	Full term
PC	*Paltelet concentration*
PCR	*Polymerase chain reaction*
PEG-IFN	*Pegylated interferon*
PIIINP	*Procollagen type III amino-terminal peptide*
PIs	*Protease inhibitors*
RBV	*Ribavirin*
RIBA	*Recombinant immunoblot assays*
RIG-I	*Retinoic acid-inducible gene I*
RNA	*Ribonucleic acid*
RT-PCR	*Reverse transcription polymerase chain reaction*
SOF	*Sofosbuvir*
SVR	*Sustained virologic response*
TGF	*Transforming growth factor*
TIMPs	*Tissue inhibitors of metalloproteinases*
TMA	*Transcription-mediated amplification*
TRIF	*TIR-domain-containing adapter-inducing interferon-β*
WBCs	*White blood cells*

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INTRODUCTION

Hepatitis C virus (HCV) is the main cause of chronic liver disease all over the world. HCV affects about 200 million people worldwide. About 350000 deaths per year are due to HCV infection. HCV is classified as a member of Flaviviridae family and Hepacivirus genus and 30% of infected people resolve their acute infection spontaneously, while about 70% turn into chronic HCV infection ***(Zaltron et al., 2012).***

Chronic HCV infection is detected by the persistence of HCV ribonucleic acid (RNA) in the blood for 6 months or more after the onset of acute infection. Many factors can affect the degree of HCV chronicity such as the age at onset of the disease, gender, ethnicity, and jaundice during the acute infection ***(Chen et al., 2006).***

Occult HCV infection (OCI) is identified by the presence of HCV RNA in the liver cells or peripheral blood mononuclear cells (PBMCs) of the patients whose serum samples test negative for HCV RNA by polymerase chain reaction (PCR) assays, with or without presence of HCV antibodies ***(Carreño et al., 2006).*** OCI can lead to liver cirrhosis and hepatocellular carcinoma ***(Zaltron et al., 2012).***

PBMCs might be considered as a long-lived HCV reservoir due to the persistence of viral RNA in the PBMCs of patients who had cleared their viremia either spontaneously or

after antiviral therapy. HCV RNA can be detected in PBMCs instead of liver biopsy in about 70% of patients with an OCI ***(Carreño et al., 2012).***

OCI can be found in some high-risk people like hemodialysis and kidney transplanted patients, cryptogenic liver disease, and immune-deficient patients. But also, some data have been reported about the presence of OCI among healthy people without any liver disease. HCV genotypes 1 and 4 are the most genotypes involved in the OCI ***(Carreño et al., 2012; Youssef et al., 2012).***

OCI has been defined in two different forms: cryptogenic and secondary. Cryptogenic OCI: if the patient has no anti-HCV antibodies but has elevated liver enzymes ***(Castillo et al., 2004).*** Secondary OCI: if the patient has anti-HCV antibodies, has normal liver enzymes and had cleared his HCV infection either spontaneously or after anti HCV therapy ***(Pham et al., 2004).***

In the last few years, the combination of pegylated interferon (PEG-IFN) and ribavirin (RBV) was the only available treatment option for HCV genotype 4 (HCV-4) infection. later on replaced by other treatment options such as direct acting antivirals (DAAs). Now, combinations of DAAs are commonly used for the treatment of HCV-4 infection due to higher cure rates, shorter treatment period, a higher genetic barrier, and minimal adverse events (AEs). One of the most

effective and commonly used combinations of (DAAs) for the treatment of HCV-4 is the combination of sofosbuvir (SOF) and daclatasvir (DCV) for 3 months ***(Abdel-Razek and Waked, 2015).*** DCV is HCV nonstructural protein NS5Ainhibitor ***(Yang and Kao, 2016),*** while SOF is a nucleotide analogue inhibitor ***(Bertino et al., 2016).***

AIM OF THE STUDY

The aim of the study is to detect the prevalence of occult hepatitis C virus infection in patients who achieved a sustained virologic response (SVR) to direct- acting antiviral agents and to outline predictors of OCI.

Chapter (1)

HCV INFECTION

Hepatitis C virus (HCV) mainly attacks the liver cells**.** The acute stage of hepatitis passes mostly without manifestations, but the chronic stage can cause hepatic fibrosis and cirrhosis, that has mostly occurred after several years. Some cirrhotic patients may develop liver failure, hepatocellular carcinoma (HCC) or fatal bleeding from esophageal or gastric varices ***(Westbrook and Dusheiko, 2014).***

1- Epidemiology:

Globally, an estimated 130–150 million people are living with HCV infection (chronically infected), and more than 700,000 were estimated to have died from HCV-related liver disease in 2013. Although the quality of epidemiologic data varies widely across countries and regions, the most recent global estimates indicate that the prevalence of HCV infection is <1.5% in many developed countries, including the United States. The prevalence is higher (≥1.5%) in several countries in Latin America, Eastern Europe and the former Soviet Union, and certain countries in Africa, the Middle East, and Asia; the prevalence is reported to be highest in Egypt. The most frequent current mode of transmission in the United States and most developed countries is through sharing drug-injection equipment. In countries where HCV is more common (≥1.5% prevalence), the predominant mode of transmission is from unsafe injections

and other health care exposures where infection control practices are poor. Travelers' risk for contracting HCV infection is generally low ***(Deborah et al., 2014).***

In Egypt, The death rate due to liver disease about 40,000 each year (near 10% of all deaths) ***(Amer and Yousef, 2015).***

Six genotypes of HCV with different subtypes have been identified. The most worldwide epidemic genotypes are 1–3 ***(Islam et al., 2015).***

HCV-4 is prevalent among Middle East and Africa causing more than 80% of HCV infections and has recently spread to several European countrie ***(Kamal and Nasser, 2008).***

Egypt has the highest prevalence of HCV genotype 4 (particularly subtype4a), which is responsible for almost 90% of the total HCV infections ***(Chaabna et al., 2018).***

2- HCV Transmission:

HCV infection is mainly transmitted by intravenous route through contact with infected blood, blood products transfusion, contaminated drug injections and intravenous drug use ***(Yang and Roberts, 2010).*** Exposure to unsafe healthcare practice, including hemodyalysis, has been reported to be one of the most important risk factors for HCV infection, even in western countries ***(Martínez-Bauer et al., 2008).*** Very few

infections transmitted by perinatal and sexual transmission are reported ***(Alter, 2007).***

3- Clinical Presentation:

A- Acute infection:

15% of the patients present with acute manifestations. The manifestations are mostly simple and non-specific as reduced appetite, fatigue, nausea, muscle or joint pains and decreased weight. Acute liver failure may occur rarely. Jaundice occurs in few cases only. The infection ends without treatment in 10-15% of patients who are young and female ***(Westbrook and Dusheiko, 2014).***

B- Chronic infection:

Chronic infection means persistent infection and viral replication more than six months. 80% of infected persons become chronically infected. Most cases had minimal or no manifestations in the first few decades of the infection. Chronic HCV infection may come with fatigue and mild cognitive symptoms. After many years, chronic infection may progress to cirrhosis or malignancy. Following manifested cure delayed relapses may occur, but this is not differentiated easily from re-infection ***(Zaltron et al., 2012).***

C- Long term complications:

10-30% of chronic infections progress to cirrhosis in thirty years. Cirrhosis risk increases with concomitant HBV