Evaluation of Hepatitis C Virus Core Antigen Titre Performance In Comparison To HCV RNA RT-PCR In Diagnosis And Follow Up Of Hepatitis C Chronic Patients Treated with Pegylated Interferon & Ribavirin

Thesis

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LIST OF ABBREVIATIONS

5'UTR:	5 'Untranslated region
Aa:	Amino Acid
AASLD:	American association of liver disease
AFP:	Alpha feto protein
Ag:	Antigen
ALT:	Alanine Amine transferase
AST:	Aspartate Amine transferase
b-DNA:	Branded DNA
CA:	Core antigen
cDNA:	Complementary Deoxyribo nucleic acid
c-EVR:	Complete early virological response
CIA:	Chemiluminescent Assay
CIs:	Confidence Intervals
CT:	Computerized topographyG
CTLs:	Cytotoxic t.lymphocyte
CTM:	Cobas TaqMan
CVs:	Coefficients of Variation
DAAs:	Direct Acting antiviral agents
EIA:	enzyme immunoassay
ELISA:	Enzyme linked Immunosorbent assay
ESRD:	End Stage Renal Disease
ETR:	End of treatment response
EVR:	Early virological response

LIST OF ABBREVIATIONS (CONT...)

FDA:	Food and drug administration
G-GT:	Gamma –Glutayl transpeptidase
HAART:	Highly active antiretroviral therapy
HC cAg:	HCV –core antigen
HCC:	Hepatocellular carcinoma
HCV G4:	HCV genotype 4
HLA:	Human Leukocytic Antigen
HPS:	High Pure System
IFN:	Interferon
IgG:	Immunoglobulin G
IMPDH:	Inosine monophosphate dehydrogenase
IRES:	Internal ribosome entry site
IRMA:	Immunoradiometric assay
LIPA:	Line probe assay
MRI:	Magnetic resonance assay
NANBH:	Non A NON B hepatitis
NAT:	Nucleic acid technique
NHANES:	Nutrition examination survey
NS proteins :	Non structural protein
ORF:	Open reading frame
ORs:	Odds Ratio
PCR:	Polymerase chain reaction
PEG:	polyethylene glycol
p-EVR:	Partial early virological response
PT:	Prothrombin time
RIBA :	Recombinant Immunoblot assay
RNA:	Ribonucleic acid

LIST OF ABBREVIATIONS (CONT...)

RT-PCR:	Reverse transcriptase polymerase chain reaction
RVR:	Rapid virological response
SD:	Standard Deviation
SIA:	Strip Immunoassay
SVR:	Sustained virological response
Th2:	Type 2 t-helper cell
TMA:	Transcription mediated amplification
TSH:	Thyroid Stimulating Hormone
ULN:	Upper Limit Normal
UTR:	Untranslated region
WHO:	World Health organization

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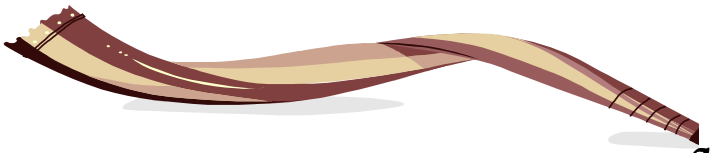

Ahmed Fouad Helmy

INTRODUCTION

The World Health Organization (WHO) estimated that worldwide 180 million people or 3% of the world's population is infected with hepatitis C virus (HCV) ***(WHO, 2000).*** In the United States, nearly 2% of the population is infected (***Armstrong et al, 2006***). In Europe, an estimated 4 million people are chronic HCV carriers with a prevalence of less than 0.1% in northern parts of Europe, increasing to >1% in the south (***Desenclos et al, 2003***). The prevalence of HCV infection is greater in Africa and Asia as infection rates exceed 5% ***(Lavanchy, 2009).*** Egypt has the highest prevalence of hepatitis C in the world with prevalence rates of 14 % of the population, equating to about 12 million infected Egyptians ***(Lavanchy, 2009; MOH, 2007).***

HCV is a leading cause of chronic liver disease in many countries ***(Chen& Morgan, 2006).*** Acute HCV infection is mostly asymptomatic and rarely recognized clinically. Spontaneous viral clearance occurs in approximately one in four individuals with acute HCV ***(Micallef et al, 2006)***. The striking feature of HCV infection is its tendency toward

persistence and development of chronic hepatitis ***(Hoofnagle, 2002)***. Some patients with chronic HCV are at increased risk of developing liver cirrhosis and hepatocellular carcinoma (HCC) and will eventually develop serious squeals ***(Seeff, 2007)***.

The diagnosis of HCV infection can be made by detecting either anti-HCV or HCV RNA. Detection of anti-HCV is recommended for routine testing of asymptomatic persons and should include use of both enzyme immunoassay (EIA). In some cases, supplemental or confirmatory testing with an additional, more specific antibody (i.e., RIBA) assay could be used, particularly in settings where detection of HCV RNA using reverse transcriptase polymerase chain reaction (RT-PCR) techniques are not provided. Antibody tests are not able to differentiate acute from chronic or resolved infection; however, the diagnosis of acute disease is suggested by seroconversion characterized by conversion from HCV negative antibody status to positive anti-HCV antibody status (***de Medina M, Schiff ER, 1995***).

The diagnosis of HCV infection can also be made through detection of HCV RNA using RT-PCR techniques. HCV RNA can be detected within one to two weeks after

exposure to the virus, weeks before the onset of ALT elevations or the appearance of anti-HCV ***(Pawlotsky et al,1999).*** In some patients, the detection of HCV RNA may be the only evidence of HCV infection. Quantitative assays for measuring the titer of HCV RNA, including a branched chain DNA assay and a quantitative PCR, are extensively used in monitoring the response of HCV patients to pegylated interferon and ribavirin therapy. Several different nucleic acid detection methods also have been developed to group isolates of HCV based on genotypes ***(Veillon, et al, 2003).*** Although polymerase chain reaction (PCR) assays for HCV RNA are available from several commercial laboratories on a research-use basis, the results may vary considerably between laboratories. Both false-positive and false-negative results can result from improper collection, handling and storage of test samples. In addition, HCV RNA may be detected intermittently during the course of infection, so a single negative PCR result is not conclusive. Furthermore, HCV-PCR is an expensive technique ***(Veillon, et al, 2003).***

Since RNA detection is labor-intensive and expensive, several attempts have been made to replace HCV RNA detection or quantification by other markers. Recently, serum HCV core antigen (CA) has emerged as an enzyme-linked immunosorbent assay-based method capable of quantifying

hepatitis C virus (HCV) core antigen (CA) in serum ***(Gaudy, et al, 2005)***. Some studies have shown that HCV- CA could be an efficient new diagnosis marker for HCV infection that is more stable than HCV RNA and needs no particular precautions for preparation and sample storage ***(Tillmann, et al, 2005)***. Several kits for detection of HCV CA have recently been developed and recently commercialized ***(Seme, et al, 2005).*** However, the sensitivity, specificity, positive and negative predictor values of this assay have not been addequatly evaluated in pretherapeutic and therapeutic follow-up of Egyptian HCV-infected patients infected with HCV genotype 4.

AIM OF THE WORK

The aim of this study is to evaluate the diagnostic performance of the HCV core antigen assay in comparison to quantitative and qualitative HCV-RNA PCR for follow up of chronic hepatitis C Egyptian patients treated with pegylated interferon alpha 2 and ribavirin.