VALIDATION AND SENSITIVITY OF HCV CORE ANTIGEN IN PATIENT WITH HCV INFECTION IN DIAGNOSIS AND MONITORING POST DAA THERAPY

Thesis

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بسم الله الرحمن الرحيم
قَالوُاسُبحَانَك لاَعلمَ لنَا إلا مَاعَلمتَناَ
إنكَ أَنتَ العَلِيمُ الحَكِيمُ
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AASLD	American Association for the Study of Liver Disease
AFP	Alpha Fetoprotein
ALT	Alanine Transaminase
APASL	Asian Pacific Association for the Study of the Liver
ART	Abbott Real-Time
AST	Aspartate Transaminase
CBC	Complete Blood Count
CHC	chronic hepatitis C virus
CS	Caesarean section
CTL	cytotoxic T lymphocytes
CTM	COBAS TaqMan
DAAs	directly acting antivirals
EDHS	Egyptian Demographic Health Survey
EHIS	Egyptian Health Issues Survey
EIAs	enzyme immunoassay
HBsAg	Hepatitis B surface antigen
HCC	hepatocellular carcinoma
HCV	hepatitis C virus
HCV-Ag	hepatitis C core antigen
HCVcAg	HCV core antigen
HCV-RNA	HCV ribonucleic acid
HOMA	Homeostatic Model Assessment
IDUs	Illicit drug users
INR	International Normalized Ratio
IR	Insulin resistance
KAP	
LFTs	Liver Function Tests
LMICs	low to middle income countries
MOC	Model of Care
NAT	nucleic acid techniques
NAT	nucleic acid testing
NCCVH	National Committee for Control of Viral Hepatitis
NPV	negative predictive value
PAT	parenteral antischistosomal treatment
PCR	Polymerase Chain Reaction
PCR	polymerase chain reaction
peg-IFNα	pegylated interferon α
PPV	positive predictive value

PT	Prothrombin Time
PTT	Partial Thromboplastin Time
RAVs	Resistance associated variants
RBV	Ribavirin
SDH	social determinants of health
SOF	Sofosbuvir
SVR	sustained virological response
SVR	sustained virologic response
T2DM	type 2 diabetes mellitus
TE	Transient Elastography
WHO	World Health Organization

ABSTRACT

Background; Successful eradication of hepatitis C virus (HCV) has great impact on the prognosis of HCV-related complications and the associated mortality. The development of the new direct-acting antiviral drugs (DAAs) has revolutionized the treatment of HCV infection. HCV core antigen (HCVcAg) is a recently developed marker that displayed a good correlation with HCV RNA assays, **Aim and objectives;** to evaluate the clinical utility of hepatitis C virus core antigen in detection of chronic hepatitis C virus at pre-treatment and eradication of HCV post DAAs treatment using core antigen ELISA and detection of its sensitivity and specificity in comparison to HCV RNA, **Subjects and methods;** This was Cross sectional study, carried out at El Galaa and Kobri Al koba military hospitals on 90 Egyptian individuals, divided into 3 groups: (Group A); included 30 healthy individual with HCV Ab and HCV RNA negative as a control, (Group B); included 30 patients with chronic HCV infection in pre-treatment, (Group C): included 30 patients who have received anti HCV treatment (DAAs) 12 weeks post treatment, **Result;** There were insignificant correlation between laboratory parameters of the patient group with HCV-cAg before treatment, **Conclusion;** Fast and immediate results, lower costs of the technique are some of the main advantages of HCVcAg. Therefore, ELISA for HCV-cAg can replace the high sensitivity HCV RNA molecular assay to confirm the presence of HCV infection and to monitor treatment outcome especially in low-income countries with limited resources, **Keyword: Hepatitis C virus; hepatitis C virus core antigen; HCV RNA; treatment monitoring.**

INTRODUCTION

Hepatitis C virus infection is one of the foremost causes of chronic liver diseases worldwide, ranging from minimal histological changes to extensive fibrosis and cirrhosis with or without hepatocellular carcinoma (HCC). The number of chronically infected persons worldwide is estimated to be about 160 million, but most are unaware of their infection **(EASL, 2015)**.

The prevalence of hepatitis C virus (HCV) infection in Egypt is the biggest within the planet, it is the most reason behind end-stage liver disease, HCC and liver-related death in Egypt, HCV-associated disease is taken under consideration the foremost indication for liver transplantation **(Gomaa et al., 2017)**.

Virological tests for the diagnosis and management of hepatitis C virus infection, screening enzyme immunoassay (EIAs) and confirmatory immunoblot assays for the detection of anti-HCV antibodies, qualitative and quantitative nucleic acid techniques (NAT) for the detection of HCV RNA and methods for the determination of HCV genotype **(Seme et al., 2005)**.

Due to the expansiveness and high technicality of PCR machinery, it's impractical for low income countries to routinely administer these tests. Thus, an alternate is required for more widespread screening of HCV especially in lower income countries, where HCV is shown to be more prevalent **(Chang et al., 2018)**.

The quantitative HCV RNA tests measure the quantity of viral load within the blood, HCV RNA detection/quantification is indicated for the patient who may undergo antiviral treatment. HCV RNA quantification

should be made by a reliable sensitive assay, and HCV RNA levels should be expressed in IU/ml **(Pawlotsky et al., 2018)**

HCV RNA is transiently negative in persons with acute infection but they're going to still persist to develop chronic infection, HCV RNA are intermittently positive in patient with chronic infection. Special handling of serum sampling collected for testing is vital for accurate results; if specimen isn't collected or transported properly false-negative results is additionally reported, false positive results can occur through contamination **(Bukh et al., 2001)**.

The hepatitis C core antigen is a viral protein, a component of hepatitis C virus, it can usually be found within the bloodstream two weeks after infection **(Bowen and Walker, 2005)**.

HCV core antigen (HCVcAg) testing could also be a possible replacement for nucleic acid testing (NAT). The HCVcAg forms the internal capsid, which is highly conserved and antigenic among all HCV genotypes and quasispecies **(Forns et al., 1999)**.

During viral assembly, nucleocapsid peptides 22 (p22) are released into plasma and can be detected before antibody and throughout the course of infection **(Easl, 2018)**.

Normal level of HCVcAg using (ELISA technique) is 0.5 PEIU/ML-200 PEIU/ML **(Frieman et al., 2016)**.

HCV core antigen testing is a simple and less expensive than viral-loading testing. Core antigen testing is going to be used to detect acute HCV infection or to verify chronic HCV infection **(Tanaka et al., 2000)**.

HCV core antigen testing is often used to identify active infection, to monitor treatment response, to monitor long term sustained virological response (SVR) and to detect re-infection **(Mannus et al., 2017)**.

The HCVcAg assays with signal amplification have high sensitivity, high specificity, and good correlation with HCV RNA levels greater than 3000 IU/ML and have the potential to replace NAT in settings with high HCV prevalence **(Freiman et al., 2016)**.

HCV core antigen testing is rapid, giving results in approximately 60 min, and less expensive than HCV RNA methods, and includes a proposed limit of detection between 1000 IU/ml and 3000 IU/ml according to HCV genotype.

Measurement of HCV core antigen level by EIA can be used as alternative to HCV RNA to monitor efficacy during and after therapy when HCV RNA assay aren't available or not affordable **(Chevaliez, 2001)**.

*Aim OF The Wor*k

The aim of this work is to evaluate the clinical utility of hepatitis C virus core antigen in detection of chronic hepatitis C virus at pre-treatment and eradication of HCV post DAAs treatment using core antigen ELISA and detection of its sensitivity and specificity in comparison to HCV RNA

Chapter (1)

Hepatitis C Virus Infection

Introduction

HCV, and its long-term resultant consequences, is a major endemic medical health problem in Egypt. Having taken a representative sample of the country, from both urban and rural areas, an Egyptian demographic health survey conducted in 2008 concluded that 14.7% of the population have been infected, making this the highest prevalence in any population in the world. In the Nile Delta and Upper Egypt, infection rates can be much higher at around 26% and 28%, respectively. With incidence rates between 2 and 6 per 1000 every year, this leads to an estimated 170,000 new cases every year to add to the 11.5 million patients suffering from the disease **(Elghitany, 2019).**

I. What is HCV?

HCV is a hepatotropic RNA virus of the genus Hepacivirus in the Flaviviridae family. The virus exists as an enveloped, positive-stranded RNA virus which is ~50 nm in size (**Figure** 1). The HCV RNA strand is made up of ~9600 nucleotide bases and is covered by an icosahedral nucleocapsid which is further surrounded by a lipid bilayer and glycoproteins. HCV is grouped into 6 major genotypes that exhibit at least 30% variation in nucleotide sequence from one another. This genetic variation within the population is a powerful selection mechanism for resistance to both medicinal drugs and evasion of the immune system. The most common HCV RNA genotype in Egypt is genotype 4, representing >85% of all HCV cases in Egypt **(Badawi et al., 2018).**