Evaluation of Serum Fibrosis Markers CTGF and IL-17 Versus Liver Biopsy for Detection of Hepatic Fibrosis in Egyptian Patients with Chronic Hepatitis C

Thesis

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Mar ian Maher

To My Dear Parents

My Beloved Husband

My Beautiful Clara and Karen

And My Supporting Brothers

Contents

List of Abbreviations

a a ..... Amino acids

ALT ..... Alanine aminotransferase

APRI ..... AST to patelet ratio index

AST ..... Aspartate aminotransferase

AT ..... ActiTest

AUC ..... Area Under Curve

BMI ..... Body mass index

CBC ..... Complete blood picture

CCN ..... Cysteine rich angiogenic protein Connective tissue growth factor Nephroblastoma blastoma overexpresssed

CD ..... Cluster of differentiation

cDNA ..... Complementary DNA

CT ..... C-terminal domain

CT ..... Cycle threshold

CTGF ..... Connective tissue growth factor

EDHS ..... Egyptian Demographic Health Survey

EIA ..... Enzyme immunoassays

ELISA ..... Enzyme-linked immunosorbent assays

ECM ..... Extracellular matrix

EMT ..... Epithelial-to-mesenchymal transition

ANOVA .... Analysis of variation

FDA ..... Food and drug adminstration

FT ..... FibroTest

GGT ..... γ-glutamyl-transferase

H & E ..... Hematoxylin and eosin

HBV ..... Hepatitis B virus

HCC ..... Hepatocellular Carcinoma

HCV ..... Hepatitis C virus

HIV ..... Human immunodeficiency virus

HSC ..... Hepatic stellate cells

IDUs ..... Injection drug users

IL-17 ..... Interleukin 17

IHA ..... Indirect heamagglutination

iTh ..... Innate T helper

IU ..... International units

INF ..... Interferons

INR ..... International normalization ratio

IGFBP ..... Insulin-like growth factor binding protein

kDa ..... kilo Dalton

mDC ..... Myeloid dendritic cells

MFB ..... Myofibroblasts

MAP kinase ... Mitogen-activated protein kinase

MRP2 ..... Multidrug resistance-associated protein 2

MS ..... Multiple sclerosis

NPV ..... Negative predictive value

O.D ..... Optical density

OR ..... Odds ratio

***P*-value .....** Probability that statistics results get by chance

PAT ..... parenteral-antischistosomal-therapy

PCR ..... Polymerase chain reaction

PPV ..... Positive predictive value

PT ..... Prothrombin time

r ..... Person correlation coefficient

RA ..... rheumatoid arthritis

Rn ..... Rox normalization

ROC ..... Receiver Operating Characteristics

ROS ..... reactive oxygen species

SD ..... Standard deviation

SPSS ..... Statistical Package for Social Science

t-test ..... Student t-test

TMA ..... Transcription mediated amplification

TGF ..... Transforming growth factor

Th ..... T helper

TMA ..... Transcription mediated amplificated

***U* .....** Mann-Whitney U Test

µg ..... Microgram

µL ..... Microlitre

µmol ..... Micromole

µM ..... Micromolar

vWC ..... von Willebrand type C repeats

WISPs ..... Wnt-induced secreted proteins

List of Tables

List of Figures

INTRODUCTION

Hepatitis C virus infection, with an estimated prevalence of more than 170 million people infected worldwide, is a major health problem ***(Lauer & Walker, 2001).***

Schistosomiasis is also of significant concern as it is endemic in Egypt ***(Gryseels et al, 2006)***. The presence of both HCV and Schistosoma is of significant concern as patients with co infections have been shown to have higher HCV RNA titers, increased histological activity, greater incidence of cirrhosis/hepatocellular carcinoma, and higher mortality rates than patients suffering from single infections ***(Kamal et al, 2001)***

For evaluation of the severity of the liver disease and diagnostic decision-making, liver biopsy remains the golden standard to date ***(Gebo et al., 2002).***

However, liver biopsy is associated with problems that sometimes limit its applicability as diagnostic procedure. Interpretation and diagnosis could be compromised by sampling errors and observer variability that may lead to under-staging particularly in diseases that exhibit a patchy rather than homogenous distribution within the liver ***(Rousselet et al., 2005).***

In addition, liver biopsy is an invasive and painful procedure, with rare but potentially life-threatening complications. Among the complications of percutaneous liver biopsy are pain (10%-30%), bleeding, biliary peritonitis, and pneumothorax. In large series, mortality has been reported to range from 0.1%-0.01%. Percutaneous liver biopsy is contraindicated in the presence of coagulopathy, thrombo-cytopenia, and ascites ***(Bravo et al., 2001).*** Thus, many patients with CHC are reluctant to undergo liver biopsy and may be discouraged from starting therapy for this reason ***(Castera et al., 2009).***

CTGF is a multi-functional protein that drives many cellular processes, but has received special focus with respect to its fibrotic actions in several organs systems. It is shown that CTGF mediates expression of fibrotic markers during HCV infection. CTGF produced in response to HCV may act locally on nonparenchymal cells, such as HSCs or myofibroblasts as well as hepatocytes to enhance expression of markers that are associated with fibrosis. Recent studies have indicated an association between CTGF and stage of fibrosis in patients with chronic HCV infection and high levels of CTGF in plasma and liver biopsy samples of HCV infected patients ***(Kovalenko et al., 2009)***. Findings demonstrating increased CTGF expression in HCV infected hepatocytes also underscore the importance of hepatocytes in producing CTGF during HCV infection ***(Nagaraja et al., 2012).*** Previous studies have indicated the

contribution of parenchymal liver cells to CTGF production in normal and diseased liver ***(Tong et al., 2009).***

Several reports have shown that the number of Th-17 cells was increased in the portal areas of livers from patients with chronic HCV infection ***(Harada et al., 2009).*** HCV antigen-specific Th17 cells were also induced in the peripheral blood from patients with chronic HCV infection, which were suppressed by virus-induced transforming growth factor-b ***(Rowan et al., 2008).*** However, the role of IL-17 in HCV infection has not been investigated. It is plausible to speculate that IL-17 may play an important role in stimulating liver inflammation during HCV infection, similar to HBV infection ***(Lafdil et al., 2010).***