# Evaluation of Serum Osteopontin as a Predictor of Liver Fibrosis in Chronic Liver Disease

*Thesis*

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بسم الله الرحمن الرحيم
قَالُواْ سُبْحَانَكَ لاَ عِلْمَ لَنَا إِلاَّ مَا
عَلَّمْتَنَا إِنَّكَ أَنتَ الْعَلِيمُ الْحَكِيمُ
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Hoda Omar Ahmed

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# List of Abbreviations

**Ab** : Antibody
**AFP** : Alpha fetoprotein
**Ag** : Antigen
**AH** : Alcoholic hepatitis
**AIH** : Autoimmune hepatitis
**ALD** : Alcoholic liver disease
**ALP** : Alkaline phosphatase
**ALT** : Alanine transaminase
**AMP** : Adenosine mono-phosphate
**ANA** : Antinuclear antibodies
**AST** : Aspartate transaminase
**AUC** : Area under curve
**BCS** : Budd–Chiari syndrome
**CBC** : Complete blood count
**CCI4** : Carbon tetrachloride
**CF** : Cystic Fibrosis
**CL** : Chemiluminescence immunoassay
**CLD** : Chronic liver diseases
**CT** : Computerize Tomography
**ECM** : Extracellular matrix
**EDHS** : Egyptian Demographic Health Survey
**EGF** : Epidermal growth factor
**EIAs** : Enzyme immunoassays
**ELISPOT** : Enzyme-linked immunosorbent spot
**ETA-1** : Early T-cell activation factor
**EtOH** : Ethanol

# List of Abbreviations *(Cont...)*

**GAD** : Glutamic acid decarboxylase
**GGT** : Gamma-glutamyltransferase
**GRDS** : Glycine-arginine-aspartic acid-serine
**GSD** : Glycogen storage diseases
**HBcAg** : Hepatitis B core antigen
**HBeAg** : Hepatitis B e antigen
**HBV** : Hepatitis B virus
**HCC** : Hepatocellular carcinoma
**HCV** : Hepatitis C virus
**HDV** : Hepatitis D virus
**HE** : Hepatic encephalopathy
**HRP** : Horseradish peroxidase
**INR** : International normalization ratio
**IRF** : Interferon regulatory factor-1
**MELD** : Model for end-stage liver disease
**MRI** : Magnetic resonance imaging
**MS** : Multiple sclerosis
N**AFLDs** : Non-alcoholic fatty liver diseases
**NASH** : Non-alcoholic steatohepatitis
**NKT** : Natural killer Tcells
**OPN** : Osteopontin
**PBC** : Primary biliary cirrhosis
**PBMNC** : Peripheral blood mononuclear cells
**PDGF** : Platelet derived growth factor
**PT** : Prothrombin time

# List of Abbreviations *(Cont...)*

**RA** : Rheumatoid arthritis
**ROC** : Receiver Operating Characteristic
**RT**-PCR : Reverse transcriptase polymerase chain reaction
**SGPT** : Serum glutamic pyruvate transaminase
**SMA** : Smooth muscle actin
**TGF** : Transforming growth factor
**TNF-α** : Tumor necrosis factor alfa
**VDREs** : Vitamin D responsive element
**VEGF** : Vascular endothelial growth factor
**WD** : Wilson disease
**WT** : Wild type

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# Introduction

Liver cirrhosis, the end stage of chronic liver diseases (CLD), is a major cause of mortality worldwide, where it accounts for approximately 850,000 death per year. Moreover, The disease is commonly complicated by the development of hepatocellular carcinoma (HCC), which is the fifth most common cancer in the world and the third most common cause of cancer related death among oncological patients ***(Zhao et al., 2008).***

Hepatitis C virus (HCV) infection, the most common cause of CLD, is estimated to affect 170 million individuals worldwide. The published Egyptian Demographic Health Survey (EDHS) in 2009 estimated an overall anti-HCV antibody prevalence of 14.7%.The number of Egyptians estimated to be chronically infected was 9.8% ***(Millera and Abu-Raddad, 2010).***

Although the majority (50-80%) of HCV-infected patients develops chronic infection, the disease progression is quite variable, where only about 15% of those chronically infected will eventually progress to cirrhosis after a median of 20–30 years post infection. Thus, identification of patients with accelerated fibrogenesis is of tremendous importance because early treatment can prevent development of cirrhosis and HCC ***(Ghany et al., 2009).***

Several studies have observed that intrahepatic inflammation, especially lobular and/or peri-portal, is one of the most important determinants of fibrosis progression. The inflammatory reaction, mediated by intrahepatic T-helper 1 (Th-1) cells, leads to activation of stellate cells with excess deposition of extracellular matrix proteins and promotes ongoing tissue damage and fibrogenesis ***(Wathmus et al., 2010).***

Liver biopsy is still the standard and most commonly used procedure in the assessment of liver fibrosis. However, it is an invasive method associated with patient discomfort and in rare cases with serious complications ***(El-Shabrawi and Isa, 2011).*** These limitations of the procedure have prompted a search for non-invasive markers of hepatic fibrosis. Non-invasive procedures such as transient elastography (FibroScan) and serum biomarkers (particularly Fibrometre, Fibrotest, Hepascore and APRI) have been developed in order to avoid biopsy, however, none of the currently available indices has sufficient accuracy to replace liver biopsy in the assessment of hepatic histology in patients with chronic HCV infection ***(Degos et al., 2010).*** The primary limitation of these indices is their inability to identify patients with intermediate stages of fibrosis ***(Zeremski et al., 2009).***

Osteopontin (OPN) is a phosphorylated acid glycoprotein, secreted by multiple cell types including bone, kidney, epithelial

cells and activated immune cells. It is a Th-1 cytokine which acts as multi-functioning factor in cell adhesion and migration, immune and inflammatory response, vascular remodeling and apoptosis. OPN also induces extracellular matrix deposition by binding to type I collagen and fibronectin, contributing to tissue fibrotic process ***(Suzuki et al., 2005)***.

Recent studies have shown that OPN is expressed in activated Kupffer cells (hepatic macrophage) and stellate cells in experimentally liver necrosis. Moreover, elevated OPN levels were found in patients with early stages of HCC and were associated with poorer prognosis ***(Sun et al., 2009).*** These data suggest that OPN is correlated with the progression of liver disease, and hence it might be used as a marker of liver fibrosis and cirrhosis.

# Aim of the Work

The aim of this thesis is to study the clinical utility of osteopontin serum level as a marker for prediction of degree of fibrosis in HCV related chronic liver diseases and to correlate its levels with the results of liver biopsy in patients with no evidence of hepatic cirrhosis or Child-Pugh classification in cirrhotic patients.

# I- Chronic Liver Diseases

## A. Definition:

Chronic liver diseases (CLDs) are characterized by progressive destruction and regeneration of the liver parenchyma leading to fibrosis and cirrhosis. Clinically it is defined as any hepatitis lasting for 6 months or longer ***(Goldberg, 2009).***

## B. Epidemiology:

Cirrhosis affects hundreds of millions of patients worldwide. The overall burden of liver disease in the United States, the vast majority of which is due to chronic disease with fibrosis, exacting an increasing economic and social cost ***(Kim et al., 2002).***

Indeed, in the US cirrhosis is the most common non-neoplastic cause of death among hepatobiliary and gastro-intestinal diseases, accounting for approximately 30,000 deaths per year. In addition, 10,000 deaths are due to liver cancer, the majority of which arise in cirrhotic livers, consistent with a steadily rising mortality rate from hepatic cancer**.** Notably, hepatocellular carcinoma is the most rapidly increasing neoplasm in the US and Western Europe ***(Lefton et al., 2009).***