Role of Anti-modified citrullinated vimentin (anti-MCV) Antibody in Chronic HCV Patients and its Correlation with HCV associated Arthritis

Thesis

Submitted for Partial Fulfillment of Master Degree in Clinical Pathology

By

Asmaa Mahmoud Mohamed
MB BCh.,
Faculty of Medicine - Ain Shams University

Supervised by

Professor/ Shahira Fathy El Fedawy

Professor of Clinical Pathology
Faculty of Medicine- Ain Shams University

Professor/ Hala Ghareeb Mohamed

Professor of Clinical Pathology
Faculty of Medicine- Ain Shams University

Doctor/ Neama Lotfy Mohamed Hassan

Lecturer of Clinical Pathology
Faculty of Medicine- Ain Shams University

Faculty of Medicine
Ain Shams University
2016

Acknowledgement

*First of all, all gratitude is due to **Allah** almighty for blessing this work, until it has reached its end, as a part of his generous help, throughout my life.*

*I wish to express my deepest thanks, gratitude and profound respect to my honored **Professor Dr. Shahira Fathy El Fedawy**, Professor of Chemical and Clinical Pathology, Faculty of Medicine, Ain Shams University. I consider myself fortunate to work under her supervision. Her constant encouragement and constructive guidance were of paramount importance for the initiation, progress and completion of this work.*

*I would like to express my sincere thanks to **Professor Dr. Hala Ghareeb Mohamed**, Professor of Chemical and Clinical Pathology, Faculty of Medicine, Ain Shams University for her great support, facilities, careful supervision, continuous advice and guidance which were the cornerstone for this work.*

*I am also deeply grateful and would like to express my sincere thanks and gratitude to **Dr. Neama Lotfy Mohamed Hassan**, lecturer of Chemical and Clinical Pathology, Faculty of Medicine, Ain Shams University for dedicating much of her precious time to accomplish this work and for her keen advices throughout the study.*

*I also want to thank **Assistant professor Dr. Noran Osama El-Azizi**, Assistant Professor of Internal Medicine and Rheumatology, Faculty of Medicine, Ain Shams University for her precious time and kind help to complete this work.*

Asmaa Mahmoud Mohamed

List of Contents

List of Abbreviations

- ACPA Anti Citrullinated Peptid Antigens
- ACR American College of Rheumatology
- AFA Antifilaggrin antibodies
- AKA Anti-keratin antibodies
- ALT Alanine aminotransferase
- ANOVA Analysis of variance
- APF Antiperinuclear factor
- AS Ankylosing spondylitis
- AST Aspartate aminotransferase
- AUCs Areas under the ROC curves
- CBC Compelet blood picture
- CCP Cyclic citrullinated peptide
- cIMT Carotid intima-media thickness
- CRP C-reactive protein
- CVD Cardiovascular disease
- DAS28 Disease activity score using 28 joint counts
- DMARDs Disease-modifying antirheumatic drugs
- DNA Deoxy nucleic acid s
- ECM Extracellular matrix
- EHM Extrahepatic manifestations
- EIM Extraintestinal manifestation
- ELISA Enzyme-Linked ImmunoSorbant Assay
- EMT Epithelial to mesenchymal transition
- ESR Erythrocyte sedimentation rate
- FSHD Facioscapulohumeral muscular dystrophy
- HCC Hepatocellular carcinoma
- HCQ Hydroxychloroquine
- HCV Hepatitis C virus

List of Abbreviations (Cont.)

- HCV-g4 HCV genotype 4
- HLA Human leukocyte antigen
- HRP Horseradish Peroxidase enzyme
- IBD Inflammatory Bowel Disease
- IF Intermediate filament
- IL Interleukin
- IMO Intermittent mono-oligoarthritis
- INF Interferon
- IQR Interquartile range
- JIA Juvenile idiopathic arthritis
- LDL Low-density lipoprotein
- MC Mixed cryoglobulinemia
- MCV Mutated Citrullinated Vimentin
- MMPs Matrix metalloproteinases
- MTX Methotrexate
- NAFL Non alcoholic fatty liver
- NH3 Ammonia
- NSAIDs Non steroidal anti-inflammatory drugs
- P Significance level
- PAD Peptidylarginine deiminase
- PCR Polymerase chain reaction
- PPV Positive Predicting Value
- PsA Psoriatic arthritis
- PTMs Post-translational modifications
- RA Rheumatoid Arthritis
- RF Rheumatoid factor
- RNA Ribonucleic acid
- ROC Receiver operating characteristic

List of Abbreviations (Cont.)

- SD Standard deviation
- SLE Systemic Lupus Erythromatosis
- SP Symmetrical polyarthritis
- SSZ Sulfasalazine
- SvH Sharp/van der Heijde
- TIMPs Tissue inhibitors of metalloproteinases
- TMB Tetramethylbenzidine
- TNF Tumor necrosis factor
- TNFα Tumor necrosis alpha
- UA Undifferentiated Arthritis
- WHO World Health Organization
- X2 Chi-Square test

List of Tables

List of Tables (Cont.)

List of Figures

Introduction

HCV is a major cause of liver associated diseases all over the world. An estimated 3% of the world's populations (more than 350 million people) are chronically infected with HCV ***(Ahmad et al., 2010)***. Egypt has by far the largest national-level HCV prevalence in the world ***(Miller and Abu-Raddad, 2010)*** and ***(Mohamoud et al., 2013)***. The estimated percentage of the Egyptian population in the 15–59 years age group who are positive for HCV antibody is 14.7%. Over 80% of HCV infections in the Egyptian population are among individuals aged 30 years and above ***(El-Zanaty, 2009)***.

HCV infection is more than just a liver disease and has been associated with numerous dermatologic, hematologic, endocrinologic, respiratory, rheumatic, and autoimmune syndromes. ***Sene et al. (2006)*** and ***Lapiński et al. (2009)*** reported that Rheumatologic complications of HCV infection are vary and include mixed cryoglobulinaemia, vasculitis, sicca symptoms, myalgia, arthritis, and fibromyalgia**.**

Nyingi et al. (2010) showed that hepatitis C-associated arthritis is one of the most common Extrahepatic manifestations (EHMs) of HCV infections. Hepatitis C arthritis can mirror rheumatoid arthritis (RA) symptoms ***(Alessandra et al., 2007)***. Consequently, HCV infection should be considered in the differential diagnosis of patients with atypical arthritis ***(Lormeau et al., 2006)***.

The worldwide prevalence of arthritis presumed to be due to HCV infection has been reported between 2.4 million and 45.9 million people ***(Rosner et al., 2004 & Ogdie et al., 2010)***

The clinical picture of HCV-associated arthritis varies widely, ranging from polyarthralgia to monoarticular or oligoarticular arthritis and symmetric chronic polyarthritis. In particular, monoarticular or oligoarticular involvement affects larger joints and is typically associated with mixed cryoglobulinemia, whereas symmetric polyarthritis associated with HCV infection frequently shows a rheumatoid arthritis (RA)-like clinical picture. However, compared with RA, HCV-associated arthritis is usually less severe and does not cause joint deformities or rheumatoid nodules **(*Abdelkader et al.*, 2012).**

Protein citrullation is involved in the pathogenesis of certain human diseases, the best example is RA. The most specific family of RA antibodies is the antibodies directed against citrullinated proteins (Anti CCP and Anti MCV). These antibodies can be detected in almost 80% of RA with a specificity of 99% making them a very useful diagnostic tool for rheumatologists ***(Van Venrooij et al., 2002 & Abdeen et al., 2011)***. The anti-citrullinated protein antibodies are produced locally in the inflammed synovium and since hepatic stellate cells, which play a pivotal role in hepatic fibrosis, contain vimentin, the scientists hypothesized that protein citrullination of vimentin may also occur in chronic hepatitis and may partly explain the fibrosis seen in this disease ***(Smeets et al., 2002 & Abdeen et al., 2011)***.

Aim of the work

To find out if anti-modified citrullinated vimentin (anti-MCV) antibodies are produced in patients with chronic hepatitis C and if such production is associated with HCV-associated arthritis.

Hepatitis C Virus

Hepatitis C virus (HCV) was discovered in 1989. It is a member of the *Hepacivirus* genus (of the family *Flaviviridae*) that causes hepatitis. The World Health Organization (WHO) estimates that about 3% of the world's population has been infected with HCV. It is well established that HCV is of global importance affecting all countries, leading to a major global health problem that requires widespread active interventions for its prevention and control. Chronic hepatitis C was linked to the development of cirrhosis and hepatocellular carcinoma ***(Lavanchy, 2011)***.

Structure of hepatitis C virus:

HCV is a small (55-65nm in size), enveloped, positive sense, single stranded ribonucleic acid (RNA) virus. The HCV particle consists of a core of genetic material "RNA", surrounded by an icosahedral protective shell of protein, and further encased in a lipid envelope of cellular origin. Two viral envelope glycoproteins, E1 and E2, are embedded in the lipid envelope ***(Moradpour et al., 2007)***, Figure (1).

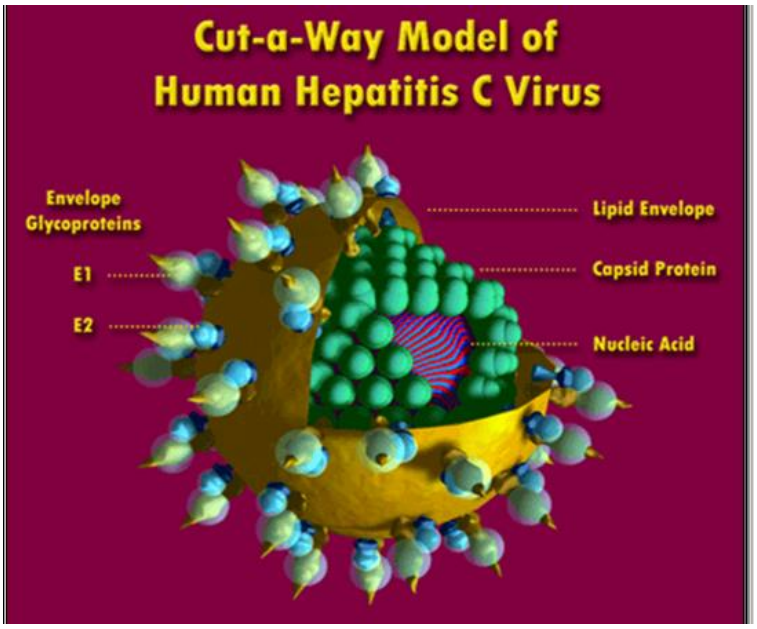

Figure (1): Structure of Hepatitis C Virus ***(www.hepcprimer.com).***

Life cycle of HCV and replication

HCV only infects humans and chimpanzees ***(Shors Teri, 2011)***. Key steps in the life cycle of HCV include entry into the host cell, uncoating of the viral genome, viral genome replication occur inside the nucleus of the host cell. Translation of viral proteins assembly and release of viron occur outside the nucleus of the host cell ***(Sklan, 2010)***.

After entering a susceptible host, HCV invades, infects and replicates within the blood stream, repeating the process in various tissues, as well as in peripheral B and T lymphocytes, as it proceeds to the liver by tropism, passing through various tissues such as those of the pancreas, thyroid, adrenal glands, spleen and bone marrow ***(MacDonald et al., 2002)***.

Since HCV can also directly infect the lymphatic tissue, its stimulation can lead to the development of B-cell

lymphoma. It is known that the liver is the principal site of HCV replication ***(Dustin and Rice, 2007)***. Infection with HCV at extrahepatic sites can promote the appearance of HCV variants, thereby decreasing the chance that the immune system will recognize the virus. Circulating HCV particles bind to receptors on the surface of hepatocytes and subsequently enter the cells ***(Lindenbach and Rice, 2005)***.

Genotypes of HCV:

Like many other RNA viruses, HCV has a high genetic heterogeneity. The estimated mutation rate in the human organism is 1.92×10^{-3} nucleotide per site per year ***(Genovese et al., 2005)***.

This genetic diversity resulted in the classification of HCV into seven genotypes (1-7) within each genotype there are at least two or three subtypes represented by letters (1a, 1b, 2a, 2b, 2c, etc.) have been identified based on the analysis of the NS5 region. Subtypes are further broken down into quasispecies based on their genetic diversity ***(Nakano et al., 2011)***. HCV circulates as a population of different but closely related genomes, known as quasispecies, whose sequences differ only by a few nucleotides. Quasispecies is defined as a heterogeneous population of individual virions, each of which may be different in at least one genomic site ***(Lau et al., 2006)***.

Different HCV genotypes are common to different areas of the world and different groups of people. For example, genotype 1a and 1b accounts for 70 to 75 % of all HCV infections in the United States; genotype 2 is most commonly found in people with HCV in Italy, North Africa and Spain; genotype 3a is believed to be the predominant