Faculty of Science
Ain Shams University
Department of Inorganic Chemistry

Evaluation of Albumin messenger Ribo Nuclear Acid (mRNA) by Polymerase Chain Reaction (PCR) in Comparison to Alpha Feto Protein (AFP) by Chemiluminescence in Liver Disease

A Thesis

Submitted for the degree of Master of Science as a partial fulfillment for Requirements of the Master of Science in Inorganic Chemistry

By

Walaa Abd ElHamid Mahsoub Ahmed

Faculty of Science

Supervisors

Prof. Dr. Eglal Mariam Raymond Souaya

Professor of Inorganic and Analytic Chemistry
Faculty of Science – Ain Shams University

Prof. Dr. Eman Saleh El-Hadidi

Professor of Clinical Pathology
Faculty of Medicine – Ain Shams University

Dr. Amr Ali Mohammed

Lecturer of Inorganic Chemistry
Faculty of science – Ain Shams University

Faculty of Science
Ain Shams University
2016

Plasma albumin m RNA as anon invasive Marker to predict liver injury in chronic Hepatitis C and Hepatocellular carcinoma patients

ABSTRACT

Background*:** Analysis of circulating nucleic acids in plasma, such as cell free RNA offers an avenue for non invasive monitoring of a variety of physiological and pathological conditions. ***Aims: Because albumin is the most abundant protein in the body and is synthesized by the liver, the current study was designed to assess plasma albumin mRNA (ALB mRNA), as a non invasive diagnostic marker of liver injury in chronic HCV (CHC) and hepatocellular carcinoma (HCC).

Patients and Methods: The study included 50 patients, 20 patients had CHC and 20 were of HCC as well as 10 healthy control subjects. Patients were subjected to clinical examination, abdominal ultrasonography, CT for HCC cases and laboratory investigations including liver function tests, AFP and plasma albumin mRNA by Real Time- PCR.

Results: Patients with CHC and HCC have a significant increase in their plasma ALB mRNA than controls; the higher level was in HCC cases. At a cut-off >935 copies/ml, plasma ALB mRNA can discriminate liver diseased from healthy subjects, with a sensitivity of 81.5%, and specificity of 96%, while, elevated serum levels of ALT had a sensitivity of 32.3%, and specificity of 92%. However, at a cut off >20 ng/ml alpha feto protein (AFP) had a sensitivity of 55.9% and sensitivity of 91.2%.in diagnosis of HCC.

Conclusion: ALB mRNA in plasma is liver specific; it is increased in liver disease suggesting liver pathology and may be more diagnostically sensitive than alpha-fetoprotein and Alanine aminotransaminase (ALT) serum levels. Thus, future studies should assess if the plasma concentration of ALB mRNA may be used as therapy monitoring.

Keywords: Albumin, HCC, CHC.


وأنزل الله عليك
الكتاب والحكمة
وعلَّمَكَ مَا لَمْ تَكُن
تَعْلَمُ وكَانَ فَضْلُ
اللَّهِ عَلَيْكَ عَظِيماً
صدق الله العظيم
سورة النساء الآية (١١٣)

Acknowledgments

First and foremost, I feel always indebted to ***Allah*** *the Most Beneficent and Merciful.*

I wish to express my deepest gratitude and thanks to **Prof. Dr. Eglal Mariam Raymond Souaya**, *Professor of Inorganic and Analytic Chemistry, Faculty of Science – Ain Shams University, for her constructive criticism, unlimited help and giving me the privilege to work under her supervision.*

My most sincere gratitude is also extended to **Prof. Dr. Eman Saleh El-Hadidi**, *Professor of Clinical Pathology Faculty of Medicine, Ain Shams University, for her enthusiastic help, continuous supervision, guidance and support throughout this work.*

I would like also to thank **Dr. Amr Ali Mohammed**, *Lecturer of Inorganic Chemistry, Faculty of Science – Ain Shams University, for the efforts and time he has devoted to accomplish this work.*

Last but not least, I can't forget to thank all members of my Family, especially my ***Parents*** *and my* ***Husband****, for pushing me forward in every step in the journey of my life.*

Candidate

Walaa Abdel Hamid

CONTENTS

LIST OF ABBREVIATIONS

No.	Abbreviations	Meaning
1	AFB1	Aflatoxin B1
2	AFP	α-Fetoprotein
3	ALP	Alkaline phosphatase
4	ALT	Alanine aminotransferase
5	AST	Aspartate aminotransferase
6	cDNA	Complementary deoxyribonucleic acid
7	CHC	Chronic Hepatitis C
8	CL	Chemiluminescence
9	CLDs	Chronic liver diseases
10	CLIA	Chemiluminescent immunoassay
11	DM	Diabetes mellitus
12	DNA	deoxyribonucleic acid
13	FNAB	Fine needle aspirative biopsy
14	GGT	γ-Glutamyl transpeptidase
15	HA	Hepatic arteriography
16	HAART	Highly Active Anti-Retoviral Therapy
17	HBV	Hepatitis B virus
18	HCC	Hepatocellular carcinoma
19	HCV	Hepatitis C virus
20	hnRNA	Heterogeneous nuclear

		ribonucleic acid
21	IA	Immunoassay
22	IARC	International Agency of Research of Cancer
23	IL	Interleukin
24	LDH	Lactate dehydrogenase
25	LOC	Lab-on-a-chip
26	MRI	Magnetic resonance imaging
27	mRNA	Messenger Ribonuclic acid
28	NASH	Non alcoholic Steatohepatitis
29	PCR	Polymerase Chain Reaction
30	PIHI	Pulse inversion harmonic imaging
31	RNA	Ribonucleic acid
32	RNAP	RNA polymerase
33	rRNA	Ribosomal ribonucleic acid
34	RT-PCR	Reverse transcriptase polymerase chain reaction
35	SD	Standard deviation
36	snRNps	Small nuclear ribonucleo-proteins
37	SPSS	Statistical package for social science
38	THI	Tissue harmonic imaging
39	tRNA	Transfer ribonucleic acid
40	U/S	Ultrasound
41	μTAS	Micro total analysis systems

LIST OF TABLES

LIST OF FIGURES

CHAPTER I

INTRODUCTION

The clinical course of untreated hepatitis C virus (HCV) infection is highly variable with the majority of patients experiencing a slow fluctuating disease that may take 20 years or more for full expression. Approximately half of HCV patients develop chronic active hepatitis and this may progress to liver cirrhosis and hepatocellular carcinoma (HCC) ***(Banker, 2003)***.

Hepatic fibrosis is the accumulation of extracellular matrix, or scar, in response to acute or chronic liver injury. Fibrogenesis represents a wound healing response to injury, and ultimately leads to cirrhosis. Both fibrosis and cirrhosis are the consequences of a sustained wound-healing response to chronic liver injury from a range of causes, including viral, autoimmune, drug induced, cholestatic and metabolic diseases ***(Rockey and Friedman, 2007).***

The development of severe fibrosis and necroinflamatory changes in liver leads to cirrhosis and worsens prognosis by enhancing the risk of HCC. Chronic HCV varies greatly in its course and outcome. The presence

of HCV RNA indicates that the patient has ongoing viral infection despite normal ALT levels ***(Berry et al., 2005).*** Serologic assays detect HCV antibodies that indicate present or previous infection, but they cannot discriminate acute from chronic or resolved infection. Occasionally immuno-compromised patients, hemodialysis patients and patients with mixed cryoglobulinemia have false negative serology results and may require HCV-RNA listing for diagnosis ***(Thio et al., 2000).*** While needle biopsy is still the mainstay in diagnosing hepatic fibrosis, its days of dominance seem limited as laboratory technology and imaging studies improve ***(Thabet et al., 2011).***

The existence of extracellular mRNA in the circulation, i.e., plasma and other body fluids has been long known ***(Swaminathan and Butt, 2006).*** The extracellular mRNA is thought to be released into the circulation from intact and viable cells as well as necrotic cells**.** The biological roles of circulating mRNA are still unclear, although its physiological significance has been investigated during the last several years ***(Fleischacker, 2006)***.

The detection of circulating RNA offers certain advantages over the detection of circulating DNA ***(Zhau et al., 2010).*** First, if both plasma RNA and DNA were derived