Diagnostic value of macrophage inflammatory protein-1 beta (MIP-1β) in serum and ascitic fluid of patients with decompensated HCV related cirrhosis with and without spontaneous bacterial peritonitis

Thesis

Submitted for Partial Fulfillment of Master Degree in Internal Medicine

By:

Sabreen Adly Mohamed

MB.B.Ch. Ain Shams University

Under Supervision of

Prof. Dr. Khaled Abd El Hamid Mohamed

Professor of Internal Medicine, Gastroenterology and Hepatology
Faculty of Medicine, Ain Shams University

Assistant Prof. Dr. Sherif Sadek Taha

Assistant Professor of Internal Medicine, Gastroenterology and Hepatology
Faculty of Medicine, Ain Shams University

Dr. Hosam Samir Elbaz

Lecturer of Internal Medicine, Gastroenterology and Hepatology
Faculty of Medicine, Ain Shams University

Faculty of Medicine
Ain Shams University
2016

سورة البقرة الآية: ٣٢

Acknowledgement

*First, I would like to thank **Allah** a lot for Blessing this work until it has reached its end, as a part of his generous help throughout our life.*

*My profound thanks and deep appreciation to **Prof. Dr. Khaled Abd El Hamid Mohamed**, Professor of Internal Medicine, Gastroenterology and Hepatology, Faculty of Medicine, Ain Shams University for his great support and advice, his valuable remarks that gave me the confidence and encouragement to fulfill this work.*

*I am also thankful to **Assistant Prof. Dr. Sherif Sadek Taha,** Assistant Professor of Internal Medicine, Gastroenterology and Hepatology, Faculty of Medicine, Ain Shams University for his valuable supervision, co-operation and direction that extended throughout this work.*

*I would like to direct my special thanks to **Dr. Hosam Samir Elbaz,** Lecturer of Internal Medicine Gastroenterology and Hepatology, Faculty of Medicine, Ain Shams University, for his invaluable help, fruitful advice, continuous support offered to me and guidance step by step till this essay finished.*

Finally my deep thanks to my family for supporting me throughout my life.

Sabreen Adly Mohamed

List of Contents

Title	Page No.
List of Abbreviations	**i**
List of Tables	**v**
List of Figures	**viii**
Abstract	
Introduction	**1**
Aim of the study	**7**
Review of Literature:	
Chapter (1): Liver cirrhosis	8
Chapter (2): Spontaneous bacterial peritonitis	52
Chapter (3): Macrophage inflammatory protein 1beta	132
Patients and Methods	**148**
Results	**167**
Discussion	**201**
Summary	**249**
Conclusion	**256**
Recommendations	**257**
References	**258**
Arabic summary	**-**

List of Abbreviations

Abb.	Full Term
AASLD	American Association for the study of Liver diseases
AF	Ascitic fluid.
AFLAC	Ascitic fluid lactoferrin.
AFP	Alpha fetoprotein.
AIDS	Acquired immune deficiency syndrome.
ALD	Alcoholic liver disease.
ALF	Acute liver failure.
ALP	Alkaline phosphatase.
ALT	Alanine aminotransferase.
APACHE	Acute Physiology and Chronic Health Evaluation.
APC	Antigen presenting cell.
AST	Aspartate aminotransferase.
ATH	Autoimmune hepatitis.
AUC	Area under curve.
BP	Bacterial peritonitis.
BSG	British society of gastroenterology.
BUN	Blood urea nitrogen.
C3	Complement 3.
CA	Community acquired.
CCL	C-C motif ligand.
CCR	Chemokine receptor.
CD	Cluster of differentiation.
CEA	Carcinoembryonic Antigen.
CK	Chemokines
CNNA	Culture negative neutrocytic ascites.
CRP	C-reactive protein.
CT	Computed tomography.
CXR	Chest X ray.
DIC	Disseminated intravascular coagulopathy.
DNA	Deoxyribonucleic Acid.

dsRNA	Double-stranded RNA.
EASL	European Association for the Study of the Liver.
ECM	Extracellular matrix.
ELISA	Enzyme linked immunosorbant assay.
ESBL	Extended spectrum beta lactamase.
ESLD	End-stage liver disease.
FN	Fibronectin.
GALT	Gut associated lymphoid tissue.
GABA	Gaba-amino butaric acid.
GE	Granulocyte esterase.
GGT	Gama glutamyl transpeptidase.
GNB	Gram negative bacteria.
GPB	Gram positive bacteria.
GTPase	Guanosine triphosphatase.
HBsAg	Hepatitis B surface antigen.
HBV	Hepatitis B virus.
HCA	Health care acquired.
HCC	Hepatocellular carcinoma.
HCV	Hepatitis C virus.
HE	Hepatic encephalopathy.
HIV	Human immune deficiency virus.
HRP	Horseradish peroxidase.
HRS	Hepatorenal syndrome.
HT4	Hydroxy tryptophan 4.
ICU	Intense care unit.
Ig	Immunoglobulin.
IL	Interleukin.
INR	International normalized ratio,
LC	Liver cirrhosis.
LDH	Lactate dehydrogenase
LE	Leukocyte esterase.
LFT	Liver function tests.

LLQ	Left lower quadrant.
LPS	Lipopolysaccharide.
LT	Liver transplantation.
MCP	Macrophage chemoattractant protein.
MLNs	Mesentric lymph nodes.
MELD	Model for End-Stage Liver Disease.
MIP-lß	Macrophage inflammatory protein 1 beta.
MMP	Matrix metalloproteases.
MODS	Multiple organ dysfunction score.
MOF	Multiple organ failure.
MPI	Mannhiem peritonitis index.
MPV	Mean platelet volume.
MR	Magnetic resonance.
MRI	Magnetic resonance imaging.
mRNA	Messenger RNA.
MRSA	Methicilin resistant staph aureus.
MSCT	Multi-Slice CT.
NAFLD	Nonalcoholic fatty liver disease.
NASH	Non alcoholic steatohepatitis.
NGAL	Neutrophil gelatinase associated lipocalin.
NK	Natural killer cell.
NO	Nitric oxide.
NOD	Nucleotide oligomerisation domain.
NSBB	Non selective beta blocker.
OD	Optical density.
OR	Odds ratio.
OS	Overall survival.
PAMP -rn.	Pathogen associated molecular pattern.
PBC	Primary biliary cirrhosis.
PCT	Procalcitonin.
PDW	Platelet distribution width.
PMN	Polymorph nuclear cells.

PNL	Polymorph nuclear leukocyte.
PPI	Proton pump inhibitor.
PRR	Pattern recognition receptor.
PSC	Primary sclerosing cholangitis.
PT	Prothrombin time.
PVT	Portal vein thrombosis.
ROC	Receiver operating characteristics.
RNA	Ribonucleic acid.
RP-nano-HPLC- ES1-MS/MS:	Reverse phase nano-high performance liquid chromatography electro spray ionization tandem mass spectrometry.
SAAG	Serum ascitic albumin gradient.
SAPS	Simplified acute physiology score.
SBP	Spontaneous bacterial peritonitis.
SNPs	Small nuclear polypeptides.
SOFA	Sepsis related organ failure assessment.
TACE	Trans Arterial Chemoembolization.
TGFß1	Transforming growth factor beta 1.
Th	T helper cell.
TLC	Total leucocytic count.
TLR	Toll like receptor.
1MB	Tetramethylbenzidine.
TNF	Tumor necrosis factor.
UA	Urine analysis.
UK	United Kingdom.
US	Ultrasonography.
USA	United States of America.
VCAM	Vascular cell adhesion molecule.
WBCs	White blood cells.
WHO	World health organization.

List of Tables

Table No.	Title	Page No.
Table (1):	Child-Pugh Score	46
Table (2):	SBP and other varieties of ascitic fluid infections.	57
Table (3):	Clinical setting of SBP, related definitions and commentary.	77
Table (4):	Differential diagnosis of ascites based on the serum - ascitic albumin gradient (SAAG)	88
Table (5):	Descriptive analysis of the study population as regard the ages.	168
Table (6):	Descriptive analysis of the study population as regard the sex.	168
Table (7):	Descriptive analysis of the study population as regard smoking status	169
Table (8):	Comparison between both groups as regard age.	170
Table (9):	Comparison between both groups as regard sex.	171
Table (10):	Comparison between both groups as regard smoking status.	172
Table (11):	Comparison between both groups as regard modified Child-Pugh score.	173
Table (12):	Comparison between both groups as regard MELD score.	174
Table (13):	Comparison between both groups as regard degree of ascites (clinical & PAUS).	175

Table (14): Comparison between both groups as regard splenomegaly. 176

Table (15): Comparison between both groups as regard long term use of PPI. 177

Table (16): Comparison between both groups as regard regular NSBB intake. 178

Table (17): Comparison between both groups as regard renal impairment. 179

Table (18): Relation between regular users of NSBB in both groups, their modified Child-Pugh score and who developed renal impairment in both groups. 180

Table (19): Comparison between both groups as regard different clinical presentations. 182

Table (20): Comparison between both groups as regard different Lab values: 183

Table (21): Comparison between both groups as regard the different inflammatory markers in serum. 185

Table (22): Comparison between both groups as regard the different values in ascitic fluid: 186

Table (23): Different clinical presentations of group A patients. 189

Table (24): Bacterial growth in ascitic fluid of group A patients 190

Table (25): Incidence of bacteremia in patients with positive ascitic culture. 191

Table (26): Comparison between incidence different types of bacteria in culture positive patients in group A. 192

Table (27): Comparison between antibiotic susceptibility of different types of bacteria in culture positive patients in group A. .. 192

Table (28): Correlation between ascitic PMN and all other parameters in SBP group. 194

Table (29): Comparison between both groups as regard the MIP-1β values in serum and ascitic fluid. 195

Table (30): Correlation between ascitic and serum MIP-1β and all other parameters in SBP group..... 197

Table (31): Diagnostic performance of ascitic MIP-1β in discriminating groups A (SBP) from group B (liver cirrhosis). 198

Table (32): Diagnostic performance of serum MIP-1β in discriminating groups A (SBP) from group B (liver cirrhosis). 199

Table (33): Comparison between serum-ascites differences of MIP-1β in both groups. 200

List of Figures

Figure No.	Title	Page No.
Figure (1):	Histopathology of cirrhotic liver.	10
Figure (2):	Gross picture of liver cirrhosis (Jonas et al., 2001)	26
Figure (3):	Spontaneous bacterial peritonitis (SBP)-associated mortality.	105
Figure (4):	Key elements driving development of bacterial resistance and risk of treatment failure.	115
Figure (5):	Evidence-based algorithm for evaluation of at-risk patients, diagnosis and management of SBP.	129
Figure (6):	Cells attraction towards concentration of chemokines.	134
Figure (7):	Three dimensional structure of chemokines.	136
Figure (8):	Structure of different chemokine classes.	138
Figure (9):	Three dimensional structure of Human Mip-1β dimer chemokine (C-C motif) ligand 4.	143
Figure (10):	10 double dilution series of MIP-1β	159
Figure (11):	Human MIP-1 beta is measured using sandwich immunoassays	163
Figure (12):	Standard curve of human Macrophage Inflammatory Protein 1 Beta (MIP1b) ELISA Kit.	164
Figure (13):	Descriptive analysis of the study population as regard the sex	169
Figure (14):	Comparison between both groups as regard age.	170

Figure (15): Comparison between both groups as regard sex. 171

Figure (16): Comparison between both groups as regard smoking status. 172

Figure (17): Comparison between both groups as regard modified Child-Pugh score. 173

Figure (18): Comparison between both groups as regard MELD score. 174

Figure (19): Comparison between both groups as regard degree of ascites (clinical & PAUS). 175

Figure (20): Comparison between both groups as regard splenomegaly (clinical & PAUS). 176

Figure (21): Comparison between both groups as regard long term use of PPI. 177

Figure (22): Comparison between both groups as regard regular NSBB intake. 178

Figure (23): Comparison between both groups as regard renal impairment. 179

Figure (24): Comparison between both groups as regard different clinical presentations. 182

Figure (25): Comparison between different clinical presentations in group A. 190

Figure (26): Bacterial growth in ascitic fluid of group A patients. ... 191

Figure (27): Receiver operating curve (ROC) curve analysis of ascitic MIP-1β between patients in group A and B. 198

Figure (28): Receiver operating curve (ROC) curve analysis of ascitic MIP-1β between patients in group A and B. 199

ABSTRACT

Background:

Spontaneous bacterial peritonitis (SBP) is a life-threatening infection and considered one of the most serious cirrhosis complications because of its frequency, morbidity and mortality rates (10:30%) and its unclear clinical picture; different laboratory diagnostics play a pivotal role for rapid and effective management of SBP patients.

Objective of the study:

The aim of the study is to assess and evaluate the potential diagnostic efficacy of macrophage inflammatory protein type 1 beta (MIP-1β) in cirrhotic patients and its usefulness in the diagnosis of SBP.

Patients and methods:

60 Egyptian patients were evaluated into two groups; Group A: 30 patients with HCV related liver cirrhosis with ascites proved to have SBP (PNL≥250/mm³ in AF) and Group B: 30 patients with HCV related liver cirrhosis with ascites but without SBP (PNL<250/mm³ in AF and negative ascites fluids culture). Different inflammatory markers and MIP-1β in sera and AF in patients of both groups were measured.

Results:

MIP-1β was significantly increased in AF in patients with SBP versus non SBP ($P \leq 0.001$).At the best cut off value 113 pg/ml, diagnostic performance of ascetic MIP-1β in discriminating SBP; the sensitivity of the test was 77.33% while the specificity was 83.33%. While at the best cut off value 123 pg/ml, diagnostic performance of serum MIP-1β in discriminating SBP from non SBP; the sensitivity of the test was 76.67% while the specificity was 80%.

Conclusion:

MIP-1β is a useful marker in the diagnosis of SBP in cirrhotic patients with satisfactory sensitivity and specificity. Chemokines should be further explored for diagnostic use.

Keywords: Spontaneous bacterial peritonitis, Macrophage inflammatory protein type 1 beta, Ascitic fluids, Chemokines.

INTRODUCTION

Ascites remains the commonest of the three major complications of advanced or decompensated cirrhosis (along with hepatic encephalopathy and variceal hemorrhage) and according to a prognostic model proposed by D′Amico and co-workers, the occurrence of varices initiates the second stage of cirrhosis, the third stage is defined by the development of ascites and variceal hemorrhage initiates the fourth stage ***(D'Amico G et al, 2013).***

The occurrence of bacterial infection, which delineates an additional fifth stage of cirrhosis, termed the "critically ill" patient with cirrhosis, as it increases mortality of patients with decompensated cirrhosis ***(Arvaniti Vet al, 2012)***.

Spontaneous Bacterial Peritonitis (SBP) is an ascitic fluid infection without a definitive, surgically treatable, intra-abdominal source such as perforation or inflammation of intra-abdominal organs, accounting for 10 to 30% of cirrhotic patients with ascites ***(Wiest R et al, 2012)***.