Evaluation of Neutrophilic Functions in Patients with Post Hepatitic Liver Cirrhosis

Thesis

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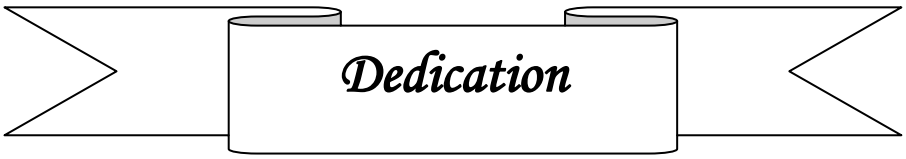


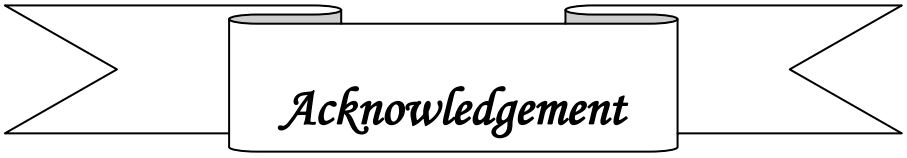
To my beloved family…….

To my father God bless his soul, who has been always a source of support, protection and encouragement, who guided me all the way, who motivated me to stay tough and strong passing every obstacle and facing everyhard situation.

To my mother may God keep her in a great shape and health, whois a source of enlightenment, love and containment, who is a great raw model for me.

To my brother and my sister…….

Wish to be good enough for you and for you I dedicate this work…….



First and for most thanks to Allah, the most Merciful.

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ALL: Acute Lymphoblastic Leukemia.
ALP: Alkaline Phosphatase.
ALT: Alanine Aminotransferase.
AML: Acute Myelocytic Leukemia.
AMM: Advia Ammonia Method.
AMPD: Amino-2-Methyl-1, 3- Propanediol.
APC: Antigen Presenting Cells.
ARDS: Acute Respiratory Distress Syndrome.
AST: Aspartate Aminotransferase.
BM: Bone Marrow.
BAP: Bone-Type Alkaline Phosphatase.
BT: Bacterial Translocation.
C. albicans: Candida albicans.
CT. Scan: Computed Tomography Scan.
CAIDS: Cirrhosis-Associated Immune Dysfunction Syndrome.
CML: Chronic Myeloid Leukemia.
CPT: Child-Pugh-Turcotte.
DIC: Disseminated Intravascular Coagulopathy.
E. coli: Escherichia coli.
EDTA: Ethylene Diaminetetra Acetic Acid.
EEG: Electroencephalogram.
FBB: Fast Blue BB Base.
FITC: Fluorescein Isothyocyanate.
FRV: Fast Red Violet.
GABA: Gamma Amino Butyric Acid.
G-CSF: Granulocyte Colony Stimulating Factor.
GI: Gastrointestinal.
GLDH: Glutamate Dehydrogenase.
HAP: Hepatic Alkaline Phosphatase.
HBV: Hepatitis B Virus.

HCC: Hepato Cellular Carcinoma.
HCL: Hairy Cell Leukemia.
HCV: Hepatitis C Virus.
HE: Hepatic Encephalopathy.
HIV: Human Immunodeficiency Virus.
HSC: Hepatic Stellate Cells.
IS: Immune System.
IAP: Intestinal Isoenzyme Alkaline Phosphatase.
INR: International Normalized Ratio.
LAP: Leucocyte Alkaline Phosphatase.
LAPA: Leucocyte Alkaline Phosphatase Activity.
LB base: Lennox L Broth Base.
LPS: Lipopolysaccharides.
LSEC: Liver Sinusoidal Endothelial Cells.
MAMPs: Microbe-Associated Molecular Patterns.
MLN: Mesenteric Lymph Nodes.
MPCs: Mesenchymal Precursor Cells.
MRI: Magnetic Resonance Imaging.
NAD: Nicotinamide Adenine Dinucleotide.
NADPH: Nicotinamide Adenine Dinucleotide Phosphate.
NAP: Neutrophil Alkaline Phosphatase.
NASH: Non Alcoholic Steato-hepatitis.
NBT: Nitro Blue Tetrazolium.
NCCLS: National Committee for Clinical Laboratory Standards.
NHL: Non-Hodgkin's Lymphoma.
NK: Natural Killer Cells.
NKT: Natural Killer T-Cells.
OB: Oxidative Burst.
P. aeruginosa: Pseudomonas aeruginosa.
PAMPs: Pathogen Associated Molecular Patterns.
PBS: Phosphate Buffered Saline.

PLAP: Placental-specific Alkaline Phosphatase.
PMN: Polymorph Nuclear Neutrophils.
PNH: Paroxysmal Nocturnal Hemoglobinuria.
PV: Polycythemia Vera.
RE: Reticuloendothelial.
RES: Reticulo-endothelial System.
ROC: Reciver operating characteristic.
ROS: Reactive Oxygen Species.
RPM: Revolutions Per Minute.
SBP: Spontaneous Bacterial Peritonitis.
SD: Standard Deviation.
TLR: Toll-Like Receptors.
TNAP: Tissue-nonspecific ALP.

Introduction

Cirrhosis of the liver refers to scarring of the liver which results in abnormal liver function as a consequence of chronic liver injury. Cirrhosis is a leading cause of illness and death Worldwide. It is expected that the number of people affected by cirrhosis will continue to increase in the near future **(Sanchez and Talwalkar, 2012).**

The liver plays an important role in the innate immune response, providing the first line of defense against microbes and toxins crossing the intestinal barrier **(Janeway and Medhitov, 2002).** Kupffer cells, the resident macrophages in the liver, are critical for the rapid clearance of microorganisms from the systemic circulation **(Gregory and Wing, 2002)** and **(Nagy, 2003).**

Although Kupffer cells by themselves are highly phagocytic and are able to remove microorganisms, they also facilitate generation of inflammatory response leading to recruitment of inflammatory cells such as neutrophils, monocytes, T and B lymphocytes, as well as natural killer (NK) cells, to the liver **(Sweet and Hume, 1996).** These inflammatory mediators will then activate neutrophils in the hepatic microvasculature (sinusoids and post sinusoidal venules) leading to a variety of events culminating in hepatocellular death **(Shashi and Hartmut, 2007).**

Patients with chronic cholestasis, particularly those with associated cirrhosis, are susceptible to infectious complications. A predictable consequence of cholestasis is malabsorption of fat-

soluble vitamins and free radical scavengers. On the other hand, it has been postulated that cholestasis affects polymorphonuclear leukocytes function by impeding chemotaxis, phagocytosis and superoxide anion release **(Salem, *et al*, 2003)**.There may be many reasons why these patients are at increased risk of infection, but impaired neutrophil function has recently been proposed as being important. Toxins that the liver can no longer efficiently excrete such as ammonia and low blood sodium levels may be implicated **(Schawcross, 2008).**

Neutrophils play a critical role in the host defense mechanism against various bacterial infections, and suggested that an impaired neutrophil chemotaxis function causes the susceptibility to infections in patients with fulminant hepatic failure. However, the most important neutrophil function, is the bactericidal function especially, oxygen-dependent bactericidal function **(Wyke *et al*, 1982).**

Neutrophils are a major innate immune cell subset involved in the first line of defense against infection. Neutrophils are produced in vast numbers in the bone marrow ($1\text{-}2\times10^{11}$ per day) and have a short half life of 12-18 hours. They are rapidly recruited to sites of infection and inflammation. Neutrophils phagocytose invading microbes and proceed to kill them but unfortunately may damage "innocent bystanders", leading to tissue destruction, inflammation, and organ failure. Neutrophils are rapidly recruited to the liver in response to hepatic injury **(Lee *et al*, 2003).**

Introduction

Neutrophils and macrophages have a reduced capacity to phagocytose and eliminate engulfed microbes and cell debris in patients with cirrhosis **(Mookerjee *et al*, 2007).**

Although neutrophils are programmed to undergo apoptosis at the time of differentiation, the rate of apoptosis is under the regulation of external factors. Therefore, changes in the rate of PMN apoptosis are likely to occur in the setting of cirrhosis. PMN from decompensated cirrhotic patients have an enhanced frequency of apoptosis, which is likely to contribute to explain the observed neutropenia in these patients **(Maria *et al*, 2004).**

Over recruitment, inappropriate activation or deregulated clearance of these cells results in the establishment of a wide variety of clinical disorders **(Rokusz and Liptay, 2003).** In the specific case of cirrhosis, neutropenia (i.e. a decreased number of circulatory PMN) may play a role in the pathogenesis of the increased rate of bacterial infections seen in these patients **(Rajkovic and Williams, 1986).**

Several hypotheses regarding the mechanisms underlying neutropenia in cirrhosis have been postulated Splenomegaly, hypersplenism, increased clearance of PMN in the spleen and the presence of serum hematopoietic progenitor cell inhibitors have been suggested as mechanisms for the presence of anemia, leukopenia and thrombocytopenia in cirrhotic patients **(Ohki *et al*, 1988).**

PMN from decompensated cirrhotic patients have a shorter life-span because of an increased rate of apoptosis. These findings

may contribute to explain the existence of neutropenia in cirrhosis **(Maria *et al*, 2004).**

Hyperammonemia is common in liver disease and its severity relates to the degree of liver dysfunction **(Clemmesen *et al*, 1999).** Recent studies suggest that in addition to ammonia, infection is a common precipitant of the syndrome of hepatic encephalopathy **(Shawcross *et al*, 2004).** Hepatic encephalopathy (HE) constitutes a neuropsychiatric syndrome which remains a major clinical problem in patients with cirrhosis **(Shawcross *et al*, 2010).**

Hyperammonemia have the ability to produce swelling of susceptible cells by altering the osmotic balance **(Cordoba *et al*, 1998).** Ammonia produces neutrophil swelling and impairs neutrophil phagocytosis **(Shawcross *et al*, 2008).**

Neutrophil phagocytic dysfunction is associated with increased risk of infection and mortality in patients with cirrhosis, Phagocytic dysfunction is universal in patients with cirrhosis and is related in part to the development of ammonia - induced neutrophil swelling, which is reversible following liver transplantation. Increasing levels of endotoxin and / or ammonia lead to neutrophil activation **(Taylor *et al*, 2010).**

Data show that in patients with liver failure and elevated arterial ammonia that neutrophil bactericidal capacity is impaired **(Nishtala *etal*, 2011).**

Stable cirrhosis is characterized by neutrophil phagocytic dysfunction which may be subtle and only revealed in inflamed

peripheral tissues where excessive inflammatory mediators continue to be released **(Tritto *et al*, 2011).**

More recently the synergistic role of inflammation and infection in modulating the cerebral effects of ammonia has been shown to be important. Furthermore, it has been recognized that infection impairs brain function both in the presence and absence of liver disease. Thus it could be postulated that in the presence of ammonia, the brain is sensitized to a systemic inflammatory stimulus and is able to elicit an inflammatory response involving both proinflammatory and neurotransmitter pathways **(Shawcross *et al*, 2010).**

Ammonia is not only directly toxic to astrocytes but induces neutrophil dysfunction with the release of reactive oxygen species, which contribute to oxidative stress and systemic inflammation. This may further exacerbate the cerebral effects of ammonia and potentially reduce the capacity of the neutrophil to fight microbial attack, thus inducing a vicious circle. This evidence supports the neutrophil in addition to ammonia as being culpable in the pathogenesis of H.E., making the neutrophil a target for future anti inflammatory therapeutic strategies in addition to ammonia lowering therapies **(Shawcross *et al*, 2010).**