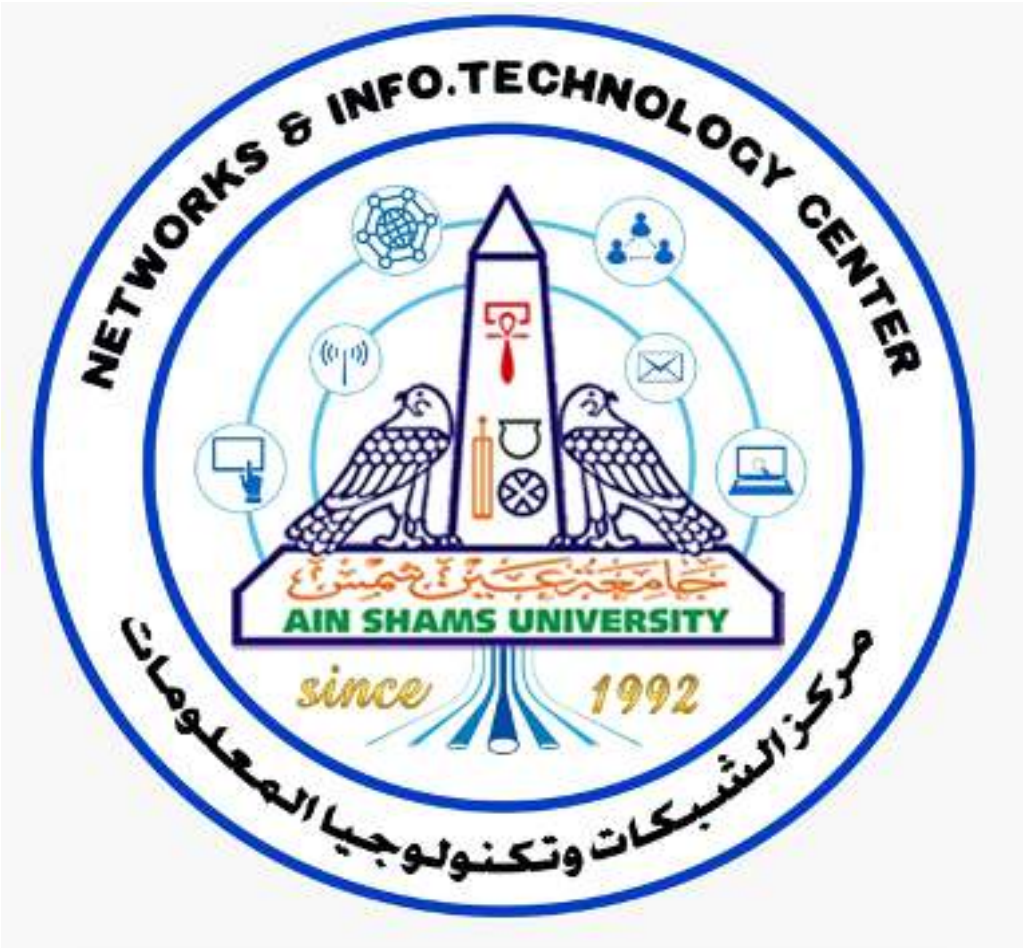
Safaa Mahmoud

بسم اللّه الرحمن الرحيم

مركز الشبكات وتكنولوجيا المعلومات

قسم التوثيق الإلكتروني



Safaa Mahmoud

جامعة عين شمس

التوثيق الإلكتروني والميكروفيلم

قسم

نقسم باللّه العظيم أن المادة التي تم توثيقها وتسجيلها

علي هذه الأقراص المدمجة قد أعدت دون أية تغيرات

Assessment of CD59 expression in Hepatitis C Virus induced thrombocytopenia

Thesis

Submitted for Partial Fulfillment of the Master Degree
in Clinical Hematology

By

Nermeen Samy Adeeb
M.B.B.CH
Faculty of Medicine – Ain Shams University

Under Supervision of

Prof.Dr. Shaza Abdelwahab
Professor of clinical hematology
Faculty of medicine, Ain Shams University

Associate professor Dr. Walla Ali El salakawey
Associate professor of internal medicine and hematology
Faculty of Medicine at Ain Shams University

Dr. Rasha Magdy Mohamed Said
Lecturer of internal medicine and hematology
Faculty of Medicine Ain Shams University

Faculty of Medicine
Ain Shams University
2019

First and foremost, All thanks return to Allah, I can do nothing without Him.

I would like to express my sincere gratitude to Prof. Dr. Shaza Abdel Wahab, Professor of Hematology and Bone Marrow Trasplantaion, Faculty of Medicine - Ain Shams University, for her encouragement, support and kindness which enable me to produce good valuable work. I really have the honor to complete this work under her supervision.

A special thanks and appreciation to Prof. Dr. Walaa Ali El salakawey, Assistant Professor of Internal medicin and Haematology, Faculty of Medicine - Ain Shams University, for her active guidance and keen supervision which were of great help throughout this work.

Special thanks to Dr. Rasha Magdy Mohammed, Lecturer of Haematology, Faculty of Medicine - Ain Shams University, for the efforts and time she has devoted to accomplish this work.

Many thanks to Prof Dr. Soha Raaof , professor of clinical pathology, Faculty of Medicin- Ain Shams University, for her generous effort and help in proceeding in this work.

I am sincerely grateful to my Family, specially my Parents for their care, support and encouragement.

Nermeen Samy Adeeb

LIST OF CONTENTS

LIST OF ABBREVIATIONS

Abb.		Full Term
AA	:	Aplastic anemia
AIHA	:	Autoimmune haemolytic anemia
ANA	:	Anti Nuclear Anti body
Anti dsDNA	:	Anti double stranded deoxyribo nucleacide
APCS	:	Antigen presenting cells
ApL	:	Ant phospholipid syndrome
APTT	:	Activated partial thromboplastin time
BAFF	:	B-lymphocyte- stimulation
BM	:	Bone marrow
CR Ig	:	Complement receptor of Immune globulin family
CR2	:	Complement Receptan2
DAAS	:	Direct acting antivirals
DAF	:	Decay accelerating factor
DCS	:	Dendritic cells
EHM	:	Extra hepatic manifestatsion
E-S	:	Evan syndrome
FH	:	Factor H
GI	:	Gastro intestinal
GP	:	Glycoprotein
GPCR	:	Glycoprotein coupled receptor
GPI	:	Glycosyl phosphate dyliosital
HCV	:	Hepatitis C Virus
HLA-DR	:	Human leucocytic antigen DR
HSC	:	Hematopoietic stem cell
HUS	:	Hemolytic uremic syndrome
IC	:	Immune complexes
IFN-alpha	:	Interferon- alpha
IgG	:	Immune globulin G
IL-8	:	Inter Lutein 8
TT	:	Thrombin time
ITP	:	Immune thrombocytopenic purpura
LKM	:	Liver kidney microsomal antibodies
LPD	:	Lympho proliferative disorders
MAC	:	Membrane attack complex

LIST OF ABBREVIATIONS

Abb.		Full Term
MC	:	Mixed Cryoglobulinemia
MCP	:	Membrane cofactor protein
MITL	:	Membrane inhibitor of reactive lysis
NHL	:	Non hodgken lymphoma
NK	:	Natural killer cell
NOSA	:	Non-organ specific– antibodies
PAIgG	:	Platelet associated immunoglobulin
PIGA	:	Phosphate tidylinostial glycan anchor biosynthesis
PNH	:	Paroxysmal nocturnal haemoglobinurea
PT	:	Prothrombin time
RBCS	:	Red blood cells
RCA	:	Regulator complement activation
RF	:	Rheumatoid factor
SDF-1	:	Stromal cell derived factor -1
SLE	:	Systemic Lupus erythromatosis
SMA	:	Anti Smooth muscle actin antibody
SVR	:	Sustained virologic response
TCR	:	T-cell receptor
Th2	:	T-helper2
TIPS	:	Trans jugular intrahepatic porto systemic shunt
TPO	:	Thrombopiotin

LIST OF TABLES

LIST OF FIGURES

Abstract

Background: A variety of pathogenic mechanisms are reported to be implicated in thrombocytopenia related to chronic HCV infection: (1) Auto-immunity (2) sequestration of platelets in the enlarged spleen secondary to portal hypertension (hyper-splenism), (3) inadequate production of thrombopoietin in advanced stage liver disease , (4) Anti-viral for HCV. The CD59 protein, formerly known as a membrane inhibitor of reactive lysis (MIRL) , inhibits the final and most important step of membrane attack complex (MAC) formation. There are many studies had been done specially on autoimmune diseases and founded that abnormalities in the expression of CD55 and CD59 surface molecules on peripheral blood cells.

Objective: to assess the expression of CD59 on the platelet surface in patients with HCV associated thrombocytopenia specially if the cause of thrombocytopenia is autoimmune.

Subjects and methods: This cross section study was conducted on 50 individual (30patient and 20 healthy control) who were subjected to flowcytometric determination of the expression of human CD59 at the platelet surface from peripheral blood sample.

Results: The groups included 30 males and 20 females with their ages ranging between 17 and 79 years with mean +SD[53.17±12.76] for group (I) and (53.3±11.85) for group (II). The ranged and mean(for group I) of HGB 10-15.9 [13.18±1.50], TLC 3.5-9.1 [5.70±1.62] and PLT 43-130 [84.27±22.36]of CBC**,** For the PCR low (26.7%) and PCR high (73.3%). For CD59 range(79-96.9%) mean (87.99±6.52%) in patient group(I), range (82.7-98.7%) mean (93.48±4.08%) in control group (II). There is significant decrease mean of patients group compared to control group as regard CD59 percent(p-value=0.002), and there is positive significant correlation between CD59% and PLT, with (r=0.924 and p-value <0.001).

Conclusion: Auto immunity is one of the mechanisms of HCV associated thrombocytopenia and in our study there is deficiency in CD59 in positive correlation between CD59 and platelet count and other studies revealed this correlation in auto immune thrombocytopenia and this denotes that CD59 may have a role in HCV associated thrombocytopenia , so thrombocytopenia could be explained by deficient CD59.

Key words: thrombocytopenia, HCV, CD59

INTRODUCTION

Thrombocytopenia in patients with chronic hepatitis C virus (HCV) infection is a major problem. The pathophysiology is multifactorial, with auto-immunogenicity, direct bone marrow suppression, hypersplenism, decreased production of thrombopoietin and therapeutic adverse effect all contributing to thrombocytopenia in different measures. ***(Dahal et al ;2017).***

In chronic autoimmune thrombocytopenic patients, thrombocytopenia occurs due to autoimmune destruction, complement mediated thrombolysis and platelet structural changes. A resistant disease course, despite immunosuppressive treatment and splenectomy, indicates that other mechanisms are responsible for the pathogenesis of the disease ***(Johnsen et al; 2012).***

The complement system helps or "complements" the ability of antibodies and phagocytic cells to clear pathogens from an organism. Over 25 proteins and protein fragments make up the complement system, including serum proteins, serosal proteins and cell membrane receptors. Three biochemical pathways activate the complement system: the classical complement pathway, the alternative complement pathway and the lectin pathway. CD55+ and CD59+ are complementary regulatory proteins; additionally, CD55+ is a glycoprotein that is expressed in peripheral blood, vascular endothelial cells and extravascular

epithelial cell surfaces. It inhibits the activity of the C3 convertase in the classical and alternative pathways. CD59+ is a phosphatidylinositol-linked membrane protein. It is expressed on erythrocytes, lymphocytes, monocytes, neutrophils, platelets, endothelial/epithelial cells, etc,. and blocks C9 binding to C5b-8, preventing the membrane attack complex formation and lysis **(Abbas et al;2010).**

A common finding in many studies is that the deficient expression of CD55 and/or CD59 was related to autoimmune hemolytic anemia autoimmune thrombocytopenia and lymphopenia in patients with systemic lupus erythematosus consequently, it is valid to propose that under expression of complement regulatory molecules plays a role in a complex mechanism that ultimately leads to cytopenia ***(Alejandro et al;2007).***

Aim of the Work

The aim of the present study is to assess the expression of CD59 on platelets in patients with thrombocytopenia induced by hepatitis C virus infection.

Review of Literature

Hepatitis C Virus Associated Thrombocytopenia

Thrombocytopenia in chronic HCV infection is a major problem, particularly in patients with advanced liver disease. The risk of serious bleeding with severe thrombocytopenia can prevent invasive procedures including biopsies for staging. Thrombocytopenia can also complicate bleeding manifestations such as variceal bleeding. It may impede the initiation and continuation of antiviral therapy, potentially decreasing the probability of successful HCV treatment. Recent studies have evaluated the underlying mechanism of thrombocytopenia in chronic HCV infection and assessed the usefulness of several therapeutic options. **(Dahal et al ., 2017).**

According to different studies, 40–80% of HCV-positive patients develop at least one extra-hepatic manifestation (EHM) during the course of the disease, which is often the first and only clinical sign of chronic HCV infection . Therefore, knowledge of EHM is also an important tool in the diagnosis of HCV infection **(Barbara & Böckle ,2010)**

Anemia, neutropenia, leucopenia, and thrombocytopenia are among the numerous side effects of currently available HCV treatments . Preliminary data suggest that the infection

itself can also induce autoimmune hemolytic anemia, leukopenia, and thrombocytopenia ,thrombocytopenia include direct bone marrow toxicity and autoimmune reactions **(Douglas et al ., 2003).**

Thrombocytopenia (platelet counts <150,000/lL) , reported in as many as 76% of cirrhotic patients . Platelets play an important role in hemostasis that has been the subject of a recent comprehensive review . The clinical significance of mild thrombocytopenia (75,000/lL–<150,000/lL) is minimal and usually does not interfere with treatment or management decisions. Moderate thrombocytopenia (50,000/lL–75,000/lL) is observed in approximately 13% of cirrhotic patients. Severe thrombocytopenia (<50,000/lL) can be associated with significant morbidity, often complicating the medical management of patients with advanced liver disease , cancer , immune thrombocytopenic purpura (ITP) , chronic hepatitis C virus(HCV) infection , and other disorders.

Severe thrombocytopenia requiring platelet transfusions occurs in 1% of patients. While mild to moderate thrombocytopenia rarely leads to spontaneous bleeding during invasive procedures including liver biopsy and liver transplantation severe thrombocytopenia can significantly increase the risk of bleeding. Cerebral hemorrhage or