Platelets and Neutrophils Cross Talk Mediating Cancer Growth and Metastasis in Urinary Bladder Carcinoma

Thesis submitted for partial fulfillment of Master Degree in Clinical and Chemical Pathology

By

Maha Mahmoud Samy Othman
(M.B; B.Ch)

Supervisors

Prof. Dr. Eman El Mahgoub
Professor of Clinical and Chemical Pathology,
Faculty of Medicine, Cairo University

Prof. Dr. Bothina Madkour
Professor Researcher of Haematology
Theodor Bilhrz Research Inistitute

Faculty of Medicine
Cairo University
2010

بسم الله الرحمن الرحيم

Dedication

I would like to dedicate this work to my family specially my ***Father*** and ***Mother*** who supported me through my entire life. I cannot express my gratitude to all they did and still doing to me. Thank you my guarding angels.

<u>*ACKNOWLEDGMENT*</u>

"First and Foremost, Thanks are Due to GOD,
The Beneficent and Merciful of All"

I would like to express my deepest gratitude and sincere thanks to ***Prof. Dr. Eman El Mahgoub,*** *Professor of Clinical and Chemical Pathology Dpt, Faculty of Medicine, Cairo University, for her expertise that she showed in the supervision of my entire academic work. Hes continuous encouragement and thoughtfulness were most valuable to me.*

I am truly grateful to ***Prof. Dr. Bothina Madkour****, Professor Researcher of Haematology, Theodor Bilhrz Research Inistitute, for her valuable support. Without her creative thinking, valuable suggestions and constructive criticism, the performance of this work would have been difficult.*

I owe much to ***Prof. Dr. Fayza Essawy****, Professor of Haematology, Theodor Bilhrz Research Inistitute, for planning the design of the work, devoting her intellectual energies and high sense of profession in the entire development of this thesis, offering all help, patience, guidance and excellent supervision.*

I would like to extend my thanks to Professor ***Dr. Salwa Toeima****, Professor of Haematology and Head of Hematology Dpt. Theodor Bilharz Research Institute, for providing all the facilities possible for the success of this work.*

I am greatly indebted to ***Ass. Prof Dr. Iman William****, for her expert guidance, valuable suggestions and all laboratory facilities throughout the entire work.*

*I am truly grateful and would like to express my deepest gratitude and sincere thanks to **Prof. Dr. Safaa El Karakessy**, Professor of Clinical and Chemical Pathology Dpt., Faculty of Medicine, Cairo University, For her support and valuable guidance.*

*I am truly grateful to **Prof. Dr. Gihan El-Fandy**, Professor of Anesthesia and President of Theodor Bilharz Research Institute, for her encouragement and kind support.*

*Many Thanks to **Prof. Dr. Hend Kamel**, Professor of Anesthesia and Head of Surgical Clinical Board, Theodor Bilharz Research Institute for approving the registration of this thesis and valuable advice.*

*I am greatly indebted to **Prof. Dr. Mamdouh Roshdy**, Professor of urology, Theodor Bilharz Research Institute for his expert guidance, valuable suggestions and encouragement.*

*Many thanks to my colleague **Dr. Ahmed Zakareya** who supported me all the way till this work has been accomplished.*

Many thanks to all staff members of Haematology Dpt. at Theodor Bilharz Research Institute who bore with me the brunt of this work.

*I cannot miss thanking the **patients** that were the mainstay of the study.*

*Finally, I really wish to thank my **"dear husband"** for his support, love and **tremendous patience**.*

List OF CONTENTS

List of Tables

List of Figures

List of Abbreviations

Abbreviation	The full term
ADP	Adenosine Diphosphate
ALT	Alanin Aminotransferase
ANC	Absolute neutrophil count
AST	Aspartate Aminotransferase
BCG	Bacillus Calmette-Guerin.
BPI	Bacterial permeability-increasing protein.
C5a	Complement 5a
cAMP	Cyclic adenosine monophsphate
CD	Cluster of Differentiation
CD24	A mucin-like adhesion molecule on cancer cells.
CD62P	P-selectin
CD162	P-selectin glycoprotein ligand-1
CIS	Carcinoma in situ
COX-2	Cyclooxygenase-2.
CRC	Colorectal carcinoma cells.
Creat.	Creatinine
CT	Computerized tomography
DIC	Disseminated Intravascular Coagulation
DNA	Deoxyribonucleic Acids
EGF	Epidermal growth factor
ELISA	Enzyme-Linked Immunosorbent Assay
FISH	Fluorescent insito hybridization
FMLP	N-formyl-1-methionyl-1-leucyl-1-phenylalanine
FucT-VII	Fucosyltrasferase enzyme
GI	Low grade malignancy
GII	Moderate grade malignancy
GIII	High grade malignancy
G-CSF	Granulocyte colony-stimulating factor
GM-CSF	Granulocyte/ macrophage colony-stimulating factor
GP	Glycoprotein.
GPCR	G-protein coupled seven transmembrane receptors.
GRO	Growth-related oncogene
H&E	Hematoxylin and eosin
Hb	Haemoglobin concentration
Histopath.	Histopathology
HIT	Heparin-induced thrombocytopenia
HIV	Human Immunodeficiency Virus
HPA	Human platelet alloantigen.
HUS	Hemolytic uremic syndrome.
IL	Interleukin

INR	International Normalized Ratio
ITP	Immune thrombocytopenic purpura.
IVP	Intravenous pyelography
kDa	Killo-Dalton
LOH	Loss of heterozygosity
LPS	Lipopolysaccharide
LRR	Leucine-rich repeated
MPO	Myeloperoxidase enzyme.
MRI	Magnetic resonance imaging
mRNA	Messenger RNA
MS	Mass spectrometry
NCI	National Cancer Institute
NADPH	Nicotinamide adenine dinucleotide phosphate hydrogen
NETs	Neutrophil extracellular traps.
NF-κB	Nuclear Factor κB
NH2-	Amino terminal
NSAIDs	Non-steroidal anti-inflammatory drugs
NSF	Nephrogenic systemic fibrosis
PAF	Platelet-activating-factor
PC	Prothrombin concentration
PDGF	Platelet derived growth factor
PECAM-1	Platelet–endothelial cell adhesion molecule-1.
PL	Phospholipase
PML	Polymorphonuclear leukocytes
PMN	Polymorphonuclear leukocytes
PSGL-1	P-selectin glycoprotein ligand-1
PT	Prothrombin Time
RBCs	Red Blood Cell Count
RNA	Ribonucleic Acid
SABC	Schistosoma-associated bladder cancer
SCC	Squamous cell carcinoma .
sP-selectin	Soluble P-selectin.
T bili	Total Bilirubin
TF	Tissue factor
TNF-α	Tumor necrosis factor-α.
TLC	Total Leukocytic Count
TNM	Tumor-node-metastasis staging system.
TP	Total Protein
TTP	Thrombotic thrombocytopenic purpura
TxA2	Thromboxane A2
VEGF	Vascular endothelial growth factor.
vWF	Von Willebrand Factor.
2-DE	2-dimensional gel electrophoresis
3-D	Three dimensional

ABSTRACT

Urinary bladder cancer is a major public health problem being one of the most common malignancies worldwide. In Egypt, this heterogeneous disease has the highest reported world wide incidence "37 per 100,000 populations" in the world due to endemic schistosomiasis. It has been believed that many cancers arise from sites of infection, chronic irritation and inflammation. It has been also suggested that the interaction between circulating platelets and neutrophils influences innate immune functions, contributing to regulate inflammation that means cell-cell interactions are crucial for the host defense mechanism. **P-selectin** is platelet activation marker that has been shown to mediate the rolling of blood cells on the surface of the endothelium and initiate the attachment of leukocytes circulating in the blood to platelets, endothelial cells, and other leukocytes. **PSGL-1** is a disulfide-bonded homodimeric mucin-like glycoprotein on leukocytes that interacts with **P-**, **L-**, and **E-selectin**. In order to investigate the role of **P-selectin** and **PSGL-1** mediated interactions in the microenvironment of the tumor; we measured **P-selectin** on platelets, **PSGL-1** on neutrophils and **sP-selectin** in plasma of 30 patients with **bladder cancer**. Patients were classified into three groups; first group with locally invasive tumor "n=10", second group with regional lymph node involvement "n=10", and third group with distant metastasis "n=10". **Platelet count** was significantly higher in patients with “invasive tumor with distant metastasis” group compared to control and the other two groups; that is due to the frequently observed thrombocytosis in cancer patients. **P-selectin** was significantly elevated in all patient groups as a part of inflammatory and immunological reactions. However among the 3 studied groups, the patients with invasive tumor and distant metastasis showed the highest elevation may be due to