DC

SERUM INTERLEUKIN-6 LEVEL AND ITS CORRELATION WITH OTHER PARAMETERS OF DISEASE ACTIVITY IN MULTIPLE MYELOMA

Thesis
Submitted in Partial Fulfillment of the requirements for
M.D. Degree in Clinical Pathology

By
Mohamed Hassan Zaki
M.B., B.Ch., M.Sc.

Under the Supervision of

Prof. Dr. Nadia Mohamed Mowafy
Prof. of Clinical Pathology
Faculty of Medicine, Ain-Shams University

Prof. Dr. Salwa Mohamed Youssef
Prof. of Clinical Pathology
Faculty of Medicine, Ain-Shams University

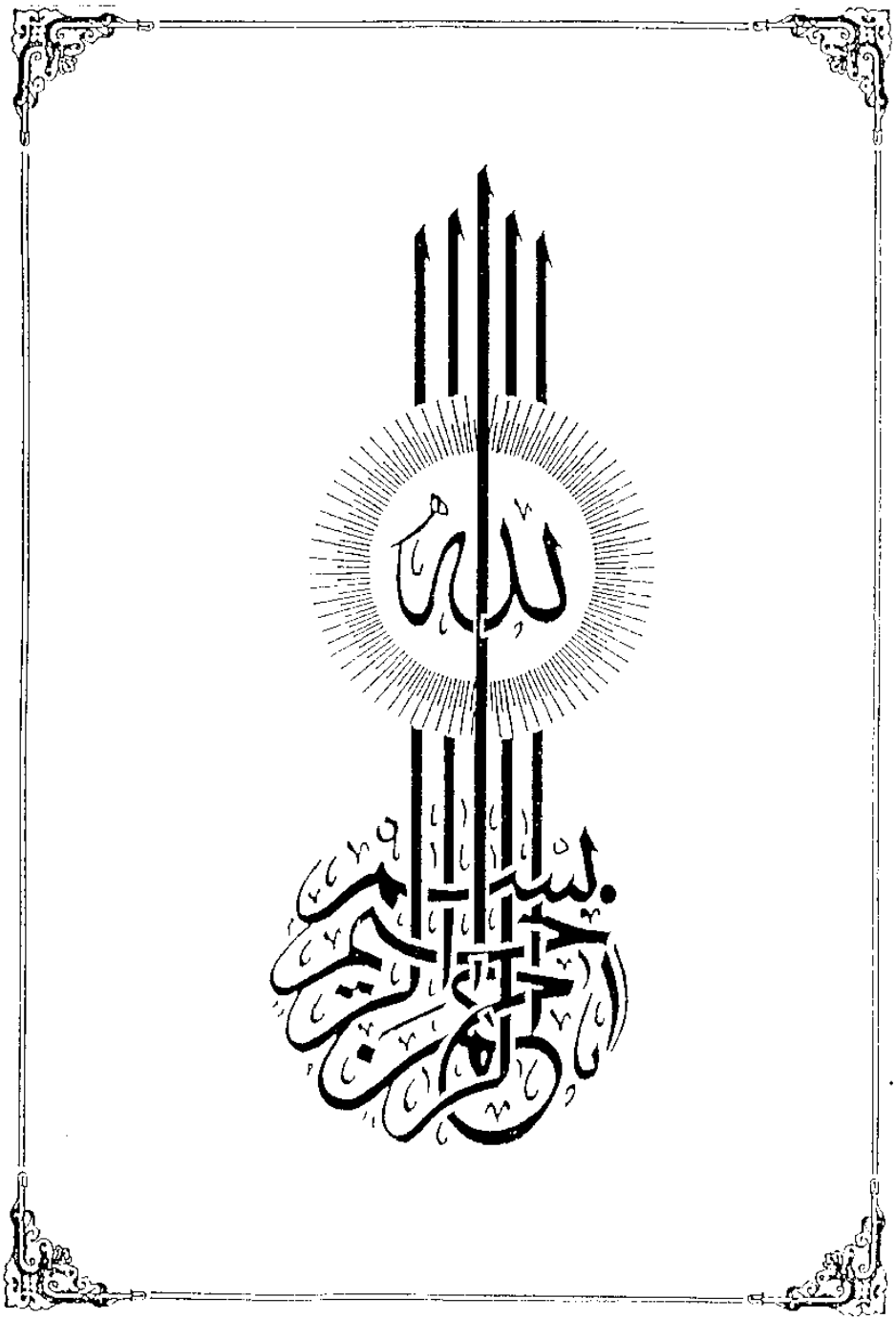
Prof. Dr. Michael Phillips
Prof. of Internal Medicine and Neurology
University of Pensylvania School of Medicine

Prof. Dr. Salwa Mohamed Abu El-hana
Prof. of Clinical Pathology
Faculty of Medicine, Ain-Shams University

Dr. Afaf Abd El-Aziz Abd El-Ghafar
Lecturer in Clinical Pathology
Faculty of Medicine, Ain-Shams University

Faculty of Medicine
Ain-Shams University
1997


بسم الله الرحمن الرحيم



ACKNOWLEDGEMENT

It is a great pleasure for me to express my gratitude, deep thanks and appreciation to **Prof.Dr. Nadia Mohamed Mowafy,** Professor of Clinical Pathology, Faculty of Medicine, Ain Shams University, *for her supervision, guidance, advice and extreme decency throughout the accomplishment of this work. Her expert supervision was invaluable.*

I have to assert my cordial thanks and respect to **Prof.Dr. Salwa Mohamed Youssef,** Professor of Clinical Pathology, Faculty of Medicine, Ain Shams University; *to whom I recognize her great help and guidance in this work. She kindly revised every part of this thesis, and her valuable comments were very helpful.*

I record my indebtedness, deep appreciation and thanks to **Dr. Michael Phillips,** Professor of Medicine and Neurology, University of Pennsylvania school of Medicine, USA, for giving me the opportunity to conduct this work at one of the oldest and most respected universities in the USA.

I would like also to thank **Dr. Salwa Mohamed Abul Hana,** Assistant Professor of Clinical Pathology, and Dr. Afaf

I would like also to thank **Prof.Dr. Salwa Mohamed Abul Hana,** Professor of Clinical Pathology, and Dr. Afaf Abd El-Aziz Abd El-Ghaffar, Faculty of Medicine, Ain Shams University, for their great help, support and valuable suggestions in revising this thesis.

My warm thanks and deep love and gratitude also go to **Dr. Peter Nowell,** Professor of Pathology and Lab. Medicine, University of Pennsylvania school of Medicine, *to whom I owe a lot for his great effort in conducting the clinical part of this work and for his guidance and encouragement and distinguished advice.*

Special thanks should go to ***Dr. Jonni Moore,*** Director of the Flowcytometry facility at University of Pennsylvania School of Medicine, *to whom I owe a lot for her great effort in conducting the practical part of this thesis.*

I extent my thanks to ***Roberta Lamb,*** *Technical Supervisor* at University of Pennsylvania School of Medicine, *for her help in conducting all technical aspects of this thesis at the* University of Pennsylvania.

Last, but not least, no words could ever thanks my ever giving family, their favour is unforgettable.

ABSTRACT

Multiple myeloma is a clonal disorder where terminally differentiated B-cells and plasma cells infiltrate the bone marrow, produce large amounts of monoclonal Igs, and secrete osteoclast-activating factor leading to bone lesions.

The present study was conducted to confirm the elevation of serum level of IL-6 in MM patients and to correlate this elevation with the levels of other parameters of disease activity such as bone marrow plasmacytosis, β2-microglobulin, CRP, LDH, Alkaline-phosphatase. Furthermore to study the effect of this elevation of IL-6 on programmed cell death (apoptosis) in myeloma cell lines and myeloma cells using a very sensitive method to detect apoptosis by flow cytometry.

We have shown that the level of IL-6 was highly elevated in all newly diagnosed patients (16 cases) using a sensitive ELISA technique. The level of this cytokine was not elevated, neither in the normal control group (10 cases) nor in the follow up patients group (8 cases).

The present study has also revealed that IL-6 has inhibited apoptosis induced by serum starvation in myeloma cell lines and myeloma cells.

The results shown in the present study are suggestive of a major role for IL-6 in MM. This role is not only as a growth factor for myeloma cells but also as a critical factor in inhibiting programmed cell death.

LIST OF ABBREVIATIONS

BM	= Bone marrow
CD	= Cluster of differentiation
CNTF	= Ciliary neurotrophic factor
CR	= Complete remission
CRP	= C-reactive protein
EFS	= Event-free survival
FISH	= Fluorescence in situ hybridization
G-CSF	= Granulocyte colony-stimulating factor
GVM	= Graft-versus-myeloma
HLA	= Human leukocytic antigen
IFN	= Interferon
Ig	= Immunoglobulin
IL	= Interleukin
kD	= Kilo Dalton
LDH	= Lactate dehydrogenase
LIF	= Leukemia inhibitory factor
LN	= Lymph nods
MAP	= Mitogen activated protein
MDR	= Multi drug resistance gene
MGUS	= Monoclonal gammopathy of unknown significance
MM	= Multiple myeloma
MoAbs	= Monoclonal antibodies
MP	= Melphalan and prednisone
MRI	= Magnetic resonance imaging
NF	= Nuclear factor
OAF	= Osteoclast activating factor
OS	= Overall survival
OSM	= Oncostatin M
PCD	= Programmed cell death
PCL	= Plasma cell leukemia

PCLI	=	Plasma cell labeling index
PEP	=	Protein electrophoresis
PLT	=	Platelets
RIA	=	Radioimmunoassay
rIL	=	Recombinant interleukin
RP	=	Retinoblastoma gene
sIL-6	=	Soluble IL-6
sIL-6R	=	Soluble IL-6 receptor
t	=	Translocations
TCL	=	Total leukocytic count
TGFβ	=	Transforming growth factor Beta
TNF	=	Tumor necrosis factor
TP	=	Total proteins
β2m	=	Beta-2-microglobulin

LIST OF GRAPHS AND FIGURES

LIST OF TABLES

CONTENTS

INTRODUCTION

INTRODUCTION

During the past few years many insights have been gained into the immunobiology of multiple myeloma. Of great importance to our understanding of the regulation of the immunobiology of multiple myeloma has been the appreciation of the roles of various biological response modifying cytokines, produced by lymphocytes. The importance of understanding how these cytokines or interleukins control the tumor growth can improve our understanding of the biology of multiple myeloma and provide insights which will facilitate the development of biologically specific therapeutic control regimens for this disease (Barlogie et al., 1989).

Interleukin-6, a pleotropic cytokine with the capacity to induce proliferation in human B cells, is a potent growth factor for murine plasmacytomas and B cell hybridomas (Van Damme et al., 1987). IL-6 also is a strong in vitro growth factor for human myeloma cells (Kawano et al., 1989).

The in vitro responsiveness of myeloma cells to IL-6 was shown to be directly and positively correlated to the proliferative capability of these cells in vivo (Zhang et al., 1989). IL-6 was shown to be over produced in bone marrow of patients with multiple myeloma (Klein et al., 1989). Consistent with this observation, serum levels of IL-6, determined in a bioassay (Bataille et al., 1989) and Radioimmunoassay (RIA) (Solary et al., 1992) were shown to be good reflectors of disease severity in plasma cell dyscrasias.