DETECTION OF BCR-ABL GENE AND IT'S EXPRESSED PROTEIN (P210) IN PERIPHERAL BLOOD CELLS OF DIFFERENT PHASES OF CML

Thesis
Submitted for Partial Fulfillment of The M.D. Degree
In
Clinical and Chemical Pathology

By

Soha Ezzel Arab Abdul Wahab
M.B., B.Ch., M.Sc. (Clinical and Chemical Pathology)

Supervisors

Prof. Dr. Osaima El Said Seliem
Professor of Clinical and Chemical Pathology
Faculty of Medicine - Ain Shams University

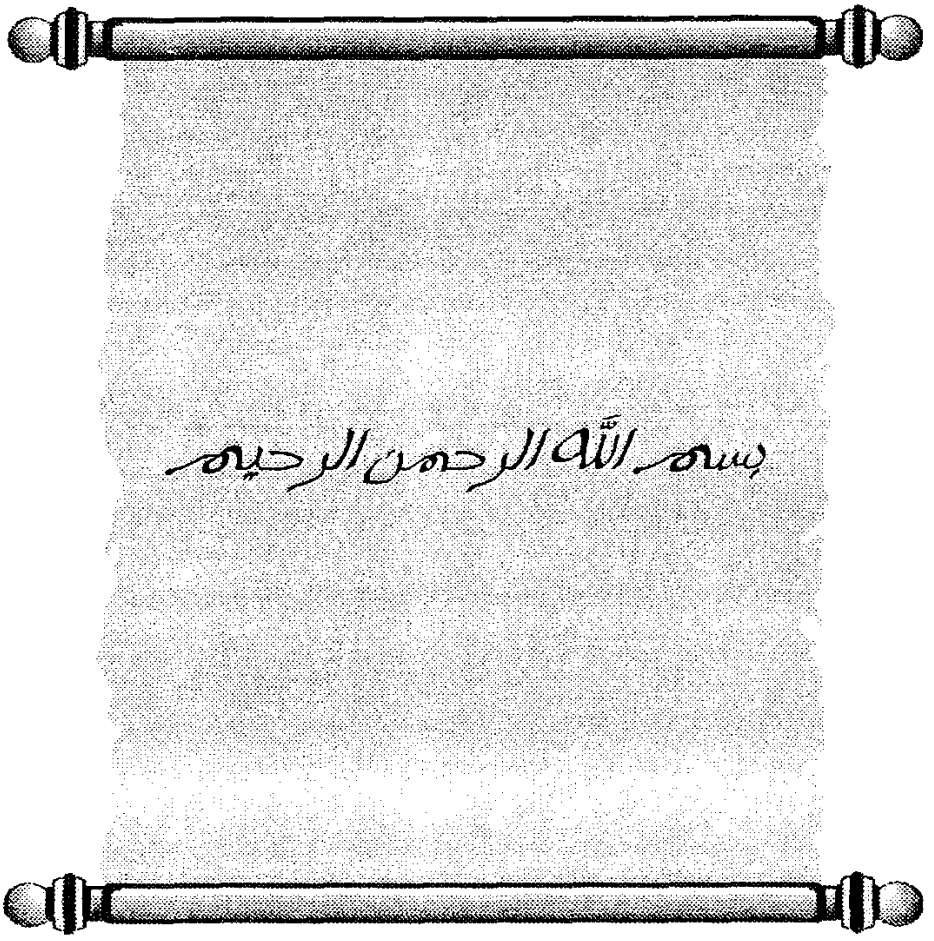
Prof. Dr. Salwa Mohamed Youssef
Professor of Clinical and Chemical Pathology
Faculty of Medicine - Ain Shams University

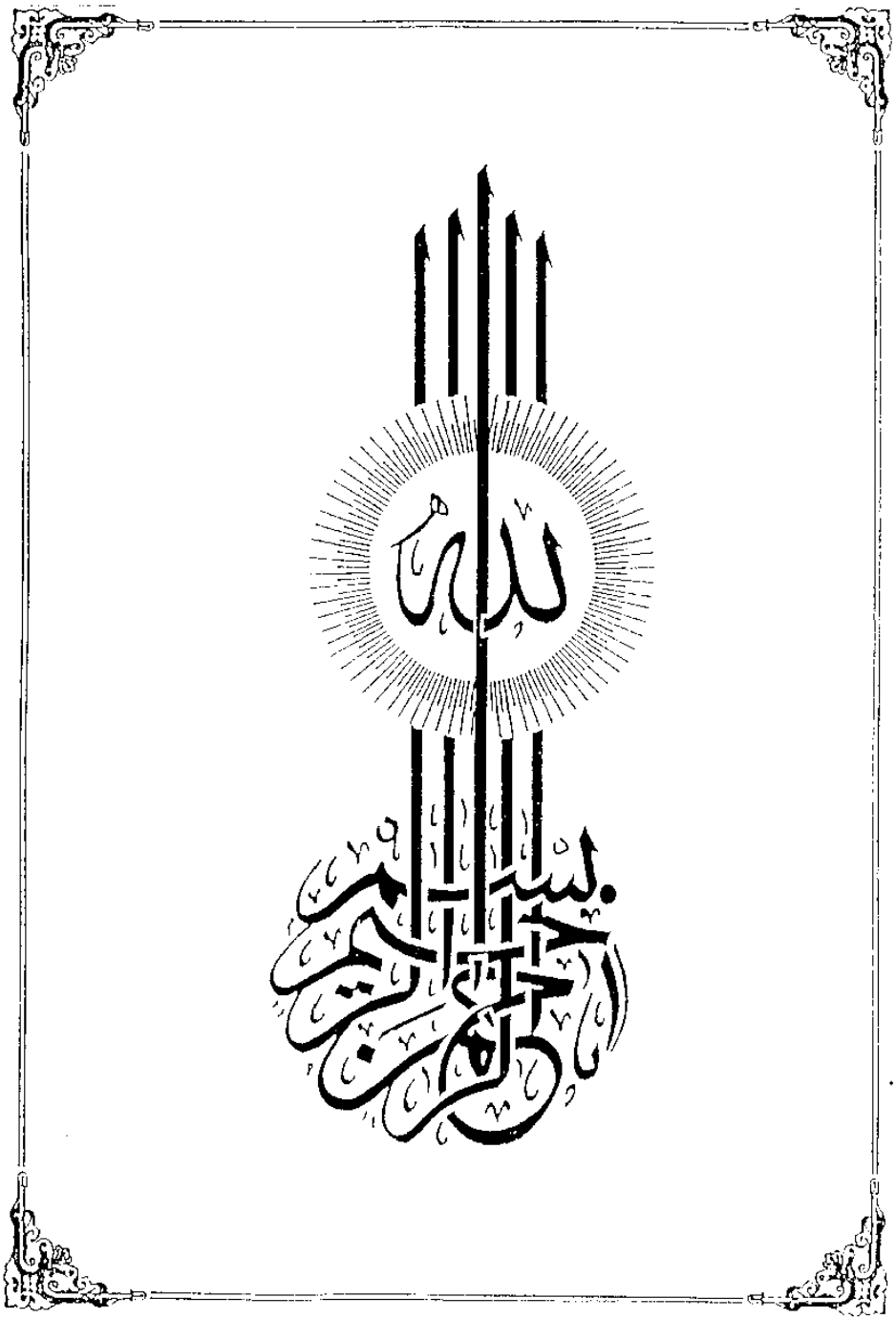
Prof. Dr. Salwa Mohamed Abu El Hana
Professor of Clinical and Chemical Pathology
Faculty of Medicine - Ain Shams University

Prof. Dr. Gamal Mohamed Mabrouk
Assistant Professor of Biochemistry and Molecular Biology
Faculty of Medicine - Ain Shams University

Prof. Dr. Afaf Abd El Azez Abdul Ghaffar
Assistant Professor of Clinical and Chemical Pathology
Faculty of Medicine - Ain Shams University

Faculty of Medicine
Ain Shams University
1998





Before all, I would like to express my deep thanks to **ALLAH** *without his great blessings, I would never accomplish this work.*

Special Acknowledgment

The work presented in this thesis was performed at the Oncology Diagnostic Unit, Ain Shams University under supervision of Prof. Dr. Gamal Mabrouk and by help of Chemist Amr Amin .During my work, I was overwhelmed by the hospitality and kind care of the staff. My deepest appreciation to the support offered to me by Prof. Dr. Aly Khalifa Aly, Professor of Biochemistry and Head of the Oncology Diagnostic Unit, Faculty of Medicine, Ain Shams University for his valuable assistance and illuminating remarks

Acknowledgment

I would like to express my deepest gratitude to Prof.Dr. Osaima El-Said Seliem, Professor of Clinical and Chemical Pathology, Faculty of Medicine, Ain Shams University who offered me the honor and chance to work under her most fruitful supervision and precious guidance.

I am much obliged to Prof.Dr. Salwa Mohamed Youssef, Professor of Clinical and Chemical Pathology, Faculty of Medicine, Ain Shams University for her most valuable assistance and illuminating remarks.

An appreciation beyond utterance to the effort, kind support and patience offered by Prof. Dr. Salwa Mohamed Abu El-Hana, Proffessor of Clinical and Chemical Pathology, Faculty of Medicine, Ain Shams University, her valuable instructions and critical mind brought out the final points of this research.

My deepest gratitude and appreciation are also due to Dr. Gamal Mohamed Mabrouk, Assistant Professor of Biochemistry, Faculty of Medicine, Ain Shams University for his valuable

instructions during the practical part of this work that brought out the final points of this research.

I wish to offer my thanks to Dr. Afaf Abd El-Aziz Abdul Ghafar, Assistant Professor of Clinical and Chemical Pathology, Faculty of Medicine, Ain Shams University.

I would like also to send many thanks to Dr. Lama Akram El-Safady, Lecturer of Clinical and Chemical Pathology, Faculty of Medicine, Ain Shams University.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF PHOTOS

LIST OF ABBREVIATIONS

A	Adenine
ABL	Abelson Leukemia
ALL	Acute Lymphoblastic Leukemia
AML	Acute Myeloblastic Leukemia
APS	Amonium Persulphate
bcr	Breakpoint Cluster Region
Mbcr	Major Breakpoint Cluster Region
mbcr	Minor Breakpoint Cluster Region
μbcr	Mu Breakpoint Cluster Region
BCR	BCR gene
BM	Bone Marrow
BMT	Bone Marrow Transplantation
BSA	Bovine Serum Albumin
C	Cytosine
CBC	Complete Blood Count
cDNA	Complementary Deoxyribonucleic acid
CFU-E	Colony Forming Unit-Erythroid
CML	Chronic Myelogenous Leukemia
CML-N	Chronic Neutrophilic Leukemia
CMML	Chronic Myelomonocytic Leukemia
CSF	Colony Stimulating Factor
DNA	Deoxyribonucleic acid
dsDNA	Double Stamded Deoxyribonucleic acid
ssDNA	Single Stranded Deoxyribonucleic acid
EDTA	Ethyline Diamine Tetra-acetic Acid
EL	Eosinophilic Leukemia
FACS	Fluoresence Activated Cell Sorting
FISH	Fluorescence in Situ Hybridization
G	Guanine
G6PD	Glucose 6 Phospharte Dehydrogenase

J-CML	Juvenile Chronic Myeloid Leukemia
kb	Kilobase
Kd	Kilo Dalton
LM	Light Microscope
MUD	Matched Unrelated Donor
PB	Peripheral Blood
PBS	Phosphate Buffered Saline
Ph`	Philadelphia
PSCT	Potential Stem Cell Transplantation
RBCs	Red Blood Cells
RNA	Ribonucleic Acid
mRNA	Messenger Ribonucleic Acid
Rt-PCR	Reverse Transcriptase Polymerase Chain Reaction
T	Tyrosine
U	Uracil
WBCs	White Blood Cells

ABSTRACT

Chronic myelogenous leukemia (CML) is a disease characterized by the presence of a unique molecular marker; i.e., BCR/ABL fusion gene and its mRNA ***(Santini et al., 1996).***

The hallmark of the disease is the Ph` chromosome which is generated by reciprocal translocation of 9,22 fusing the ABL oncogene of q arm of chromosome 9 to the breakpoint cluster region within the BCR gene on chromosome 22. The BCR/ABL gene is transcribed into a BCR/ABL specific mRNA that is translated into P210 kd protein possessing enhanced tyrosine kinase activity as compared to that of normal protein ***(Melo, 1996).***

A Western blotting as well as dot blot assays for the BCR/ABL fusion protein of circulating WBCs, in addition to Rt-PCR amplification for BCR/ABL gene were performed for 27/33 patients in different phases of the Ph` positive CML patients to assess their clinical utility in diagnosis and monitoring the disease.

Forty five blood samples were collected from 33 patients in different phases of the disease, at different times in addition to two control samples from patients with high total leukocytic count. However, only 27 of them gave a reproducible results.

We observed that, all studied cases 27/27 showed a positive result by dot blot assay in the form of bluish colored dots with different intensities corresponding to different phases of the disease.

On the other hand, Western blotting assay for chronic phase patients revealed a major band for