

Cytochrome P₄₅₀ 3A4 gene Polymorphism among Egyptian Children with Acute lymphoblastic Leukemia

Thesis

Submitted for Partial Fulfillment of Master Degree
In Pediatrics

Presented by

Nancy Abdo Elrazik Abdo Elfatah Moawad

(M.B; B.Ch.), Ain Shams University (2007)

Under Supervision of

Prof. Mohamed Abo-El-Asrar Mohamed El-Bayoomy Afify

Professor of Pediatrics, Faculty of Medicine
Faculty of Medicine – Ain Shams University

Dr. Nancy Samir El Barbary

Lecturer of Pediatrics Faculty of Medicine
Faculty of Medicine – Ain Shams University

Dr. Gamal Thabet Ali

Lecturer of Clinical Pathology
National Cancer Institute

**Faculty of Medicine
Ain Shams University
2012**



وَأَنْزَلَ اللَّهُ عَلَيْكَ
الْكِتَابَ وَالْحِكْمَةَ
وَعَلَّمَكَ مَا لَمْ تَكُنْ
تَعْلَمُ وَكَانَ فَضْلُ اللَّهِ
عَلَيْكَ عَظِيمًا

صدق الله العظيم

سورة النساء
الآية
(113)



*Thanks to **ALLAH** who helped me to accomplish this work*

*My deepest and warmest gratitude to my great supervisor Prof Dr. **Mohamed Abo-El-Asrar Mohamed El-Bayoomy Afify**, Professor of Pediatrics, Faculty of Medicine, Ain Shams University, who in addition to his valuable guidance and supervision, has provided me with a great deal of support, encouragement and Knowledge.*

*The present work could not have been done without the help of Dr. **Nancy Samir El Barbary**, Lecturer of Pediatrics, Faculty of Medicine Ain Shams University, for her guidance and continuous support.*

*I would like to express my great appreciation and thanks to Dr. **Gamal Thabet Ali**, Lecturer of Clinical Pathology, National Cancer Institute, Cairo University. It was an honor to me to carry out this work under his continuous guidance, encouragement and expert supervision.*

Lastly, I would like to thank all of my family for their endless support to me.

Nancy Abdel Razik Zidan

List of Abbreviations

| | |
|-----------------------|---|
| ALL | : Acute lymphoblastic leukaemia |
| AML | : Acute myolegenous leukemia |
| AML | : Myeloid leukemia |
| BFM | : Berlin-Frankfurt-Muenster |
| CCG | : Children's Cancer Group |
| CNS | : Central nervous system |
| CNS | : Central Nervous System |
| COG | : Children's Oncology Group |
| CR | : Complete remission |
| CSF | : Cerebrospinal fluid |
| CYP3A4 | : Cytochrome P450, family 3, subfamily A, polypeptide 4 |
| DFCI | : Dana-Farber Cancer Institute |
| DFS | : Disease-free survival |
| EBV | : Epstein-Barr virus |
| EFS | : Event-free survival |
| FAB | : French-American-British |
| FCM | : Flow cytometry |
| FISH | : Fluorescence in situ hybridization |
| GST | : Glutathione S-transferase |
| HIV | : Human immunodeficiency virus |
| HTLVI & II | : Human lymphotropic viruses I and II |
| IM | : Intramuscular |
| M : E | : Myeloid to erythroid ratio |
| MILL | : Mixed lineage leukemia |
| MRC | : Medical Research Council |
| MRD | : Minimal residual disease |
| NG2 | : Neural-glial antigen 2 |
| PCR | : Polymerase chain reaction |
| POG | : Pediatric Oncology Group |
| RB | : Retinoblastoma protein |
| SJCRH | : St. Jude Children's Research Hospital |
| SNPs | : Simple nucleotide polymorphisms |
| WBC | : White blood cell count |

List of Tables

| Table No. | Title | Page No. |
|--------------------|---|----------|
| Table (1): | French-American-British classification of lymphoblastic leukemia | 13 |
| Table (2): | Clinical and laboratory features at diagnosis in children with ALL..... | 36 |
| Table (3): | Selected substrates, inducers and inhibitors of CYP3A4..... | 64 |
| Table (4): | Data collected serially from patients during this study | 79 |
| Table (5): | The clinical characteristics of the enrolled patients..... | 87 |
| Table (6): | Frequency of CYP3A4 genotype in patints and controls..... | 89 |
| Table (7): | Comparison between wild type group, heterozygotic group and homozygotic group as regards age at diagnosis. | 90 |
| Table (8): | Comparison between wild type group, heterozygotic group and homozygotic group as regards TLC at diagnosis. | 90 |
| Table (9): | Comparison between wild type group, heterozygotic group and homozygotic group as regards sex. | 91 |
| Table (10): | Comparison between wild type group, heterozygotic group and homozygotic group as regards clinical risk degree. | 91 |
| Table (11): | Comparison between wild type group, heterozygotic group and homozygotic group as regards Immunophenotyping | 92 |

List of Tables (Cont.)

| Table No. | Title | Page No. |
|--------------------|--|----------|
| Table (12): | Comparison between wild type group, heterozygotic group and homozygotic group as regards FAB classification..... | 92 |
| Table (13): | Comparison between wild type group, heterozygotic group and homozygotic group as regards bone marrow remission at BMA day d15 | 93 |
| Table (14): | Comparison between wild type group, heterozygotic group and homozygotic group as regards Bone Marrow Remission at BMA d42 | 94 |
| Table (15): | Comparison between wild type group, heterozygotic group and homozygotic group as regards bone marrow remission at BMA d49 | 94 |
| Table (16): | Comparison between wild type group, heterozygotic group and homozygotic group as regards relapse. | 95 |
| Table (17): | Comparison between wild type group, heterozygotic group and homozygotic group as regards secondary malignancy..... | 95 |
| Table (18): | Adriamycine toxicity..... | 96 |
| Table (19): | Vincristine toxicity..... | 96 |
| Table (20): | VP16 toxicity | 96 |
| Table (21): | Comparison between patients who developed secondary malignancy and those who did not as regards CYP3A41*B polymorphism and sex. | 97 |

List of Tables (Cont.)

| Table No. | Title | Page No. |
|--------------------|--|----------|
| Table (22): | Comparison between patients who developed secondary malignancy and those who did not as regards CYP3A41*B polymorphism and risk stratification. | 98 |
| Table (23): | Comparison between patients who developed secondary malignancy and those who did not as regards cyp3a4 (v) polymorphism and IPT. | 99 |
| Table (24): | Comparison between patients who developed secondary malignancy and those who did not as regards cyp3a4 (v) polymorphism and FAB classification. | 100 |
| Table (25): | Comparison between who developed relapse and who did not as regards cyp3a4 (v) polymorphism and sex. | 101 |
| Table (26): | Comparison between who developed relapse and who did not as regards cyp3a4 (v) polymorphism and clinical risk deree..... | 102 |
| Table (27): | Comparison between who developed relapse and who did notas regards cyp3a4 (v) polymorphism and IPT..... | 103 |
| Table (28): | Comparison between who developed relapse and who did not as regards cyp3a4 (v) polymorphism and FAB classifications..... | 104 |

List of Tables (Cont.)

| Table No. | Title | Page No. |
|--------------------|---|----------|
| Table (29): | Comparison between who developed relapse and who did not as regards cyp3a4 (v) polymorphism and bone marrow remission at BMAd15. | 105 |
| Table (30): | Comparison between FAB classifications (L1 and L2) as regards cyp3a4 (v) polymorphism and sex. | 106 |
| Table (31): | Comparison between FAB classifications (L1 and L2) as regards cyp3a4 (v) polymorphism and clinical risk degree. | 107 |
| Table (32): | Comparison between FAB classifications (L1 and L2) as regards cyp3a4 (v) polymorphism and IPT. | 108 |
| Table (33): | Comparison between FAB classifications (L1 and L2) as regards cyp3a4 (v) polymorphism and bone marrow remission at BMAd15. | 109 |
| Table (34): | Comparison between IPT as regards CYP3A41*B polymorphism and sex. | 110 |
| Table (35): | Comparison between IPT as regards CYP3A41*B polymorphism and clinical risk degree. | 111 |
| Table (36): | Comparison between risk types as regards CYP3A41*B polymorphism and sex. | 112 |

List of Figures

| Figure No. | Title | Page No. |
|---------------------|---|----------|
| Figure (1): | Cytogenic abnormalities in patient with ALL. | 22 |
| Figure (2): | Philadelphia chromosome. A piece of chromosome 9 and a piece of chromosome 22 break off and trade places..... | 25 |
| Figure (3): | Cytochrome P450, family 3, subfamily A, polypeptide 4 | 60 |
| Figure (4): | CYP3A4 Gene in genomic location: bands according to Ensembl, locations according to GeneLoc | 61 |
| Figure (5): | Cartoon diagram of two doxorubicin molecules intercalating DNA, from PDB1D12 | 67 |
| Figure (6): | 4'-demethyl-epipodophyllotoxin 9-[4,6-O-(R) -ethylidene-beta-D-glucopyranoside], 4' - (dihydrogen phosphate)..... | 68 |
| Figure (7): | Methyl (1R,9R,10S,11R,12R,19R) - 11-(acetyloxy)..... | 71 |
| Figure (8): | CYP3A4 A290G after Digestion with Mps 1 | 84 |
| Figure (9): | Distribution of patients as regards clinical risk degree. | 87 |
| Figure (10): | Distribution of patients as regards sex. | 88 |
| Figure (11): | Distribution of patients as regards immunophenotyping. | 88 |

List of Figures

| Figure No. | Title | Page No. |
|---------------------|---|----------|
| Figure (12): | Frequency of cyp3a4 genotype in patients and control | 89 |
| Figure (13): | Comparison between wild type group, heterozygotic group and homozygotic group as regards bone marrow remission at BMA day d15. | 93 |

List of Contents

| Subjects | Page |
|---------------------------------------|-----------|
| No | |
| List of abbreviations | <i>i</i> |
| List of tables..... | <i>ii</i> |
| List of figure | <i>vi</i> |
| Introduction..... | i |
| Aim of the Study..... | 3 |
| Review of Literature | |
| Acute Lymphoblastic Leukemia..... | 4 |
| Cytochrome P450 3A4 | 60 |
| Cyp3a4 Polymorphism and leukemia..... | 74 |
| Subjects and Methods | 77 |
| Results | 86 |
| Discussion | 113 |
| Summary and Conclusion | 125 |
| Recommendations | 129 |
| References | 130 |
| Appendix | I |
| Arabic Summary | — |

Introduction

Acute lymphoblastic leukaemia (ALL) is the most common paediatric cancer with a cure rate of approximately 80%. Relapse occurs despite treatment stratification based on clinical criteria (*Aplenc et al., 2003*).

Several biological risk factors for relapse, such as age, sex, initial white blood cell count and translocation (9;22) modify relapse risk (*Pui & Evans, 1998*). Other factors, such as ethnicity, have a controversial impact on relapse risk (*Pui et al., 1995*), (*Bhatia et al., 2002*).

Relapse risk in ALL may be also related to simple nucleotide polymorphisms (SNPs) of enzymes that metabolize chemotherapeutic agents (*Aplenc et al., 2003*). Simple nucleotide polymorphisms (SNPs) in drug metabolizing enzymes may alter the metabolism of chemotherapy agents and modify the risks of relapse and toxicity (*Relling & Dervieux, 2001*).

Cytochrome P450, family 3, subfamily A, polypeptide 4 (CYP3A4), a member of the cytochrome P₄₅₀ mixed-function oxidase system, is the most abundant hepatic and intestinal P450 enzyme and is involved in the metabolism of more than 50% of all drugs used in humans including glucocorticoids such as dexamethasone, phenobarbital, and anticancer drugs such as ifosfamide, etoposide, doxorubicin and taxol (*Li et al., 1995*).

Recently CYP3A4 has also been identified in the brain, however its role in the central nervous system is still unknown (*Robertson et al., 2003*).

CYP3A4 protein is encoded by the CYP3A4 gene (*Hashimoto et al., 1993*). This gene is part of a cluster of cytochrome P₄₅₀ genes on chromosome 7q21.1 (*Inoue et al., 1992*). Most drugs undergo deactivation by CYP3A4, either directly or by facilitated excretion from the body. Also, many substances are bioactivated by CYP3A4 to form their active compounds, and many protoxins being toxicated into their toxic forms.

While over 28 single nucleotide polymorphisms (SNPs) have been identified in the CYP3A4 gene, it has been found that this does not translate into significant inter individual variability *in vivo*. It can be supposed that this may be due to the induction of CYP3A4 on exposure to substrates.

Most frequent CYP3A4 polymorphism is a -392A>G substitution (CYP3A4*1B) in a 5regulatory element that exhibits frequencies ranging from 0.0% among Chinese-Americans to 54.6% among black Americans (*Lamba et al., 2002*), (*Rebbeck et al., 1998*), (*Kuehl et al., 2001*), (*Paris et al., 1999*).

Studies of the relationship of this polymorphism to gene expression and substrate metabolism have been inconclusive (*Lamba et al., 2002*). However, CYP3A41B has been associated with a higher clinical stage and grade of prostate cancer (*Plummer et al., 2003*), the presence of distant metastatic disease in osteosarcoma (*Dhaini et al., 2003*), increased risk of small cell lung cancer (*Dally et al., 2003*) and indeed the occurrence of treatment related leukemia (*Felix et al., 1998*).



Aim of the Study

The aim of this study is:

- 1- To clarify the prevalence of CYP3A4 genotype among Egyptian children with acute lymphoblastic leukemia in comparison to healthy control.
- 2- To explore whether the polymorphism of CYP3A4 is related to the response to chemotherapy and risks of relapse.

Acute Lymphoblastic Leukemia

Definition:

Acute lymphoblastic leukemia (ALL) is a malignant proliferation of lymphoid cells blocked at an early stage of differentiation. ALL is a biologically heterogeneous disorder, so that morphologic, immunologic, cytogenetic, biochemical, and molecular genetic characterizations of leukemia lymphoblasts are needed to establish the diagnosis or to exclude other possible causes of bone marrow failure and, finally, to classify ALL subtypes. This heterogeneity reflects the fact that leukemia may develop at any point during the multiple stages of normal lymphoid differentiation (*Conter et al., 2004*).

Epidemiology:

Acute leukemia, the most common form of cancer in children, comprises approximately 30 percent of all childhood malignancies, with acute lymphoblastic leukemia (ALL) being five times more common than acute myeloid leukemia (AML). Each year in the United States approximately 2500 to 3500 new cases of ALL are diagnosed in children. Survival rates for leukemia have improved dramatically since the 1980s, with a current five-year survival rate of approximately 78 percent. This improvement is in large part because of treatment of large numbers of children with sequential standardized research protocols. Approximately 75 to 80 percent of children with newly diagnosed ALL participate in clinical research trials, the