MDR-1 POLYMORPHISMS (G2677T & C3435T) IN B-CHRONIC LYMPHOCYTIC LEUKEMIA

Thesis

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Wafaa Wageh

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List of Abbreviations

α …… Alpha

µg ..... Microgram

µL ..... Microlitre

µ ..... Mu

ABCB1…… ATP-binding cassette sub-family B member 1

ADP ……Adenosine diphosphate

AEI …… ..…Allelic expression imbalance

ALC……..Absolute lymphocyte count

ALL……Acute lymphoblastic leukaemia

AML ..... Acute myeloblastic leukaemia

ATP ……Adenosine triphosphate

Bad ..... Bcl-2/BCL-XL-associated death promoter

Bak ..... Bcl-2 homologous antagonist/killer

Bax ..... Bcl-2-associated factor X

Bcl ..... B cell leukemia/lymphoma

B-CLL ..... B cell chronic lymphocytic leukemia

Bik ..... Bcl-2 interacting kille**r**

β2MG..... Beta 2 microglobulin

CBC ..... Complete blood count

CCL21..... Chemokine ligand 21

CCR7 ..... Chemokine receptor type 7

CD..... Cluster of differentiation

CHOP……..…Cyclophosphamide, doxorubicin, vincristin and prednisolone

CLL……...Chronic lymphocytic leukemia

CML…….…. Chronic myloid leukemia

DNA ..... Deoxyribonucleic acid

DW..... Distilled water

dL……..Decilitre

dsDNA…….Double stranded DNA

EBV……..Epstein Barr virus

EDTA……...Ethylene diamine tetraacetic acid

FAB……..French American British classification

FC……....Fludarabin with cyclophosphamide

FCL……...Follicular cell lymphoma

FCR……..….Fludarabin, cyclophosphamide and rituximab

FISH……...Fluorescence in situ hybridization

FMC7…….Mouse Anti-Human B-Cells

FR……..….Fludarabin with rituximab

g..... Gram

Hb ..... Hemoglobin

HCL ..... Hairy cell leukemia

HCV ..... Hepatitis C virus

HIV……Human immunodefiency virus

Hrk ..... Harakini, Bcl-2 interacting protein

HSCT……Haematopoietic stem cell transplantation

HSM ..... Hepatosplenomegaly

Ig..... Immunoglobulin

IgVH..... Immunoglobulin variable region heavy chain gene

IU ..... International unit

Kb……Kilo bite

kD ..... Kilo Dalton

L ..... Litre

LDH ..... Lactate dehydrogenase

LDT..... Lymphocyte doubling time

M..... Molar

Mcl-1..... Myeloid cell leukemia sequence 1

MCL……..….Mantle cell lymphoma

MDR….....Multiple drug resistance

MHC..... Major histocompatibility complex

mAb……..Monoclonal antibody

mL ..... Millilitre

mRNA..... Messenger RNA

N ..... Normal

NCI..... National Cancer Institute

NK……..Natural killer

ng ..... Nanogram

OD…….Optical density

P53 ..... (P) means protein and the number after it represents the molecular weight of this protein

PB..... Peripheral blood

PCR ..... Polymerase chain reaction

PCR-RFLP……Polymerase chain reaction restriction fragment length polymorphism

Pgp…….P glycoprotein

PLL..... Prolymphocytic leukemia

q ..... Long arm of the chromosome

RNA..... Ribonucleic acid

RT-PCR ..... Reverse transcriptase PCR

REs……..Restriction enzymes

rpm..... Revolution per minute

sCD23..... Soluble CD23

sIg ..... Surface immunoglobulin

SLL..... Small lymphocytic lymphoma

SLVL..... Splenic lymphoma with villous lymphocytes

SNPs……..Single nucleotide polymorphisms

sTK……serum thymidine kinase

TCR……..T cell receptor

TLC..... Total leukocytic count

t..... Translocation

UV……Ultraviolet

VAD……..Vincristin,adriamycin and dexamethazone

VH..... Variable region heavy chain gene

WBC……..White blood cell

WHO ..... World Health Organization

ZAP70..... Zeta-chain associated protein kinase

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Abstract

The human multidrug resistance (MDR-1) gene encodes P-glycoprotein (Pgp), which affects the pharmacokinetics of many drugs. Here, we investigated whether common MDR1 single nucleotide polymorphisms (SNPs) (C3435T and G2677T) affect predisposition to B-chronic lymphocytic leukemia (B-CLL). Genotyping was performed in 65 patients with CLL and 70 controls using polymerase chain reaction- restriction fragment length polymorphism (PCR-RFLP). We observed a higher frequency of carriers of 3435CT gene among B-CLL patients as compared to normal individuals (58.5% vs. 22.9%, p=<0.001). The genotypes 3435CT was associated with B-CLL, [odds ratio=4.8, 95% confidence interval=2.3-10.0]. Moreover, patient and control groups did not differ significantly regarding MDR1 genotype (G2677T). Furthermore, no correlation was shown between the MDR1 (3435 or 2677) genotypes and clinical and laboratory data of patient group. In conclusion, these data indicate that the MDR1 C3435T SNP may carry an increased risk of developing B-CLL.

Key Words:

PgP – MDR-1 – B-CLL .

INTRODUCTION

B-Chronic lymphocytic leukemia is one of the most common hematological malignancies that results in significant morbidity and mortality. It is caused by the clonal expansion of B cells with a distinctive morphological appearance and surface immunophenotype. One of the most striking features of CLL is the extent of its clinical variability. Some studies have been developed to understand this variability in terms of biological heterogeneity ***(Pettitt, 2007).***

The incidence of CLL is higher among whites than blacks. The incidence of CLL is higher in males than in females, CLL is a disease that primarily affects the elderly***(Gribben,2010).***

Most people are diagnosed without symptoms as the result of a routine blood test that returns a high white blood cell count, but as it advances CLL results in swollen lymph nodes, spleen, and liver, and eventually anemia and infections. Early CLL is not treated, and late CLL is treated with chemotherapy and monoclonal antibodies. It is now possible to diagnose patients with short and long survival more precisely by examining the DNA mutations, and patients with slowly-progressing disease can be reassured and may not need any treatment in their lifetimes ***(Chiorazzi et al.,2005).***

The prognosis of patients with CLL varies widely at diagnosis. Some patients die rapidly, within 2-3 years of diagnosis, because of complications from CLL. Most patients live 5-10 years,

with an initial course that is relatively benign but followed by a terminal, progressive, and resistant phase lasting 1-2 years. During the later phase, morbidity is considerable, both from the disease and from complications of therapy ***(Rai & Keating,1997).***

Prognosis depends on the disease stage at diagnosis as well as the presence or absence of high-risk markers ***(Kristinsson et al.,2009).***

The multidrug resistance 1 gene (MDR1) product P-glycoprotein (Pgp) is an important member of ATP-binding cassette transporter family that functions as an energy dependent xenobiotic efflux pump ***(Gervasini et al., 2006).***Therefore, the most important physiological role of Pgp is the protection of the organism against toxic xenobiotic agents and environmental carcinogens ***(Jamroziak et al., 2004 and Gervasini et al., 2006).*** Moreover, as a wide range of important anticancer agents such as drugs used in chemotherapy can be actively extruded by this protein, Pgp expression in tumor cells is associated with multidrug resistance phenotype in some malignancies, including leukemia ***(Jamroziak et al., 2004, 2005).***

Pgp is expressed at the highest level in adrenal gland; at an intermediate level in breast, stomach,colon, jejunum, rectum, liver, kidney, and placenta; and at a low level in testis, brain, bone marrow, and heart in humans. Its wide tissue distribution suggests that Pgp has a fundamental role in normal cellular metabolism ***(Hitchins et al., 1988).***

MDR1 is a polymorphic gene with more than 40 single-nucleotide polymorphisms (SNPs). The most important MDR1 gene polymorphism is C3435T SNP, which is located on exon 26 and considered to be associated with decreased tissue protein expression and activity ***(Hoffmeyer et al., 2000).***

A number of studies indicated that the presenceof the 3435T allelic variant and particularly the homozygous mutant 3435TT genotype seems to be associated with a greater susceptibility to renal epithelial tumors ***(Siegsmund et al.,2002),*** colon cancer ***(Kurzawski et al., 2005),*** ulcerative colitis***(Schwab et al., 2003; Glas et al., 2004 and Farnood et al., 2007),*** and acute lymphoblastic leukemia ***(Jamroziak et al., 2004).***

Aim of the Work

The aim of the present study was to evaluate the role of two MDR1 gene polymorphisms (G2677T polymorphism) in exon 21 and (C3435T polymorphism) in exon 26 as risk factors for development of B-CLL and their relation to the clinical presentation of the patients.