The Impact of P-53 pathway genes polymorphism on the Clinical Outcome in Adult Patients with Acute Myeloid Leukemia

Thesis for Fulfillment of Master Degree in Medical Oncology

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List of Abbreviations

AL: Acute leukemia

ALFA: Acute Leukemia French Association

ALL: Acute Lymphocytic Leukemia

ALSG: Australian Leukemia Study Group

AMKL: acute megakaryoblastic leukemia

AML: Acute Myeloid Leukemia

AMML: acute myelomonocytic leukemia

AMoL: acute monocytic leukemia

APL: Acute promyelocytic leukemia

ATLS: Acute tumor lysis syndrome

ATP: adenosine triphosphate

ATRA: all-trans retinoic acid

AUL: acute undifferentiated leukemia

BAALC: brain and acute leukemia, cytoplasmic

CALGB: Cancer and Leukemia Group B

CBF: Core binding factor

CDK: cyclin-dependent kinases

CLL: chronic lymphocytic leukemia

CMV: cytomegalovirus

CNS: Central Nervous System

CSF: cerebrospinal fluid

DBD: DNA binding domains

DFS: disease free survival

DIC: disseminated intravascular coagulopathy

DN: de novo

DNA: deoxyribonucleic acid

DS: Down Syndroms

EBMT: European Bone Marrow Transplant

ECOG: Eastern Cooperative Oncology Group

EFS: event-free survival

EGIL: European Group for Immunologic Classification of Leukaemia

FA: Fanconi anemia

FAB: French American British

FDA: Food and Drug Administration

FISH: fluorescent in situ hybridization

FL: FLT3 ligand

G-CSF: Granulocyte Colony-Stimulating Factor

GIMEMA: Italian group for adult hematological diseases

GM-CSF: Granulocyte-Macrophage Colony-Stimulating Factor

GO: Gemtuzumab ozogamicin

GVL: graft-versus leukemia

HiDAC: high dose AraC

HLA: human leucocyte antigen

HOVON: The Dutch-Belgian Hemato-Oncology Cooperative Group

HSCT: hematopoietic stem cell transplantation

IL3: interleukin 3

IR: infra-red

IRB: Institutional Review Board

ITD: Internal tandam duplicate

JM: juxtamembrane

JMML: juvenile myelomonocytic leukemia

kDa: kilodalton

LDAC: low-dose cytarabine

LDH: lactate dehydrogenase

Mdm: murine double minute 2

MDR: multidrug resistance

MDR: myelodysplastic related

MDS: Myelodysplastic syndrome

MLL: mixed lineage leukemia gene

MM: multiple myeloma

MPO: myeloperoxidase

MRC: Medical Research Council

MRC: Medical Research Council (UK)

NCI: National Cancer Institute

NPM: Nucleophosmin

NSE: nonspecific esterase

OS: overall survival

PAS: periodic acid- Schiff

PETHEMA: Program for the study and treatment of malignant hemopathies

RARα: retinoic acid receptor α

RAS: Reticular activating system

RFLP: reaction fragment length polymorphism

RIC: reduced-intensity conditioning

RNA: ribonucleic acid

RT-PCR: reverse transcriptase–polymerase chain reaction

SAKK: Swiss Group for Clinical Cancer Research

SBB: Sudan black B

SNP: single nucleotide polymorphisms

SWOG: South West Oncology Group

TKD: Tyrosine Kinase Domain

TMD: Transient myeloproliferative disease

t-MDS, t-AMLL: therapy-related disease

TRM: transplant related mortality

UV: ultraviolet

WBC: white blood cells

WHO: World Health Organization

Abstract

Introduction: Acute myeloid leukemia (AML) is a highly heterogeneous disease, with biologically and prognostically different subtypes. Many cytogenetic studies have been implicated to clinical outcome in AML. p53 gene pathway has been proved in many cancers to have an impact on survival.

Aim of work: To study the impact of p53, p21 and mdm2 gene polymorphism on clinical outcome in adults with AML treated at National Cancer Institute -Cairo University.

Methods: All patients presented to Medical Oncology department-NCI from April 2010 till November 2011. Clinical data and bone marrow samples were obtained. Molecular genetic analysis involving P53, MDM2 and P21 single nucleotide gene polymorphism will be done using PCR-RFLP coupled analysis. Clinical follow up was done for a period of 1 year (0.5 - 12 months)

Results: 48 cases were enrolled in the study. The mean age was 35.7 years (20-60yrs). More than half of the patients were AML M1 and M2 subtypes (52%). 48% of the patients presented with fever of more than 38. Most cases presented with hepatomegaly (56%), and splenomegaly was 39% of total cases. Initial total leucocytic count (TLC) at diagnosis ranged from less than 11,000/mm³ (17%) to more than 50,000 (33%) (50,000–183,000/mm³). All of the patients were subjected to standard of care treatment in National Cancer Institute. Adriamycin 45mg/m² short infusion over 30 minutes was given for 3 consecutive days. Ara C 100mg/m² continuous infusion was given from day 1 to day 7 of induction therapy. Patients who achieve CR were subjected to high dose Ara-C plus mitoxantrone regimen (HAM). ATRA 45 mg/m2/day was given in M3 cases continuously for 90 days plus Adriamycin 45 mg/m² for 3 days during induction. After a median follow up period of 11 months, 28 patients (58.3%) achieved CR while20 cases (41.6%) had noCR. Median OS was 8.7 months. Patients with GG genotype P53 had a median OS of 13.4 months while heterozygous variant p53 had a median OS of 8.4 months. Homozygous variant p53 patients showed a median OS of 1.5 month (p=0.0045). Neither p21or mdm2 polymorphism alone showed an impact on OS (p=0.310) and (p=0.846). DFS was not affected by any of the 3 gene polymorphisms alone. P53/p21 combination showed a median OS of 12.1 months for 12 cases who had both genes of non-polymorphic type compared to 1.1 month (0-5.3 months) median OS for 6 patients with both genes of variant type (P=0.037).DFS for the same combination was also significant with 13.7 months for nonpolymorphic p53/p21 and 1.9 months for both variant type (p=0.004). Other gene combinations failed to show an impact on clinical outcome.

Conclusion: p53 gene polymorphism is an independent predictive of poor outcome in AML. Combined gene polymorphism of p53/p21 carries a poor prognosis on survival. We recommend further larger studies on p53 gene pathway with correlation with clinical outcome in AML patients.

Key words: acute myeloid leukemia, p53 gene, mdm2, p21, polymorphism, clinical outcome, National Cancer Institute, Egypt.

Introduction & Aim of the Work

Acute myeloid leukemia (AML) is a highly heterogeneous disease, with biologically and prognostically different subtypes distinguished by cytogenetic and molecular genetic analysis, as recognized in the revised WHO classification system (WHO 2008). However, since the publication of this schema, application of various high throughout technologies including whole genome and exome sequencing of AML cases has revealed a plethora of recurrent mutational targets, including a number of genes encoding transcriptional regulators, not previously implicated in leukemogenesis. Deciphering the combinations of mutations that cooperate to induce AML and determining which particular alterations (or combinations) confer independent prognostic information, which will necessitate analysis of large cohort sizes involving international cooperation ***(Grimwade D et al.,2009).***

It has become increasingly possible to predict likely clinical outcome in patients with newly diagnosed AML. This is not only important for the counseling of the affected individuals and their families, but is becoming increasingly relevant to determine post remission treatment approaches, well-recognized adverse risk factors include older age, secondary disease, and higher presenting white blood cell (WBC) count ***(Grimwade D et al.,2009),*** however, major steps forward in understanding disease pathogenesis and predicting likely response to therapy have been explored, showing that a significant proportion of AML cases harbor recurrent chromosomal abnormalities, including balanced translocations that were found to disrupt key genes involved in normal haematopoiesis ***(Grimwade D et al., 2011).*** These include the t(15;17)(q22;q21), which leads to fusion of the PML and RARA genes in acute promyelocytic leukaemia (APL) and rearrangements disrupting components of the core binding factor (CBF) transcription factor complex, i.e. t(8;21)(q22;q22)/RUNX1-RUNX1T1 and inv(16)(p13q22)/t(16;16)(p13;q22)/CBFB-MYH11.

Such balanced chromosomal rearrangements are now considered to be critical initiating events in leukemogenesis and it is clear that cytogenetic

analysis provides a very powerful approach to distinguish biologically and prognostically distinct subsets of AML. The importance of cytogenetics in AML provides the framework for risk stratification schemes employed by many trial groups to guide treatment approach ***(Swerdlow SH et al., 2008).*** Testing of NPM1, CEBPA and FLT3 mutation are recommended at least in patients with cytogenetically normal AML (CN-AML) who will receive treatment other than low-dose chemotherapy or best supportive care **(Dohner et al., 2010).**

Cell cycle control is a crucial event in normal hematopoiesis, and abnormalities of regulatory cell cycle genes have been found to contribute to the development of many hematologic malignancies. Neoplastic cells originate from a single cell that has lost the ability to maintain the balance between proliferation and apoptosis. Cell cycle progression is regulated by coordinated interactions of cyclins, cyclin dependent kinases (CDKs), CDK inhibitors (CKIs), oncogenes and tumor suppressor genes ***(K. Hiromura et al.,1999)***. The p53 and the Rb pathways are two major growth regulatory pathways. The p53 tumor suppressor is a transcription factor that mediates cellular responses to DNA damage by regulating cell-cycle arrest, senescence, and apoptosis ***(Levine AJ, 1997).*** P53-dependent G1 arrest is mediated, at least in part, through induction of p21 (member of the Cip/Kip CKI family). P21 functions by inhibiting and inactivating a number of cyclin/CDK complexes involved in the G1–S transition. It has been shown to inhibit G2–M phase transition as well ***(G. Delsal et al., 1996)***. Several studies have shown that p53 mutations are poor prognostic indicators for leukemia ***(J. Imamura et al., 1994),*** whereas others suggest that structural alterations of the p53 gene do not play an important role in the initiation and progression of the disease ***(B. Seliger et al., 1996)***. The activity and the stability of p53 are regulated by interaction with mdm2. mdm2 functions by directly blocking the activity of p53 as a transcription factor and by targeting it for degradation. In AML, overexpression of mdm2 was associated with shorter survival ***(S. Faderl ET AL., 2000).***

Single nucleotide polymorphisms (SNP) in codon Arg72Pro of P53 results in impairment of the tumor suppressor activity of the gene. A similar effect is caused by a SNP in codon 31 of P21. In contrast, a SNP in position 309 of MDM2 results in increased expression due to substitution of thymine by guanine. All three polymorphisms have been associated with increased risk of tumorigenesis ***(Onel K ET AL.,2000).***

Aim of Work

To study the impact of **p53** arg72pro gene polymorphism (G-C or C-C), **p21** codon 31 Ser/arg polymorphism and **mdm2** T309G genotyping (T-G or G-G) gene polymorphisms on clinical outcome in adults with acute myeloid leukemia treated at National Cancer Institute (NCI) -Cairo University.

Epidemiology

Incidence

AML accounts for approximately 25% of all leukemias in adults in the West and constitutes the most frequent form of leukemia. Worldwide, the incidence of AML is highest in the USA, Australia, and Western Europe ***(Dohner H et al., 2010)***. AML represented approximately 30 percent of the annual new cases of leukemia in the United States of America, where 13790 new cases of AML occur annually and were divided into 7350 male cases and 6430 female cases, with estimated deaths about 10200 in both genders included 5790 male deaths and 4410 female deaths. The higher incidence rate of leukemia was in California**,** Texas and Florida **(Siegel, 2012)**

In the National Cancer Institute (NCI) Cairo University during the years 2002-2003, out of a total of 18496 new cancer cases, 349 patients (1.9%) were diagnosed as AML with a median age of 22 and male to female ratio of 1.37. Leukemia ranked 4th most common site among males and 3rd among females. ***(Elattar et al.,2003)***

Etiology and Risk Factors

Most cases of AML have no apparent cause. Risk factors associated with increased incidence include Genetic factors, environmental factors and acquired diseases.

a. Genetic factors

Genetic factors are implicated in the pathogenesis of AML by virtue of its high incidence in patients with syndromes characterized by chromosomal abnormalities or instability or defective DNA repair ***(Schwartz CL et al.,1988).***

These disorders have variable inheritance patterns and can be divided according to the type of genetic defect ***(Lichtman MA et al.,1982)*** as shown in Table 1.

Table1. Genetic Disorders Implicated in the Pathogenesis of Acute Myeloid Leukemia

Congenital Defects	Marrow Failure Syndromes
Down syndrome	Fanconi anemia
Bloom syndrome	Dyskeratosis congenita
Monosomy 7 syndrome	Schwachman-Diamond syndrome
Klinefelter syndrome	Amegakaryocytic thrombocytopenia
Turner syndrome	Blackfan-Diamond syndrome
Neurofibromatosis	Kostmann agranulocytosis
Congenital dysmorphic syndromes	Familial aplastic anemia

(Lichtman MA et al., 1982)

Down syndrome child have 50 folds increased risk for developing leukemia, if less than 5 years old age, and usually acute megakaryoblastic leukemia M7 **(Tandonnet et al., 2010).**

Fanconi anemia is an inherited autosomal recessive condition that results from defects in genes that modulate the stability of DNA, with an inability to repair RNA (**Dokal, 2008).** Overexpression of TNF in the marrow may play a role in the suppression of erythropoiesis **(Dufour et al., 2008).** The syndrome is characterized by short stature, absent or supernumerary thumbs, dysplastic radii and abnormal skin pigmentation. Many patients may diagnosed at the fifth decade of life by the onest of progressive marrow failure and conversion to MDS or AML, approximately 10% of patients progress to AML **(Rosenberg, 2008).**

Dyskeratosis congenita is a recessive X-chromosome-linked disorder, the telomeres are markedly shortened resulting in genomic instability and marrow cell apoptosis **(Savage, 2008).** This syndrome is characterized by cutaneous macules, alopecia of scalp, eyelashes, and eyebrows, hyperkeratosis of palms and soles and mucosal leukoplakia. Aplastic anemia usually develops in late childhood or early adulthood, with increased risk for AML **(Roth, 2008).**