INTRODUCTION

Acute myeloid leukemia (AML) is a malignancy originating in a multipotential hematopoietic cell characterized by clonal proliferation of abnormal blast cells in the marrow and impaired production of normal blood cells ***((Hoffbrand et al., 2006)***.

During the last few decades the classification of AML has shifted from a morphologically based classification to a scheme including cytogenetics and accordingly three major risk groups [low, intermediate and high] have been distinguished depending on AML karyotype at diagnosis ***(Moreno et al., 2003).***

However, the so called intermediate risk group which represents approximately 2/3 of cases comprises a heterogeneous group of patients with apparently normal karyotypes or patients with a variety of other aberrations whose prognostic outcome is uncertain. Therefore, better identification of prognostic indicators in this group has been of particular interest in the past few years ***(Moreno et al., 2003)***.

Molecular mutations are of increasing importance for stratification and risk assessment in AML. Investigations of the genetic aberrations lead to a better understanding of molecular lesions in the pathogenesis of AML, thus enabling new AML subgroups and prognostic factors to be defined ***(Kuchenbauer et al., 2005).***

fms-like tyrosine kinase (FLT3) is a class III receptor tyrosine kinase (RTK) expressed in normal hematopoietic stem cells and its dimerization by FLT3 ligand expressed on bone marrow stroma, induces growth control signals in normal hematopoiesis ***(Boissel et al., 2002)***.

FLT3 was also found to be expressed on the majority of AML blast cells, acute lymphoblastic leukemia and blastic crisis of chronic myeloid leukemia. The FLT3 ligand stimulates proliferation and induces inhibition of apoptosis in AML cells expressing functional FLT3. Therefore, alterations in the structure and signaling could potentially contribute to leukomogenesis ***(Kainz et al., 2002)***.

Mutations of the FLT3 have been reported in adult AML, myelodysplastic syndrome and occasionally in chronic myeloid leukemia and lymphoproliferative disorders. The most frequent FLT3 mutations found in AML are internal tandem duplications (ITD). These mutations affect the juxtamembrane domain and can be of variable length although all cases are in-frame mutations. ITD of the FLT3 gene are observed in all AML subtypes according to the French/American/British (FAB) classification ***(Muñoz et al., 2003)***.

ITDs lead to dimerization and constitutive activation of the receptor in absence of its ligand resulting in autonomous proliferation and differentiation block in leukemia cells and this effect can be related to the high peripheral white blood cell counts found in patients with FLT3 mutations ***(Kiyoi et al., 2006)***.

Clinical studies investigating FLT3-ITD in patients with AML have indicated a strong association with poor prognosis or adverse risk factors. Thus FLT3-ITD seem to add considerable prognostic information in patients within the intermediate risk group especially those with normal karyotype while its prognostic value in various cytogenetic risk groups is still a matter of debate ***(Kainz et al., 2002)***.

Since FLT3-ITD is the most frequent genetic aberration in AML, the aberrant signal transduction pathways from the mutant FLT3 would serve as an important molecular target for therapy ***(Kiyoi and Naoe, 2006)***.

AIM OF THE WORK

The aim of this work is to:

- Investigate FLT3 expression levels by real time PCR in adult AML patients.
- Correlate its levels with different cytogenetic subgroups, immunophenotyping and other prognostic factors.

ACUTE MYELOID LEUKEMIA

Acute leukemia (AL) is a heterogeneous group of neoplasms affecting uncommitted or partially committed hematopoietic stem cells ***(Chan, 2002)***. They are broadly classified into non-lymphoblastic (commonly referred to as myeloid) and lymphoblastic according to the cell of origin whether myeloblast or lymphoblast ***(Hoffbrand et al., 2006)***.

Acute myelogenous leukemia (AML) or acute non-lymphoblastic leukemia (ANLL) is a marrow-based clonal malignant disease of hemopoietic tissue that arises as a result of somatic mutation in a pluripotential stem cell or a slightly more differentiated progenitor cell. It is characterized by the proliferation of abnormal leukemic (blast) cells, principally in marrow and impaired production of normal blood cells ***(Hoffbrand et al., 2006)***.

The cell of origin in AML is a blast that most often show myeloid or monocytic differentiation. In approximately 5 to 10% of patients blasts have erythroid or megakaryocytic differentiation; for this reason, ANLL has been considered a more precise term but AML is more commonly and is the recommended term ***(Chan, 2002)***.

The AML is the predominant form of leukemia during the neonatal period, but it represents < 15% of cases of leukemia in children under 10 years and 25 to 35% between ages 10 and 15 years. While in adults it represents 80-90% of cases of acute leukemia. The incidence is higher in males than in females ***(Löwenberg and Burnett, 2005)***.

I- AETIOLOGY:

The cause of AML is unknown, but studies suggest that environmental, occupational and genetic factors may contribute to the aetiology in some patients (Table 1) ***(Lichtman and Liesveld, 2006)***.

Table (1): Conditions predisposing to the development of acute myelogenous leukemia.

ENVIRONMENTAL FACTORS:

- Radiation.
- Benzene.
- Alkylating agents and other cytotoxic drugs.

PREDISPOSING DISEASES :

Acquired Diseases :

Clonal hematopoietic diseases :

- Chronic myelogenous leukemia.
- Idiopathic myelofibrosis.
- Primary thrombocythemia.
- Polycythemia vera.
- Acquired sideroblastic or non-sideroblastic anemia.
- Bi-or tricytopenia with hyperplastic marrow.
- Paroxysmal nocturnal hemoglobinuria.

Other Hematopoietic Disorders :

- Aplastic anemia.
- Eosinophilic faciitis.
- Myeloma.

Inherited conditions :

- Identical sibling with AML.
- Non-identical sibling with AML.
- Down syndrome.
- Fanconi anemia.
- Bloom syndrome.

Table (1): Cont.

- Ataxia-telangiectasia.
- Wiskott-aldrich syndrome.
- Dyskeratosis congenita.
- Combined immunodeficiency syndrome.
- Familial AML.
- Werner syndrome (progeria).
- Neurofibromatosis
- Schwachman syndrome.
- Chromosome 21q disorder.

(Lichtman and Liesveld, 2006)

II-LEUKEMOGENESIS:

Acute myeloid leukemia is believed to begin in a single somatic hematopoietic progenitor or multipotential stem cell that transforms to a cell incapable of normal differentiation, in addition many of these cells no longer possess the normal property of apoptosis resulting in a cell with prolonged life span and unrestricted clonal proliferation ***(Hoffbrand et al., 2006).***

The pathogenesis of AML involves an array of molecular alterations that disrupt almost every facet of cell transformation. These include dysregulation of cell proliferation, differentiation, self-renewal, survival, cell dissemination, and escape from apoptosis**.** Some of the features of AML and their potential causes are listed in (Table 2) ***(Licht and Sternberg, 2005).***

Table (2): The molecular lesions in AML associated with malignant characteristics.

Property	Autonomous Cell proliferation	Differentiation Block	Increased Self-Renewal	Escape from Apoptosis	Loss of Cell Cycle Control	Dissemination
Molecular Lesion	**Activating mutations:** Flt3, Ras, c-kit, c-FMS, Jak2, ***Inactivating* mutation**;-NF1,	**Fusion transcription factors** -Retinoic acid receptor PML-RARα, PLZF-RARα, - Core binding factor: RUNX1-MTG8, CBFβ-MYH11, RUNX1-EVI1 -MLL-fusion: -Point mutations of transcription factors: Pu1, C/EBPα, RUNIX1	**B-catenin mutations** **Activated RTK pathways cooperate to induce self-renewal**	-AKT pathway activation following RTK activation leads to Bad deactivation -P53 mutation in AML of the elderly -P53 dysregulation by fusion proteins NPM mutation -Bcl2 over expression -Survivin (IAP) over expression	-P53 dysfunction -Loss of Rb	-TNF secretion by leukemic blasts stimulates endothelium. -Increased selectin, cadherin and integrin expression encourage adhesion and egress through vessels

(Licht and Sternberg, 2005)

1. Inappropriate Proliferation: The Role of Signaling Molecule

Activation of receptor and intracellular protein tyrosine kinases stimulates a cascade of phosphorylation-driven protein docking and recruitment events that leads to the alteration of transcription in the cell nucleus and the stimulation of cell cycle progression. Although cell proliferation is regulated by the presence of growth factors and adhesion signals in normal cells, it can be triggered in leukemic cells in a cell autonomous manner. This abnormal proliferation is often the result of mutations affecting proliferative signaling pathways ***(Licht and Sternberg, 2005)***.

Many dysregulated tyrosine kinase receptors were found in AML eg. FLT3, C-KIT.

The FLT3 tyrosine kinase is expressed in almost all patients with AML. It is constitutively activated by internal tandem duplication within the juxtamembrane domain or by mutation within the activation loop of the kinase in approximately 30% of AML ***(Wadleigh et al., 2005).***

The c-KIT tyrosine kinase is expressed in 60%–80% of AML patients, and this kinase is activated by mutation in mast cell leukemia and some cases of AML ***(Kaushansky, 2005)***.

The JAK2 kinase is activated by the V617F point mutation in the overwhelming majority of patients with polycythemia vera and in a significant proportion of patients with essential thrombocythemia or idiopathic myelofibrosis, all

disorders that can evolve into AML. Also, the V617F JAK2 mutation is found in 5% of patients with myelodysplastic syndrome, suggesting that the mutation will also be found in patients who transform to frank AML ***(Kaushansky, 2005)*** and ***(Steensma et al., 2005).***

Activated tyrosine kinases transmit proliferative signals by engagement of the RAS family of small G-proteins, and mutation of genes encoding these proteins can mimic the effects of receptor tyrosine kinase (RTK) mutations. N-RAS is mutated and constitutively activated in 10%–20% of AML, K-RAS in 5%–15% of patients, while H-RAS is rarely mutated ***(Valk et al., 2004).***

AML samples that contain RAS mutations do not have kinase fusions or activating mutations, suggesting that Ras and tyrosine kinase molecules fall in a single complementation group in AML. In general the RTK pathways in AML are activated by gain of function mutations and have been documented in nearly 50% of cases of AML ***(Licht and Sternberg, 2005).***

2. Differentiation Blockade: The Role of Transcription Factors in AML

Transcription factors are commonly disrupted in AML either by their fusion as a result of chromosomal translocation or by point mutation. Factors affected by chromosomal rearrangement include the core binding factor complex, the retinoic acid receptor (RAR), and the MLL protein. Point

mutations in myeloid transcription factors, including C/EBPα and PU.1, may also lead to loss of normal myeloid differentiation in AML. Chimeric transcription factors often work as dominant negative forms of the original factor. CBF and RAR fusions are prime examples of this ***(Licht and Sternberg, 2005).***

3. Escape from Programmed Cell Death

The ability to evade apoptosis is critical to the development of a malignancy. Protein tyrosine kinase activation can have the dual effect of promoting cell proliferation and in addition enhancing cell survival by activating phosphatidylinositol 3-kinase (PI 3-kinase) signaling. The phospholipid products of PI 3-kinase activate the AKT serine/threonine kinase, and this kinase in turn phosphorylates BAD and releases the BCL-2 pro-survival molecule ***(Licht and Sternberg, 2005).***

The p53 protein is a focal point in the regulation of apoptotic signaling and cell cycle regulation. Mutations within p53 are associated with adverse response to chemotherapy in patients with AML. Moreover, the function of this protein is often compromised in AML ***(Falini et al., 2006)***.

4. Self-Renewal

Unlike normal progenitor cells that are committed to a particular hematopoietic lineage, leukemic cells from patients with AML can undergo self-renewal rather than lineage-specific commitment. Moreover, the leukemic stem cell population in AML is functionally heterogeneous with differing capacities for self-renewal ***(Hope et al., 2004).***

NPM is mutated in approximately one-third of newly diagnosed AML, and the expression of this cytoplasmic NPM variant is associated with expression of genes thought to support maintenance of the stem cell phenotype ***(Alcalay et al., 2005).***

The FLT3-ITD mutant of AML, which activates proliferative and survival pathways, also confers the property of self-renewal in human $CD34^{+}$ cells. Thus, the expression of mutated and fusion genes in AML seems to underlie some aspects of enhanced self-renewal, although such findings do not exclude the possibility that the progenitor cell in AML might itself have intrinsic self-renewal properties independent of a leukemogenic insult ***(Chung et al., 2005)***.

5. Loss of Cell Cycle Control

Deregulation of cell cycle control in AML may occur through multiple mechanisms. First, constitutive Ras/MAP kinase signaling leads to the activation of nuclear transcription factors that induce expression of cyclins. Rb mutations and deletions occur in AML as in other forms of malignancy. Hypermethylation of growth suppressor genes is a recurrent theme in many malignancies and is well described in AML. In particular, the $p15^{Ink4a/ARF}$ locus as well as the $p16^{Ink4b}$ locus encoding cyclin-dependent kinase inhibitors can be methylated and silenced in AML ***(Toyota et al., 2001)***.

6. Leukemia Cell Dissemination

High level of expression of selectin on surface of leukemic cells is a negative prognostic marker in AML.

Secretion of tumor necrosis factors and other cytokines by the leukemic blast can lead to increased expression of selectins, cadherins and other adhesion proteins on vascular endothelium, resulting in increased leukemia cell adhesion ***(Stucki et al., 2001).***

III- CLASSIFICATION:

Different classification systems were proposed to classify AML depending on morphological, cytochemical immunological and cytogenetic features.

1. The French-American-British (FAB) Classification

It is the most widely used classification system since it was established in 1976. It is a lineage-based system that had defined eight variants of AML depending on morphology and cytochemistry (Table 3). The FAB group's purpose was to subdivide AML according to their predominant cell type and the maturation sequence. The FAB classification requires a blast count of 30% or more in the bone marrow for the diagnosis of AML ***(Pui et al., 2000)***.

The FAB classification doesn't include biphenotypic or hybrid leukemia that can't be identified on the basis of morphology or histochemical stains alone. Moreover, the FAB group classification did not contain clinical characteristics, cytogenetic patterns, response to chemotherapy or prognosis in its formulation ***(Pui et al., 2000) and (Arber, 2001)***.

Other classification systems have been proposed in an attempt to address these issues. But despite its limitations, the FAB classification is used almost universally.

Table (3): Morphologic (FAB) classification of AML

Subtype	Morphology features
Acute myeloblastic leukemia minimally differentiated (M0)	≥ 30% myeloblasts without granules
Acute myeloblastic leukemia without maturation (M1)	≥ 30% myeloblasts, with or without scanty granules, <10% show maturation beyond blast stage.
Acute myeloblastic leukemia with maturation (M2)	≥30 myeloblasts with granules: promyelocytes or mature cells ≥10%; monocytic cells <20%
Acute promyelocytic leukemia (M3)	≥ 30% myeloblasts and promyelocytes with prominent granules.
M_3V (APL-V)	Microgranular (hypogranular) cells promyelocytic leukemia
Acute myelomonocytic leukemia (M4)	Myeloblasts, monoblasts and promyelocytes >30% marrow cells; monocytic cells > 20%
$M4_{EO}$	Acute myelomonocytic leukemia with increased marrow eosinophil
Acute monoblastic leukemia without differentiation (M5a)	>80% monocytic cells; >80% are large monoblasts with lacy nuclear chromatin and abundant cytoplasm
Acute monoblastic leukemia with differentiation (M5b)	80% monocytic cells with monoblasts, promonocytes, monocytes; promonocytes predominate blood monocytosis.
Acute erythroleukemia (M6)	Megaloblastic erythroid presursors (>50%): myeloblasts (>30%)
Megakaryocytic leukemia (M7)	Megakaryoblasts "lymphoid" morphology (L1, L2, M1) cytoplasmic budding.

MPO : Myeloperoxidase ; SBB : Sudan Black B; CAE : Chloroacetate esterase; NSE : nonspecific esterase
+ : Usually present; ++ : Present in abundance, - : Usually absent, ± : May or may not be present.
† : Inhibited by sodium fluoride, ‡ : May be inhibited by sodium flouride.

(Miller and Daoust, 2000)

2. World Health Organization (WHO) Classification

The WHO proposed a classification which had integrated molecular genetics to the morphological, cytochemical and immunophenotypic features since the molecular genetic abnormalities are of increasing importance for prognosis and choice of treatment (Table 4)

The WHO has introduced a reduction in the percentage of blasts required for diagnosis of AML from 30% to 20% ***(Swirsky and Richards, 2001)***.

In addition, patients with the clonal, recurring cytogenetic abnormalities t (8;21) (q22;q22), inv (16) (p13;q22) or t (16;16) (p13;q22), and t(15;17) (q22;q12) should be considered to have AML regardless of the blast percentage ***(Vardiman et al., 2002).***

The classification is hierarchical with cases with certain recurrent cytogenetic abnormalities being categorized first, followed by cases with multi-lineage dysplasia then therapy related cases. Finally, the remaining cases are assigned on the basis of cytological features to categories that have similarities to the FAB categories of AML ***(Swirsky and Richards, 2001).***

Also, other categories have been added which are:

- Acute basophilic leukemia.
- Myeloid sarcoma.
- Acute panmyelosis with myelofibrosis
- Myeloid proliferation related to down syndrome.

- ***Acute basophilic leukemia***

Acute basophilic leukemia is an AML that exhibits a primary differentiation to basophils. This acute leukemia is relatively rare, comprising less than 1% of all cases of AML. Most cases evolve from the chronic phase of CML, but de novo