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Introduction

In recent years, the development of several novel technologies help in explaining the genetic heterogeneity of acute myeloid leukemia (AML). Genetic analysis becomes an essential tool firstly, as routine work for disease classification, and secondly, determined treatment strategies. The recurrent AML-associated gene fusions as well as mutations in *DNMT3A, FLT3,* and *NPM1A* genes are used as rapid pretherapeutic genetic analysis, and prognostic sensor **(Döhner *et al.*, 2015).**

Recent studies reported a new prognostic marker, which is DNA methyltransferase 3A (*DNMT3A*) gene, it controls DNA methylation. DNA methylation is one of the most important epigenetic player, and it takes part in gene expression. Previous studies showed that the most common mutations of *DNMT3A* is hotspot of arginine in 882th codon (R882). Several studies reported that the patients with *DNMT3A* mutations have bad prognosis **(Park *et al.*, 2015).**

Fetal liver tyrosine kinase 3 (*FLT3*), is a member of receptor tyrosine kinases (RTKs) class III subfamily, that is expressed by early hematopoietic progenitors, and it participates in growth regulation of hematopoietic progenitor cells. Some studies reported that *FLT3* expressed in AML cells and stimulated survival and proliferation of leukemic blasts. Internal tandem duplication mutation of *FLT3* (*FLT3-ITD*) is the most

prevalent mutation in AML patients **(Wang *et al.*, 2014).** Several studies showed that the correlation between the presence of *FLT3-ITD* mutation and poor prognosis is an increased risk of relapse, decreased disease-free survival, and overall survival, so it is important to identify *FLT3* mutations to determin therapy road map in the future **(Zhong *et al.*, 2012)**.

Besides mutations described above, the *NPM1* gene was a partner in the chromosomal translocation of leukemias and lymphomas that result in the formation of fusion protein containing only the N-terminal region namely, *NPM1*-Anaplastic lymphoma kinase (*NPM1*-ALK), *NPM1*-Retinoic acid receptor alpha (*NPM1*-RARα) and *NPM1*- myeloid leukemia factor1 (*NPM1*-MLF1). *NPM1* appeared to contribute to oncogenesis by activating the oncogenic potential of the fused protein partner ***(Falini et al., 2005).*** Recently, nucleophosmin (*NPM1*) exon 12 mutations which resulted in shift of the nucleophosmin protein from the nucleus to the cytoplasm causing cytoplasmic localization were found in patients with AML. Also *NPM1* gene mutations were even more prominent in those with normal karyotype ***(Pastore et al., 2014).***

Aim of the work

The aim of the present study was to assess the frequency and prognostic impact of *DNMT3A*, *FLT3-ITD*, and *NPM1A* gene mutations in *de novo* AML patients and to correlate these mutations with clinical picture, and disease outcome.

Reviewof literature

1. Acute Myeloid Leukemia (AML):

1.1. Definition:

The terms acute myeloid leukemia (AML), refer to a group of marrow-based neoplasms that have clinical similarities but distinct morphologic, immuno-phenotypic, and cytogenetic features **(Churchill, 1904)**.

Acute myeloid leukemia represent a clinically and biologically heterogeneous group of diseases caused by the malignant transformation of a hematopoietic stem cell or myeloid progenitor cell. The proliferative advantage of the leukemic stem cell, coupled with impairments in differentiation and inhibition of apoptosis, is thought to arise from acquired genetic alterations that lead to accumulation of immature or bone marrow blast cells. The blasts eventually suppress normal hematopoiesis and infiltrate other organs and tissues ***(*Yagi *et al.*, 2003 and Lacayo *et al.*, 2004)**.

1.2. Pathophysiology:

The hematopoietic system is responsible for producingbody needs of blood cells over an personal's life time through all body process as differentiation, proliferation and self-renewal. When the genetic and epigenetic alterations are taken place, these mutations lead to deregulation of the vital processes and the

development of acute leukemia. Numerous studies reported the AML arises from epigenetic and genetic mutations which occur in the normal stem cells or in more differentiated myeloid cell progenitor ***(*Misaghian *et al.*, 2009).**

The pathogenesis of AML is uncertain, but cytogenetic abnormalities are present in most patients. Chromosome translocations are a common pathway in leukemogenesis. New diagnostic tools, including polymerase chain reaction (PCR), comparative genomic hybridization, and micro-array analysis, have improved the sensitivity of detection of genetic abnormalities and the ability to sub-classify AML **(Yunis *et al.*, 1981*).***

World Health Organization (WHO) has incorporated cytogenetic findings into AML classification due to the importance of cytogenetics in diagnosis and prognosis of AML **(Brunning *et al.*, 2001).** Genetic syndromes and toxic exposures contribute to the pathogenesis in some patients. The underlying pathophysiology consists of a maturation arrest of bone marrow cells in the earliest stages of development. This developmental arrest results in 2 disease processes. First; marked decrease in normal blood cells production, which results in varying degrees of anaemia, thrombocytopenia, and neutropenia. Second; the rapid proliferation of these cells, along with a reduction in their ability to undergo programmed cell death (apoptosis), results in

their accumulation in the bone marrow, blood, and frequently the spleen and liver ***(*Henderson *et al.*, 2002*)***.

1.3. Epidemiology:

Acute leukemia arerare disease but have a disproportionately large effect on cancer survival statistics among children and younger adults. The reported frequency of leukemia increased in the first half of the twentieth century, began slowing in its rate of acceleration in the 1940 and has stabilized over the last 30 or 50 years. Improved diagnostic technology presumably accounts for this peculiar trend ***(*Scheinberg *et al.*, 2005*).***

1.3.1. General incidence:

Although account of acute leukemia for less than 2% of all cancer, these diseases are the first and second leading causes of death due to cancer in the United States in men and women, under 40 years of age. AML accounts for approximately 15% to 30% of acute leukemia in children and adolescents and 90% in adults. The overall annual incidence is 3.4 per 100, 000 ***(*Ries *et al.*, 2002*)***.

In National Cancer Institute (NCI), Cairo University, there were 6417 cases of leukemia attending between January 2002 and December 2010. These cases account for 7.7% of all 83500

newly diagnosed proven malignant cases. AML accounts for approximately 41.5% of all 840 newly diagnosed acute leukemia and for 4.6% of all incident cancers ***(*NCI Cancer Registry, 2010)**.

1.3.2. Age incidence:

The age-incidence of AML rises exponentially after the age of 45, exceeding 15 cases/105 populations by age 75. AML has been extensively characterized using cytogenetic analysis since the mid-1970. The increased incidence of AML in the elderly is probably related to improved diagnosis, the recognition of AML after MDS and longer life expectancy, resulting in increased environmental exposures ***(*Tallman, 2005*)***. In NCI, Cairo University the median age of AML patients was 22 years, with a range from less than 10 years and up to 80 years ***(*NCI Cancer Registry, 2010*)***.

1.3.3. Sex incidence:

The incidence of AML is higher in males than in females (1.3:1.0) **(Scheinberg *et al.*, 2005*)***. In NCI, Cairo University, the male: female ratio in AML is 1.37:1.0 ***(*NCI Cancer Registry, 2010*)***.

1.3.4. Geographical variations:

For most types of cancers, striking differences in frequency exist between countries. This is less true for hematological malignancies, but some international variation is seen for each of the main diagnostic groups of leukemia. The leukemias seem to occur at the highest rates in certain areas of Canada (Quebec, Ontario, Saskatchewan and Yukon), among whites in the USA, Australia and Denmark. Leukemias are also relatively more common in other Western countries than registered in most parts of the world ***(*Olsen, 2005)**.

1.4. Etiology:

The precise molecular origins of AML are unknown. The patho-physiologic mechanisms are multiple, act in concert, and probably are distinct in different types of AML. Environmental, inherited genetic and occupational factors play a pathogenicrole of AML **(Linet & Devesa, 2002 and Deschler & Lubbert, 2006).** Table 1 and 2 represent different environmental and genetic factors that contribute to AML.

Table 1: Environmental factors contributing to Acute Myeloid Leukemia **(Henderson *et al.*, 2002).**

- **Solvents (benzene)**
- **Smoking**
- **Ionizing radiation**
  - Atomic bomb exposure
  - Nuclear power exposure
  - Medical radiation
- **Non-ionizing radiation**
- **Chemotherapy**
  - Alkylating agents
  - Topoisomerase II inhibitors
- **Other drugs**
  - Chloramphenicol
  - Phenylbutazone

Table 2: Genetic Disorders Implicated in the Pathogenesis of Acute Myeloid Leukemia **(Taylor and Birch, 1996).**

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

1.5. Diagnostic workup:

Essential steps aredeterminated bymorphological assessment, cytochemical analysis, flow cytometric study,

molecular and cytogenetics to recognize better diagnosis and pathobiology of AML **(Wiernik, 2003, and Gorczyca, 2006).**

1.6. Classification:

AML classification has been widely used since it has included French-American-British (FAB) classification, this classification is a lineage-based system that defines the categories primarily on cytochemical andmorphologic frame work as shown in Table 3 ***(*Bennett *et al.*, 1985*)***, and WHO classification.WHO classification was incorporated the FAB classification and adding several distinctive AML cytogenetic categories as shown in Table 4 ***(*Vardiman *et al.*, 2008)**.

Table 3: FAB classification **(Bennett *et al.*, 1985).**

FAB Classification	
M0:	Undifferentiated leukemia
M1:	Myeloblastic leukemia without differentiation
M2:	Myeloblastic leukemia with differentiation
M3:	Promyelocytic leukemia
M4:	Myelomonocytic leukemia With marrow eosinophilia (M4Eo)
	Monoblastic leukemia
M5:	Monoblastic without differentiation(M5a)
	Monoblastic differentiated (M5b)
M6:	Erythroleukemia
M7:	Megakaryoblastic leukemia

Table 4: Acute myeloid leukemia and related myeloid neoplasms (the WHO classification) **(Vardiman *et al.*, 2008).**

I-Acute myeloid leukemia (AML) with recurrent genetic abnormalities
AML with t(8;21)(q22;q22), RUNX1-RUNX1T1
AML with inv(16)(p13.1q22) or t(16;16)(p13.1;q22), CBFB-MYH11
Acute promyelocytic leukemia (APL) with t(15;17)(q22;q12), PML-RARA
AML with t(9;11)(p22;q23); MLLT3-MLL
AML with t(6;9)(p23;q34); DEK-NUP214
AML with inv(3)(q21q26.2) or t(3;3)(q21;q26.2): RPN1-EVI1
AML (megakaryoblastic) with t(1;22)(p13;q13); RBM15-MKL1
Provisional entity: AML with mutated NPM1
Provisional entity: AML with mutated CEBPA
II-Acute myeloid leukemia with myelodysplasia-related changes
III-Therapy-related myeloid neoplasms
IV-Acute myeloid leukemia, not otherwise specified
AML with minimal differentiation
AML without maturation
AML with maturation
Acute myelomonocytic leukemia
Acute monoblastic/monocytic leukemia
Acute erythroid leukemias
Pure erythroid leukemia
Erythroleukemia, erythroid/myeloid
Acute megakaryoblastic leukemia
Acute basophilic leukemia
Acute panmyelosis with myelofibrosis
V-Myeloid sarcoma
VI-Myeloid proliferations related to Down syndrome
Transient abnormal myelopoiesis
Myeloid leukemia associated with Down syndrome
VII-Blastic plasmacytoid dendritic cell neoplasms

1.7. Prognosis:

Clinical features, morphology, surface markers, and cytogenetics are combined to describe clinicopathologic syndromes in AML, and prognosis is usually determined by a combination of specific factors. A single factor can not reliably predict prognosis but must be correlated with all available information, Table 5 summarizes the prognostic factors affecting AML ***(*Buchner and Heinecke, 1996)**.