INTRODUCTION

Acute myeloid leukemia (AML) is a malignant clonal disorder of immature cells in the haemopoietic hierarchical system. These cells have abnormal function characterized by a failure to progress through the expected differentiation program and / or to die by the process of apoptosis. This leads to the accumulation of a clone of cells which dominates bone marrow activity and leads to marrow failure ***(Bullinger et al., 2004)***.

MicroRNAs (miRs) are a class of small, noncoding RNAs. They are epigenetic regulators that regulate gene expression by either degradation of mRNA or inhibition of translation ***(Yeh et al., 2016)***. Their physiological roles start from development, differentiation, cell cycle regulation and end by aging and metabolism. Consequently, they are frequently deregulated in human cancers ***(Cammarata et al., 2010).***

The roles of microRNAs in carcinogenesis are very complex. Numerous microRNAs promote tumorigenesis and cancer progression by enhancing tumor growth, angiogenesis, invasion and immune evasion, while others have tumor suppressive effects ***(Hayes et al., 2014).*** As a result, the expression profile of cancer microRNAs can define tumor type, susceptibility, prognosis and response to treatment ***(Bouchie, 2013*)*.***

Different microRNA expression profiles are seen in various cytogenetic groups of AML. Moreover, AMLs with specific gene mutations also harbor distinct sets of microRNA signatures ***(Marcucci et al., 2011).*** This may play a part in the pathogenesis and prognosis in AML ***(Lavrencic et al., 2008).***

Evidence is emerging that micro RNA-9 is an important regulator of normal development and diseases. Its aberrant expression levels have been reported in many types of cancers, having opposing effects on proliferation through regulation of various mRNA targets. It is over expressed in Hodgkin lymphoma, primary brain tumors as Neuroblastoma and Medulloblastoma. In contrast, it is down regulated in ovarian tumor ***(Yuva-Aydemir et al., 2011).***

Regarding hematological malignancies, miR-9 is an important player in scope of AML prognosis. It is overexpressed in Mixed Lineage Leukemia (MLL) rearranged AML, causing a significant increase in cell viability and a decrease in apoptosis ***(Chen et al., 2013)***. On the other hand, miR-9 repress the oncogenic *LIN28B*/*HMGA2* axis, thus, acting as a tumor suppressor-miRNA which acts in a stringent cell context ***(Emmrich et al., 2014).***

Aim of the Work

This present study aims to:

Measure miR-9 expression levels in AML patients group and to correlate different expression levels of miR-9 to clinical and laboratory data to evaluate its role on treatment strategy in AML patients.

Chapter (1): Acute Myeloid Leukemia

Definition

Acute myeloid leukemia (AML) is a type of myeloid lineage malignancy. It is a heterogeneous disease characterized by abnormal proliferation and differentiation of precursor cells in the bone marrow leading to accumulation of blast cells in bone marrow or peripheral blood resulting in impaired hematopoiesis ***(Shahjahani et al., 2015).***

Epidemiology

Several studies have been conducted to determine the incidence of AML across the world. Meta-analysis and systematic review of these studies helped to reach accurate incidence of AML from 2011 to 2015. Worldwide the incidence is approximately 2-3 cases per 100,000 population per year in children, rising to 15 per 100,000 in older adults ***(Khan et al., 2016).***

Pathophysiology

AML is a biologically diverse and complex cancer, the oncogenic transformation in AML cannot be attributed to single causative agent alone. The overwhelming evidence suggests high level of interaction among genetic events contributing to AML pathogenesis ***(Ley et al., 2013).***

There is a direct relation between AML phenotype and gene expression, the expression of specific oncogene in AML is a result of interplay between genetic, epigenetic and cytogenetic factors. The combination of data on every single molecular parameter into phenotypic signatures is crucial in disease diagnosis and risk stratification in AML ***(Eppert et al., 2011).***

I. Chromosomal aberrations:

For more than three decades, the molecular pathogenesis of AML has been studied using cytogenetic analysis. Recurrent chromosomal structural variations are well established as diagnostic and prognostic markers, suggesting that somatic mutations have an essential role in pathogenesis ***(Rowley, 2008).***

AML has broad and variable spectrum of chromosomal abnormalities as demonstrated in **Figure 1**, in comparison to chronic myeloid leukemia and other myeloid neoplasms**.** According to WHO classification of myeloid neoplasms 2016; diagnosis of AML, regardless of blast count from peripheral blood or bone marrow, can be confirmed if associated with the following chromosomal abnormalities t(8:21), t(15:17) and inv16 ***(John et al., 2016).*** There are also Hundreds of other uncommon chromosomal aberrations present ***(Harrison et al., 2010).***

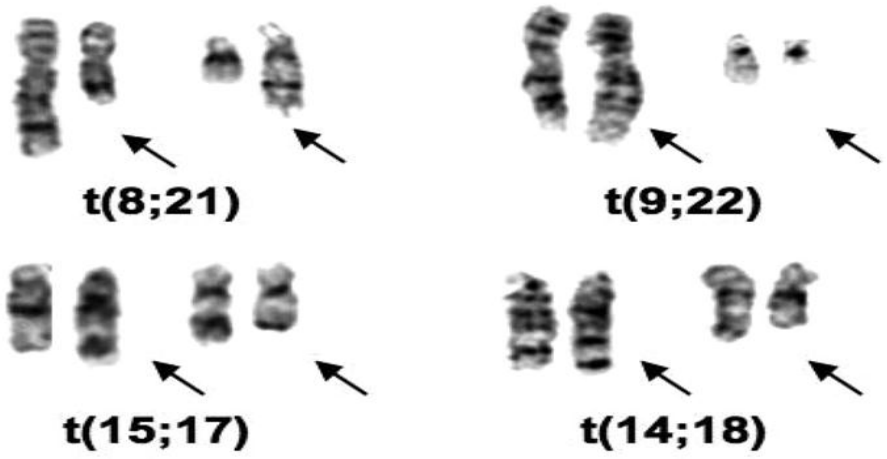

Figure (1): Common chromosomal abnormalities in AML ***(Harrison et al., 2010).***

On the other hand, nearly 50% of AML cases have a normal karyotype, and many of these genomes lack structural abnormalities, even when assessed with high-density comparative genomic hybridization or single-nucleotide polymorphism (SNP) arrays ***(Bullinger et al., 2010).***

II. Genetic mutations:

The impact of genetic mutations on pathogenesis of AML has been attributed to their ability to affect DNA methylation. Targeted sequencing has identified recurrent mutations in Fms related tyrosine kinase 3(FLT3) and Neucleophosphomin (NPM1) comprising about 50% of all genetic mutations in AML **(Figure 2)** ***(Islam et al., 2017).*** In addition to multiple other mutations like CCAAT enhancer binding protein (CEBPA), Ten-Eleven-Translocation 2 (TET2) and DNA Cytosine-5-Methyltransferase 3 Alpha (DNMT3A) **(Figure 2)** ***(Ley et al., 2010).***

a- NPM1:

Nucleophosphomin (NPM1) mutation has been reported in 15% of AML cases. It is widely expressed nucleocytoplasmic shuttling protein that plays an active role in ribosomal protein assembly, chromatin remodeling and DNA repair, replication and transcription **(Figure 2)** ***(Lindstrom, 2011).***

The favorable prognosis conferred by NPM1 mutations was most recently confirmed in a meta-analysis, which demonstrated NPM1 mutations to be associated with higher complete remission (CR) rates and prolonged disease-free and overall survival (OS) ***(Liu et al., 2014).***

b- FLT3:

Receptor tyrosine kinases (RTKs) are integral part of intracellular signaling. Their distribution has been reported to cause tumor development and transformation. FMS-like tyrosine kinase 3 (FLT3) is a type III RTK expressed in approximately 90% of acute myeloid leukemia (AML) and plays a critical role in normal hematopoiesis **(Figure 2)** ***(Wang et al., 2016).***

(FLT3-ITD) Internal tandem duplication of juxta membrane domain is the most prevalent genetic aberration in AML, it is present in 20-30% of cases and associated with a poor prognosis. FLT3-ITD expressing cells cause DNA damage because they express elevated levels of NADPH

oxidase 4 (NOX4)-generated pro-survival hydrogen peroxide (H_2O_2) contributing to increased levels of DNA oxidation and double strand breaks ***(Moloney et al., 2017)***.

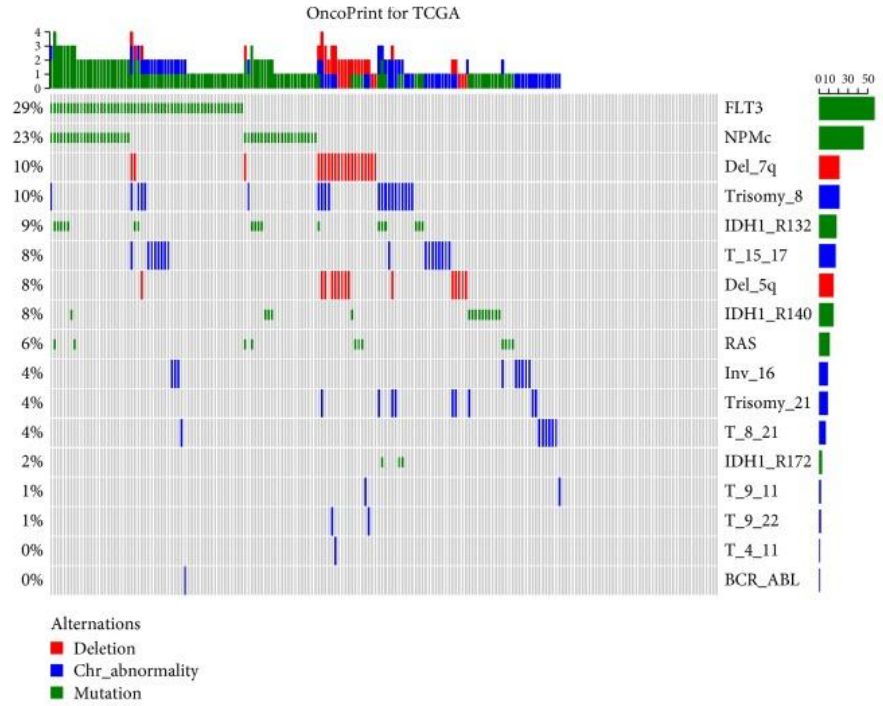

Figure (2): Distribution of genetic and cytogenetic abnormalities in AML ***(Islam et al., 2017).***

Recurrent mutations, such as FLT3, NPM1, CEBPA, and DNMT3A, are not sufficient to explain diverse clinical AML subtypes; epigenetic alteration are abundant and common and might explain the biology behind various AML groups. The mutation of NPM1 was associated with four slightly distinct epigenetic signatures that include clusters 12, 13, 14 and 16. All these clusters were detected by Quantitative DNA Methylation Sequencing which showed variation in methylation patterns from

hypomethylation to hypermethylation and cannot be explained by concurrent FLT3-ITD mutation ***(Figueroa et al., 2010).***

III. Epigenetic alteration:

Epigenetics is the study of different factors that modulate genetic function resulting in stimulation or inhibition of gene expression. Recent cumulative studies have reported that epigenetic modifications are involved in the regulation of hematopoietic development ***(Bock et al., 2012).***

Recently, the advent of massive parallel sequencing has revealed that >70% of AML cases have mutations in DNA methylation-related genes or mutations in histone modifiers, alterations have been described in several AMLs, and in some cases their origin has been studied in details (such as in acute promyelocytic leukemia, caused by the promyelocytic leukemia–retinoic acid receptor-a fusion protein) indicating that epigenetic modification are key players in the development of most, if not all, AMLs, ***(Mehdipour et al., 2015).***

DNA methyltransferases and histone methyl-transferasesare reported to be repeatedly mutated in AML. They regulate gene expression in myeloid progenitor lineages thus they are epigenetic modifiers that contribute to cellular identity ***(Lokody, 2014).***

Classification

Classification of AML is critical for physicians to choose specific treatment protocol to avoid minimal residual disease and risk of relapse ***(Christopeit et al., 2014).*** AML consists of different subtypes, with diagnosis, prognosis and treatment varying among them .The classification of AML considering all possible aberrations is not only a complex and challenging problem but also a matter of clinical validation that was never done before ***(Dohner et al., 2010).***

There are two main systems for classification of AML

A. French–American–British (FAB) classification

B. World Health Organization (WHO) classification

A. FAB classification:

According to the French–American–British (FAB) classification, which was developed in the 1970s, the AML subtypes are grouped in nine categories from M0 to M8 based on the maturation of AML cells and their morphology as represented in **Table 1*(Bennett et al., 2011).*** In particular, M0 to M5 and M8 comprise leukemias deriving from precursors of white blood cells, while M6 is derived from erythroid lineage and M7 derived from megakaryoblastic lineage ***(Gibson et al., 2011).***

Table (1): FAB Classification of AML

M0	Minimally differentiated acute myeloblastic leukemia
M1	Acute myeloblastic leukemia, without maturation
M2	Acute myeloblastic leukemia, with granulocytic maturation
M3	Promyelocytic or acute promyelocytic leukemia (APL)
M4	Acute myelomonocytic leukemia
M4eo	Myelomonocytic together with bone marrow eosinophilia
M5	Acute monoblastic leukemia (M5a) or Acute monocytic leukemia (M5b)
M6	Acute erythroidleukemias, including erythroleukemia (M6a) and very rare pure erythroid leukemia (M6b)
M7	Acute megakaryoblastic leukemia
M8	Acute basophilic leukemia

(Bennett et al., 2011; Gibson et al., 2011)

The FAB classification required a blast count of 30% or more in the BM for the diagnosis of AML ***(Miller and Pihan, 2009).***

The FAB classification provided a consistent morphologic framework in which the significance of the genetic lesions could be appreciated. However, morphologic-genetic correlations are not always perfect, and as the genetic findings may predict the prognosis and biologic properties of the leukemia more consistently than does morphology. All these highlight the role of incorporating genetic, cytogenetic and epigenetic factors in the WHO classification ***(Vardiman et al., 2002)***. Although the WHO classification may be more useful, the FAB system is still widely used ***(Bennett et al., 2011).***

B. WHO Classification:

In 2001, a new classification for hematopoietic and lymphoid neoplasms including acute leukemias was published. The World Health Organization (WHO) in conjunction with the Society for Hematopathology and the European Association of Hematopathology, integrated genetic information with morphologic, cytochemical, immuno-phenotypic and clinical information into diagnostic algorithms for the myeloid neoplasms ***(Jaffe et al., 2001).*** Since 2001, the WHO classification of myeloid neoplasms has been oriented towards categorization of disease entities according to underlying genetic alterations as they are usually associated with distinctive clinic-pathological features and may serve as specific diagnostic and prognostic markers ***(Falini et al., 2010).*** The 2008 WHO classification added new distinct and provisional entities as demonstrated in **Table 2.** The most significant difference from the FAB classification is that the WHO requisite blast percentage at least 20% blasts in the blood or bone marrow ***(Maassen et al., 2013).***

The WHO focused on significant cytogenetic and molecular genetic subgroups including a large number of recurring, balanced cytogenetic abnormalities that are recognized in AML ***(Grimwade et al., 2010).***

Since 2008, multiple studies have been published confirming the clinical relevance of the new classification. Although data are still preliminary, it showed the impact of integration of genetic information on the response to treatment. For example, studies showed that mixed phenotype acute leukemia (MPAL) with the t(9;22) can respond favorably to treatment that includes a Tyrosine Kinase Inhibitor (TKI) ***(Kawajiri et al., 2014).***

Table (2): The 2016 Revision to WHO Classification of Acute Myeloid Leukemia (AML) and related neoplasms

AML with recurrent genetic abnormalities:

- AML with t(8;21)(q22;q22); *RUNX1-RUNX1T1**(2008)***
- AML with inv(16)(p13.1q22) or t(16;16)(p13.1;q22); *CBFB-MYH11* ***(2008)***
- AML with t(15;17) (q22;q12); *PML-RARA**(2008)***
- AML with t(9;11) (p22;q23); *MLLT3-MLL**(2008)***
- AML with t(6;9) (p23;q34); *DEK-NUP214**(modified 2008)***
- AML with inv (3) (q21q26.2) or t(3;3) (q21;q26.2); *RPN1-EVI1* ***(modified, 2008)***
- AML with t(1;22)(p13;q13); *RBM15-MKL1**(***modified 2008)**
- Provisional entity: AML with mutated RUNX1**(2016)**
- Provisional entity: AML with BCR-ABL1**(2016)**
- Provisional entity: AML with mutated NPM1**(modified 2008)**
- Provisional entity: AML with mutated CEBPA **(modified 2008)** Biallelic mutation **(2016)**

AML with myelodysplasia-related changes: (2008)

- Well-documented history of myelodysplasia or myelodysplastic/ myeloproliferative neoplasm
- MD-related cytogenetic abnormality
- Multiline age dysplasia in bone marrow and peripheral blood smears (defined as dysplasia in >50% of cells in two or more lineages)

Therapy-related myeloid neoplasms:(2008)

This category includes therapy related acute myeloid leukemia (t-AML), myelodysplastic syndrome (t-MDS) and myelodysplastic syndrome/ myeloproliferative neoplasms (t-MDS/ MPN) occurring as late complications of cytotoxic chemotherapy and/or radiation therapy administrated for a prior neoplastic or non-neoplastic disorder. Excluded from this category is transformation of MPN since it is often not possible to determine if this is disease evolution or therapy related.

AML, not otherwise specified (NOS):(2008)

- AML with minimal differentiation (M0)
- AML without maturation (M1)
- AML with maturation (M2)
- Acute myelomonocytic leukemia (M4)
- Acute monoblastic/monocytic leukemia (M5a/ M5b)
- Acute erythroid leukemia (M6)
  - Pure erythroid leukemia (M6b)
  - Erythroleukemia, erythroid/myeloid (M6a)
- Acute megakaryoblastic leukemia (M7)
- Acute basophilic leukemia (M8)
- Acute panmyelosis with myelofibrosis

Myeloid sarcoma (2008)

Myeloid proliferations related to Down syndrome (modified 2008)

- Transient abnormal myelopoiesis
- Myeloid leukemia associated with Down syndrome

Blasticplasmacytoid dendritic cell neoplasm(2008)

Acute leukemias of ambiguous lineage(2008)

Acute undifferentiated leukemia
Mixed phenotype acute leukemia with t(9;22)(q34.1;q11.2); *BCR-ABL1*
Mixed phenotype acute leukemia with t(v;11q23.3); *MLL* rearranged
Mixed phenotype acute leukemia, B/myeloid, NOS
Mixed phenotype acute leukemia, T/myeloid, NOS

(Arber et al., 2016)

To summarize updates on 2016 modification of 2008 WHO classification we need to mention few points 1) biallelic mutation of *CEBPA* was added to meet criteria for recurrent genetic abnormality 2) *De novo* AML with *BCR ABL1* rearrangement is recognized as a new provisional entity with a targetable genetic change 3) cases with *RUNX1* (but without myelodysplastic-type cytogenetics) will be a provisional entity with worse prognosis. 4) It is clarified that myelodysplastic morphologic changes alone do not exclude a diagnosis of AML with mutated *NPM1* or *CEBPA*. Cases with germ line mutations that predispose to the development of myeloid malignancies will be separately recognized. Finally, blastic-plasmacytoid dendritic cell neoplasm is now an entity separate and distinct from AML ***(George and Czuchlewski, 2016).***

Diagnosis of AML

A- Clinical Picture:

AML can present with various symptoms and signs but the majority are related to failure of normal hematopoiesis and organ infiltration resulting in anaemia, neutropenia, thrombocytopenia and organomegaly ***(Greer et al., 2014).***

1. Symptoms due to failure of normal hematopoiesis:

The most common complaint is nonspecific fatigue or malaise, pallor and weakness that are caused by anemia. Fever is common and is the presenting feature in 15- 20% of patients. Hemorrhagic signs and symptoms, including

petechiae, epistaxis and easy bruising may be found in up to one half of patients at diagnosis. Weight loss is present in up to 50% of patients, but is usually not severe. Bone pain occurs in less than 20% of patient ***(Lichtman et al., 2010).***

2. Symptoms due to organ infiltration:

Extra-medullary involvement is most common in monocytic or myelomonocytic leukemia. Organomegaly and adenopathy have been reported in up to one half of patients with AML. Skin involvement may be of three types: non-specific lesions, leukemia cutis and granulocytic (myeloid) sarcoma of skin and subcutaneous tissue. Non-specific lesions, such as macules, papules, vesicles or pyoderma gangrenosum are generally painful and respond to steroids ***(Pastore et al., 2014).***

B- Laboratory diagnosis:

The diagnosis of AML requires examination of peripheral blood and bone marrow samples. Workup should comprise morphological examination, cytochemistry, immuno-phenotyping, cytogenetics and molecular genetics ***(Fey and Dreyling, 2008).***

1. Complete blood picture:

Blasts are usually present in the peripheral smear or in a buffy coat smear. Auer rods and Phi bodies are considered pathognomonic of M3AML **(Figure 3)**. *Phibodies* are fusiform or spindle-shaped rods similar to