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Evaluation the Role of MicroRNAs and some Immunological Biomarkers in Acute Myeloid Leukemia

A thesis

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By

Hind Saleh Mhaimeed Al-Hamadani

B.Sc.Biology/Microbiology/2011

M.Sc.Biology/ Zoology /2014

Supervised by

Prof.
Dr. Alaa Jawad Hassan

Assist. Prof.
Dr.Hussein Naji Abdulla

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﴿ بِسْمِ اللّهِ الرَّحْمَنِ الرَّحِيمِ ﴾

((وَيَسأَلونَكَ عَنِ الرّوحِ قُلِ الرّوحُ مِن أَمرِ رَبّي
وَما أوتيتُم مِنَ العِلمِ إِلّا قَليلًا))

﴿صَدقَ اللهُ العظيمُ﴾

سُورةُ الاسراء
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Hind

Dedication

To my mother .

To all patients with AML in the world .

Hind

Summary

Acute myeloid leukemia (AML) is the most common leukemia among the adult population and accounts for about 80% of all cases , and its characterized by clonal expansion of immature “blast cells” in the bone marrow and peripheral blood .

In this study, the following parameters were determined: complete blood (CBC) , CD34 levels by ELISA technique; RNA yield and quality; and quantification of miRNA-203, miRNA-143, and miRNA-495 expression by Quantitative Real-Time PCR (qPCR) in the blood of acute myeloid leukemia (AML) patients and control groups.

The study was conducted in period between December 2020 and September 2021 at the University of Babylon , College of Science, department of biology . In this case-control study, blood samples were collected from 115 AML patients (38 male and 77 female) and their ages ranged between 18 and 66 years , then, 60 patients were selected based on the quantity and natural color of their samples (28 male and 32 female), in addition to the 30 samples from healthy apparently subjects as a control group (11 male and 19 female), and this group matched with the patients groups.

The characteristics of acute myeloid leukemia (AML) patients revealed that the mean age was (37.52) years, ranged(18-66) years, and there were insignificant differences between the means of age in patients and control groups . The percentage of female patients was higher (58.3%) than the percentage of male patients (41.7%). The majority of patients presented with subtype M3 (N = 23, 38.3%) of total patients,

while the M4 subtype represented 20.0% (N = 12) of total patients, but M2 and M5 represented 15.0% (N =9). In addition, M1 and M7 subtypes represented 6.7% (N = 4) and 5% (N =3) respectively of total patients, and there were no patients with subtypes M0 and M6 (0.0%).

The results of present study showed a significant differences ($P<0.05$) between apparently healthy control and AML groups in blood parameters. The mean of WBC in control group were 7.60 $\times 10^3$(cell/mm^3) , whereas its count in the patients group were significantly decreased to 5.67×10^3(cell/ mm^3) .The mean of Blast (%)in the patients group were 62.63 whereas this percentage in the control group were significantly decreased to 0.00 . The mean percentage of neutrophils (%)in the patients group were 17.35 , whereas this percentage in the control group were significantly increased to 70.14. The mean of Lymphocytes (%) in the patients group were 20.30 , whereas this percentage in the control group were significantly increased to 30.19 . The mean count of RBC in the patients group were 2.92 $\times 10^6$ (cell/ mm^3) , whereas in the control group it was significantly increased to 4.76 $\times 10^6$ (cell/ mm^3) .Furthermore , the mean of Hemoglobin (Hb) in patients group were 8.12 (g/dl) , whereas it was significantly increased to 13.09 (g/dl) in the control group . The results revealed that the mean of Hematocrit (HCT) in the patients group were 24.51 , but in the control group it was significantly increased to 41.01 (%) , in addition , the mean of Mean corpuscular hemoglobin concentration (MCHC) in the patients group were 32.81 (g/dl) , whereas it was significantly decreased to 32.06 (g/dl) in the control group. Meantime ,the mean count of Platelets (PLT) in the patients group were

86.48 $\times 10^3$(cell/ mm^3) , while its count in the control group were significantly increased to 264.67 $\times 10^3$(cell/ mm^3).

On the other hand , there were insignificant differences ($P<0.05$) between c and control ML patients groups in other blood parameters. The mean of Mean corpuscular volume (MCV) in the patients group were 84.44 (FL) , whereas its insignificantly increased to 87.17 (FL) in the control group , in addition the mean of corpuscular hemoglobin (MCH) in the patients group was 28.09 (pg) , but it was insignificantly decreased to 27.85 (pg) in control group .

The biomarkers of current study including ; CD34 (pg/µl), miRNA-203 (ng/µl), miRNA-143 (ng/µl) and miRNA-495 (ng/µl) in patients and control groups shows a significant differences in relation with AML patients at p-value (< 0.001). The levels of CD34 in the patients groups were 0.55 (pg/µl) , whereas it's significantly increased to 0.81 (pg/µl) in the control group . The biomarker level of miRNA-203 was 0.17 (ng/µl) in the patients group , whereas its level in the control group were significantly increased to 12.02 (ng/µl) .Furthermore the levels of miRNA-143 (ng/µl) in AML patients group were 0.15 (ng/µl) , while its level in the control group were significantly increased to 2.49 (ng/µl) . As well as , the level of miRNA-495 in the patients group were 0.04 (ng/µl) , but it was significantly increased to 0.87 (ng/µl) in the control group .

The results of current study revealed that there were insignificant differences between subtypes of AML according to age ($p=0.214$) , but there was a significant differences between subtypes of AML according

to Blast (%),Neutrophils (%) and Lymphocytes (%) . Furthermore, , there are insignificant differences ($P<0.05$) between subtypes of AML according to the complete blood picture ,WBC (cell/µl),RBCs (cell/µl), Hb (g/dl), HCT (%), MCV (FL), MCH (pg), MCHC (g/dl) and PLT (cell/µl)). The present study showed a relationship between AML subtypes and the biomarkers CD34 and miRNAs , in which there are insignificant differences between subtypes of AML according to the CD34 level ($p=0.525$), whereas there was a significant differences between subtypes of AML according to the levels miRNAs ,miRNA-203 , miRNA-143 , and miRNA-495 (ng/µl) at a value $p= 0.001$, 0.023, and <0.001, respectively.

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List of Abbreviations

Abbreviation	Meaning
ALL	Acute lymphoblastic leukemia
AML	Acute myeloid leukemia
APL	Acute promyelocytic leukemia
BM	bone marrow
CD34	Cluster of differentiation
cDNA	Complementary Deoxyribonucleic acid
CLL	Chronic lymphocytic leukemia
CML	Chronic myelogenous leukemia
CT	Cycle threshold
ELISA	Enzyme Linked Immunosorbent Assay
EDTA	Ethylene diamine tetra acetic acid
FAB	French-American-British
Hb	Haemoglobin
HCT	Hematocrit
KIR	killer immunoglobulin-like receptor
LAA	leukemia-associated antigen
LSCs	Leukemia stem cells
MCH	Mean Corpuscular Hemoglobin
MCHC	Mean Copuscular Hb Concentration
MCV	Mean Corpuscular Volume
miRNAs	MicroRNAs
NCRs	Natural cytotoxicity receptors
NKs	Natural killer cells NKs
PB	Peripheral blood
PLT	Platelet count
RBC	Red Blood Cell Count
RNA	Ribo nucleic Acid
RT – PCR	Quantitative reverse transcription polymerase chain reaction
TAA	Tumor-associated antigen
T-regs	T-regulatory cells
WBCs	Total White Blood Cell Count
WHO	World Health Organization
µL	Microliter

Introduction

Acute myeloid leukemia (AML) is the most common leukemia among the adult population and accounts for about 80% of all cases , and it is characterized by clonal expansion of immature "blast cells" in the bone marrow and peripheral blood resulting in ineffective erythropoiesis and bone marrow failure (Bain and Bene ,2019). Depending upon the etiology, genetics , immune-phenotype , and morphology, there are many different classification systems for AML , the most common risk factor for AML is myelodysplastic syndrome. Other hematological disorders that increase the risk of AML include myelofibrosis and aplastic anemia .In addition, several congenital disorders like Down syndrome and Bloom syndrome are increasing the risk of AML, which tends to present in the early 20s. Also, the environmental exposures like radiation, tobacco smoke and benzene are considered among the risk factors for AML . Finally, previous exposure to chemotherapeutic agents is also a risk factor for AML subtypes (Hartmann and Metzeler ,2019 ; Boddu and Zeidan ,2019).

Previously, AML was classified according to the French-American-British classification system using morphology and immune-phenotype/cytochemical criteria to define eight major AML subtypes (FAB M0 to M7) (Bonnet and Dick ,1997). AML is a heterogeneous disease characterized by the increased proliferation and survival of immature myeloid cells and is the result of a number of genetic abnormalities, including chromosomal rearrangements and mutations (Ferrara and Schiffer,2013) . Early studies characterizing the role of miRNAs in AML focused on identifying AML-specific

miRNA expression patterns , therefore distinctive miRNA profiles were identified for many cytogenetic subtypes of AML (Dixon-McIver *et. al*., (2008) and Li *et. al.* ,2008) .

MicroRNAs (miRNAs) are short non-coding single-stranded RNAs (~19–22 nucleotides) (Vitsios *et. al*.,2017; Wallace and O'Connell,2017) , that will negatively regulate mRNA stability (Svoronos *et. al*.,2016; Wallace and O'Connell,2017; Fernandez *et. al*.,2017) , and this biomarker play an important role in many biological functions, such as cell growth, differentiation, proliferation, and apoptosis (Pichiorri *et. al*.,2011; Vitsios *et. al*.,2017). Moreover, miRNAs can act as tumor suppressors or oncogenes , contributing to malignant transformation in solid and hematological tumors, including AML(Wong *et. al*.,2010; Senyuk *et. al*.,2013; Svoronos *et. al*.,2016) . Since , there are many studies showed that microRNAs act as diagnostic and prognostic biomarkers in AML (Maki *et. al.*,2012; Lin *et. al*.,2015; Caivano *et. al*.,2017).

CD34 is a transmembrane phosphoglycoprotein, first identified in 1984 on hematopoietic stem and progenitor cells . Although the structure of CD34 is well-investigated, there is still relatively little known about its function , but certain studies on hematopoietic cells suggest its role in cytoadhesion , regulation of cell differentiation and proliferation (Nielsen and McNagny ,2008) . The stem cell marker CD34 is expressed by leukemia blasts only for a subset of patients with acute myelogenous leukemia (AML). It is still controversial as to whether CD34 expression defined as at least 10–20% positive cells has any prognostic effect in patients with AML who receive intensive chemotherapy(Anne *et. al*.,2005).

Aim of study:

the aim of the current study was to investigate the role of microRNAs and some immunological biomarkers in patients with acute myeloid leukemia as a new markers for establishing AML diagnosis and prognosis . In addition, to investigating the differences between gender and age categories in patients compared to healthy subjects according to the parameters of the current study, and this would be achieved by the following objectives:

1. Estimation the complete blood count (CBC) and blood film examination.
2. Measurement of the gene expression of miR- 143, miR- 203 and miR- 495 by RT-PCR.
3. Measurement of the serum levels of CD34.

2.Literature review

2.1: Leukemia

Leukemia is an amalgam of cancers and arises due to the malignancy of the any elements of bone marrow and blood . In other terms, they are abnormal white blood cells, which are not fully developed and are called blasts or leukemia cells. The growth of the Leukemia cells are rapid than normal cells, but with time, they replace the population of the normal WBCs and RBCs and may spread to the lymph nodes and other organs(Chapalamadugu *et. al*.,2015).

The geographic distribution of leukemia is universal, with higher prevalence and overall mortality in the more developed countries, however, the mortality rate is higher in developing countries (Bray *et al*.,2018). While for the majority of leukemia cases there are no obvious known predisposing factors, some genetic and acquired germ line mutations and clonal chromosomal abnormalities are associated with increased incidence of leukemia (Stieglitz and Loh,2013).

2.2: Classification of leukemia

In general , leukemia can be classified into :-

1.Acute vs. chronic: Acute leukemia's are characterized by abnormal cells that are less mature, develop quickly, and leave the bone marrow as dysfunctional cells called “blasts.” These blasts crowd out healthy cells in the bone marrow, causing the rapid onset of symptoms. Blasts normally make up 1% to 5% of marrow cells, and having more than 20% blasts in the bone marrow is required for a diagnosis of acute leukemia. In contrast, chronic leukemia's develop slowly and may take years to develop symptoms. They are composed primarily of more mature and

functional cells, and there are generally not elevated numbers of blasts (Brunning ,2003 ; Vardiman,2010 ; Arber *et. al*.,2016) **.**

2.Myeloid vs. lymphoid: Hematopoietic stem cells give rise to two types of blood cells: myeloid and lymphoid. Myeloid cells include monocytes, macrophages, neutrophils, basophils, eosinophil's, erythrocytes, and megakaryocytes. Lymphoid cells include T cells, B cells, and natural killer cells (Vardiman,2010; Arber *et. al*.,2016). As such, the four major subtypes of leukemia are:

A. Acute lymphoblastic leukemia (ALL): ALL occurs when primitive white blood cells of lymphoid origin reproduce without developing into normal B and T cells. It is the most common leukemia in pediatrics, accounting for up to 80% of cases in this group vs. 20% of cases in adults.

B. Acute myelogenous leukemia (AML): AML is also characterized by the hyperplasia of blasts, but in this case, of myeloid origin. It accounts for half of the leukemia cases diagnosed in teenagers and people in their 20s. It is the most common acute leukemia in adults.

C. Chronic lymphocytic leukemia (CLL): CLL occurs when mature but abnormal white blood cells of lymphoid origin undergo hyperplasia, leading to a monoclonal population of dysfunctional lymphocytes. Most cases occur in people between ages 60 and 70.

D. Chronic myelogenous leukemia (CML): A monoclonal population of self-renewing, dysfunctional myeloid cells (e.g., neutrophils, basophils, eosinophil's, macrophages) characterizes CML. Most cases occur in people between ages 25 and 60.(Yamamoto and Goodman,2008; Vardiman,2010; Arber *et. al*.,2016; Siegel *et. al*.,2017)

There are several risk factors associated with a higher risk of developing leukemia including :

1. Exposure to ionizing radiation is associated with an increased risk of multiple subtypes of leukemia(Miranda-Filho *et. al*.,2018; Bispo *et. al*.,2020).

2. Exposure to benzene is a risk factor for leukemia in adults, particularly AML (Snyder,2012)

3. Previous exposure to chemotherapy, especially alkylating agents and topoisomerase inhibitors, increases the risk for acute leukemia later in life (Miranda-Filho *et. al*.,2018; Bispo *et. al*.,2020).

4. A history of any hematologic malignancy is a risk factor for subsequently developing another subtypes of acute lymphatic leukemia (ALL)(Friedman *et. al*.,2010).

5. Viral infections (e.g., human T-cell leukemia virus, Epstein Barr virus) are linked with subtypes leukemia(Davis *et. al*.,2014)

6. Several genetic syndromes such as Down syndrome, Fanconi anemia, Bloom syndrome and Li-Fraumeni syndrome are associated with an increased risk of AML and ALL.(Stieglitz and Loh ,2013).

2.3: Acute myeloid leukemia (AML):

AML is a disease of the bone marrow, a disorder of hematopoietic stem cells due to genetic alterations in blood cell precursors resulting in overproduction of neoplastic clonal myeloid stem cells. While , extra medullary manifestations can occur (e.g. myeloid sarcomas and leukemia cutis), the underlying disease is due to abnormalities in hematologic cellular production. A small subset of cases have identified causative factors such as prior chemotherapy or certain chemical exposures, but the large majority are due to genetic alterations, through chromosomal abnormalities or isolated gene mutations, without clear causative agents(Grimwade and Ivey ,2016).

AML accounts for about 80% of acute leukemia in adults and 20% of acute leukemia in pediatrics(Appelbaum *et. al*.,2006).Since , the prognosis for AML patients varies greatly, ranging from very short survival even for a few days to complete cure clinical outcome can be in part predicted by age, performance status, and cytogenetic findings(Juliusson *et. al*.,2009). However, the prognosis of an individual AML patient can't yet be estimated precisely, therefore , it is important to find out new biomarkers for the prediction of prognosis, treatment response, detection of relapse, and monitoring for minimal residual disease(Estey *et. al*.,2001).AML is a highly heterogeneous disease at molecular and clinical levels; well-identified genetic and cytogenetic aberrations hold a pathogenetic and prognostic relevance in this neoplasm (De Kouchkovsky and Abdul-Hay ,2016), and this disease is characterized by a number of recurrent cytogenetic abnormalities and mutations that influence disease phenotype, response to conventional therapies, risk of relapse, and survival (Dohner *et. al*.,2017). For example, t(8;21) and inv(16)/t(16;16),

which lead to the balanced translocations RUNX1–RUNXT1 and CBFB–MYH11, respectively, constitute a cytogenetically favorable risk group that is highly curable with cytotoxic combination chemotherapy, whereas the presence of a complex karyotype (defined as ≥3 cytogenetic abnormalities) or specific chromosomal aneuploidies (e.g., -5/-5q. -7, and -17/ -17p) is associated with a relatively chemo resistant phenotype and poor prognosis (Grimwade *et. al*.,2010) .

The number of mutations in the AML genome is significantly lower than most solid-tumor malignancies, with an average of only 5 recurrent mutations per genome. However, at least one driver mutation is identified in 96% of patients with de novo AML, with 86% harboring ≥2 driver mutations (Papaemmanuil *et. al*.,2016; Cancer Genome Atlas Research Network *et. al*.,2016).

The majority of AML patients have a down regulated NK cell surface expression of the activating natural cytotoxicity receptors (NCRs; that is, NKp30, NKp44 and NKp46)(Costello *et. al*.,2002 , Szczepanski *et. al*.,2010), also the expression of DNAX accessory molecule-1 (DNAM-1) was shown to be reduced on NK cells from AML patients younger than 65 years compared with age-matched controls(Sanchez-Correa *et. al*.,2011) .Together with the activating NK cell receptor NKG2D, these are the main receptors involved in NK cell-mediated recognition and killing of leukemic target cells(Pende *et. al*.,2005).

2.4:Classification of AML

Acute myeloid leukemia (AML) comprises a heterogeneous group of neoplastic disorders in which ≥20% of cells in the bone marrow or blood are myeloblasts . it has a number of subtypes and precursor neoplasms that are distinguished from each other by morphology,

immunophenotype, cytochemistry, and genetic abnormalities (Daniel *et. al*.,2016) .

There are two major systems that are used to classify AML into subtypes:

A. The World Health Organization (WHO) classification:

The World Health Organization (WHO) classification reviews chromosome translocations and evidence of dysplasia (Papaemmanuil *et. al*.,2016) , Seven classes are described in the WHO classification, including:

- AML with recurrent genetic abnormalities
- AML with myelodysplasia-related changes (AML-MRC)
- Therapy-related AML (t-AML)
- AML, not otherwise specified (NOS)
- Myeloid sarcoma
- Myeloid proliferations related to Down syndrome
- Blastic plasmacytoid dendritic cell neoplasm.

B. The French-American-British (FAB) classification:

The French-American-British (FAB) classification of AML was developed in the 1970s by a group of French, American, and British leukemia experts.They classified AMLs into subtypes from M0 to M7, and this was based on the type of cell from which the leukemia developed and the level of maturity of the cells. The FAB classification relied on appearance of leukemia cells under the microscope after routine staining

(Angelescu *et. al*., 2012) .According to the FAB classification the subtypes M0 to M5 start in precursors of white blood cells. M6 AML originates in very early forms of red blood cells and M7 AML starts in early forms of cells that form platelets. The FAB classification also defines symptom differences, for example, uncontrolled bleeding is seen in the M3 subtype of AML, also known as acute promyelocytic leukemia (APL) as in table (2.1) and figure(2.1). The subtypes also predict prognosis and identify the best suitable treatment.

Table (2.1) : FAB classification of AML (Vakiti *et. al.*, 2021)

FAB subtype	**Name**
M0	Undifferentiated acute myeloblastic
M1	Acute myeloblastic leukemia with minimal maturation
M2	Acute myeloblastic leukemia with maturation
M3	Acute promyelocytic leukemia (APL)
M4	Acute myelomonocytic leukemia
M5	Acute monocytic leukemia
M6	Acute erythroid leukemia
M7	Acute megakaryoblastic leukemia

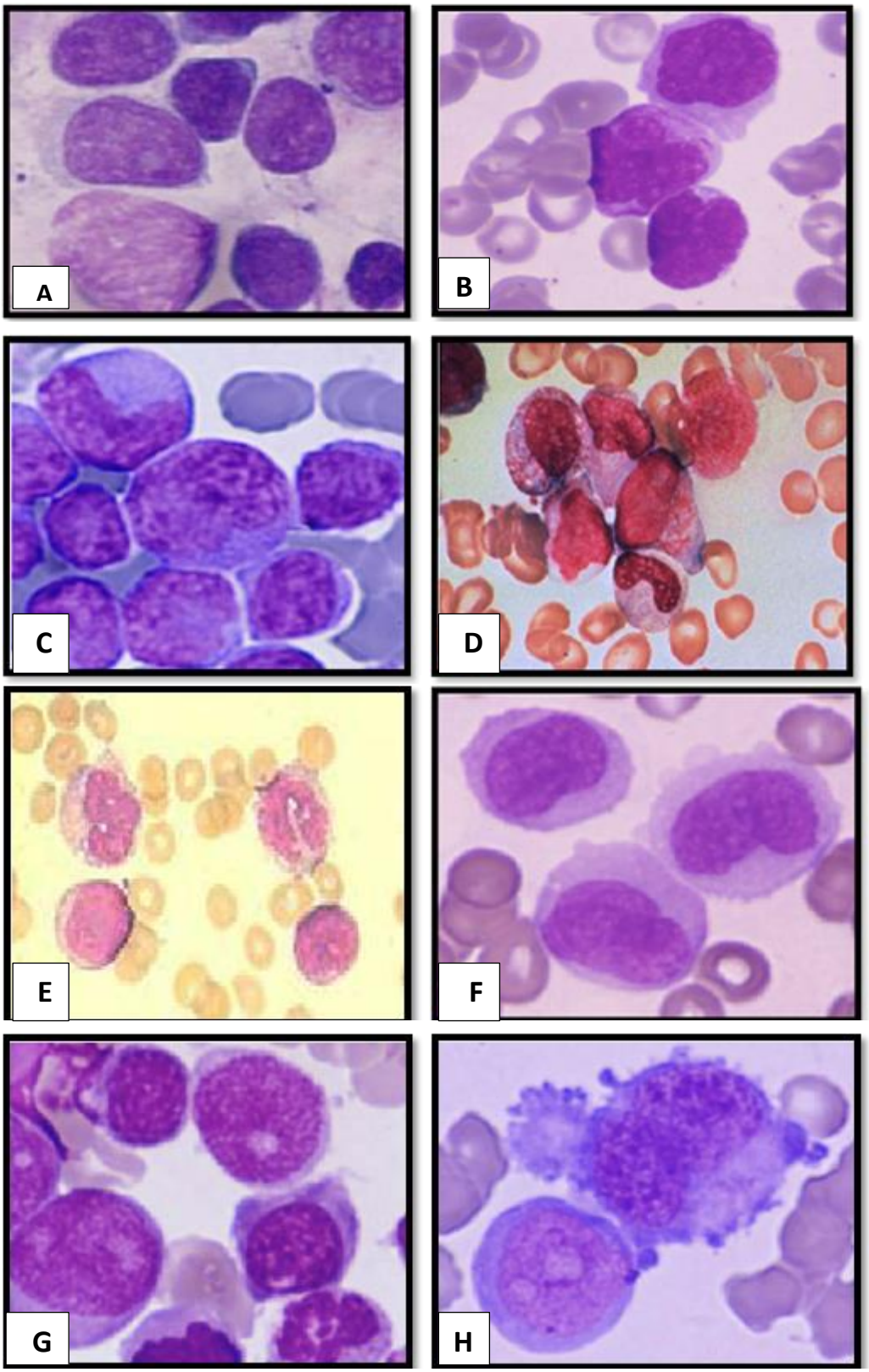


Figure (2.1): Classification of acute myeloid leukemia.(A) Acute myeloblastic leukemia AML –M0. (B) Acute myeloblastic leukemia AML-M1.(C) Acute myeloblastic leukemia AML-M2.(D) Promyelocytic leukemia AML-M3.(E) Acute myelomonocytic leukemia M4.(F) Acute monoblastic leukemia M5 (AMoL) .(G) Erythroleukemia M6 .(H) Acute megakaryoblastic leukemia (AMkL) M7.(Abdul-Hamid ,2011).

2.5: Immunity of AML

In AML, cytotoxic T cells fail to eliminate leukemic blasts and become senescent through the activity of immunosuppressive cells such as regulatory T cells (Treg) (Szczepanski *et. al*.,2009 ; Le Dieu *et. al*.,2009), furthermore, macrophages have been shown to become avidly M2 polarized, and the cytokine profile in peripheral blood (PB) of AML patients contributes in preventing T-cell activation and proliferation(Buggins *et. al*.,2001 and Al-Matary *et. al*.,2016) .

In numerous studies, presence of circulating T cells recognizing AML correlates with continuing disease control, while their disappearance can herald relapse. T cells recognizing known Tumor-associated antigen (TAA) , such as WT1, PRAME proteinase 3 and survive is occur in patients who remain in remission after SCT, validating these antigens as useful targets for both allogeneic and autologous T-cell attack (Rezvani and Barrett, 2008). In addition, there is a strong evidence for a NK cell-mediated immune control of AML. Donor–recipient combinations where the donor NK cell is mismatched with the killer immunoglobulin-like receptor (KIR) and MHC molecules of the recipient, as well as certain 'favorable' donor KIR alleles, permit an allogeneic NK reaction associated with a significantly higher progression-free survival after SCT (Velardi *et. al*., 2009;Stringaris *et. al*., 2010).

The immunotherapeutic strategies to maintain remission after patients receive intensive chemotherapy are logical and being tested in clinical trials (Lichtenegger *et. al*.,2015) , so there is an incomplete description of the state of the adaptive immune system in AML patients who have completed chemotherapy. Previous study on

peripheral blood lymphocytes recovery in AML after induction demonstrated a skewing of the T-cell compartment towards peripherally expanded oligoclonal activated T-regulatory cells (T-regs) in the time immediately following chemotherapy (Kanakry *et. al*.,2011).The immunological microenvironment of AML can be shown in figure (2.2).

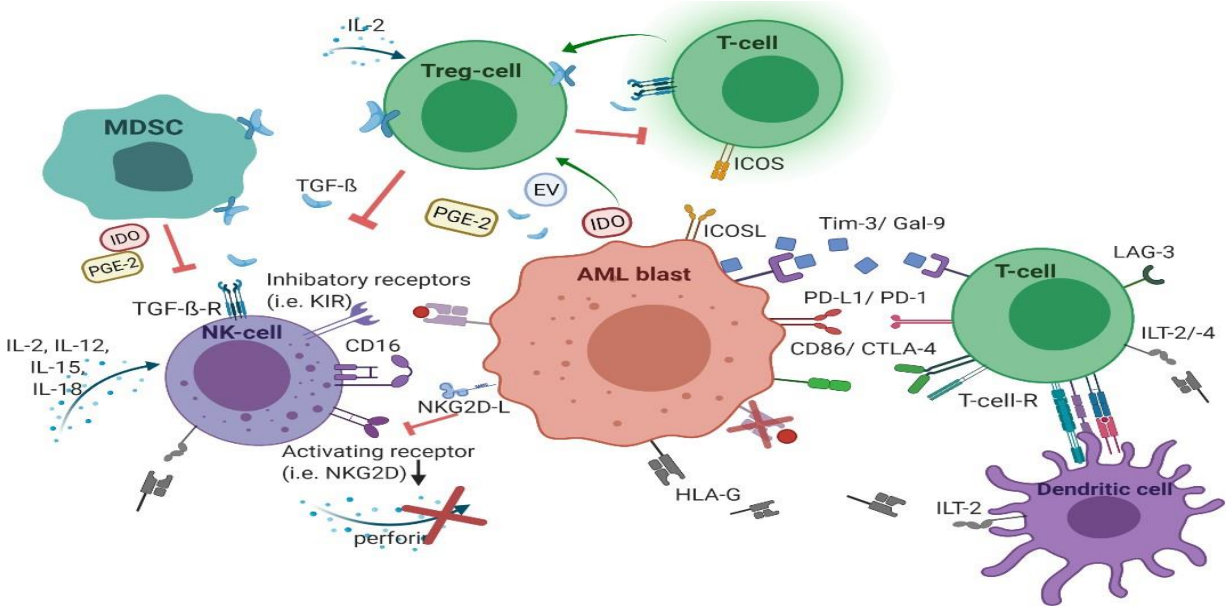


Figure (2.2) :The immunological microenvironment in acute myeloid leukemia (AML). AML blasts reduce antigen-presentation through down regulation of classical human-leukocyte-antigen (HLA)-presentation. Non-classical HLA-G is supposed to suppress immunogenicity. Checkpoint molecules promote immune evasion (Gal-9/Tim-3, PD-L1/PD-1, CD86/CTLA-4, and LAG-3). Secretion of TGF-β and indoleamine 2,3-dioxygenase (IDO), as well as inducible T-cell-co-stimulator ligand (ICOS)/ICOS-ligand interplay induces T-cell conversion into immunosuppressive T-regulatory cells (Treg) cells. Myeloid- derived suppressor cells (MDSC) suppress natural killer (NK)-cell-mediated cytotoxicity, i.e., via IDO, prostaglandin-E2, and TGF-β. (Sendker *et. al*.,2021) .

2.6: CD34 as biomarker for AML

CD34 is a cluster of differentiation that was described for the first time by Civin and colleagues as a cell surface glycoprotein . It works as a cell-to-cell adhesion promoter molecule , and it may play a role to mediate the attachment of bone marrow stem cells to BM extracellular matrix or directly to stromal cells (Nielsen and McNagny ,2009). CD34 is a glycosylated trans membrane protein and it is a well-established marker for human hematopoietic stem and early progenitor cells(Lei *et. al*.,2018) , in addition, the CD34 family proteins are included in enhancing proliferation, blocking differentiation, trafficking, and cell adhesion(Nielsen and McNagny ,2008, AbuSamra *et. al*.,2017).Furthermore, the expression of CD34 might be a marker for predicting outcome and survival in AML leukemia patients. Positive CD34 is a marker for less differentiated AML FAB subtypes, also positive CD34 may be regarded a marker for less complete remission (CR) achievement. CD34 positivity is linked to unfavorable cytogenetic risk group and it might be associated with worse outcome in all cytogenetic risk subgroups (Amer *et. al*.,2020).

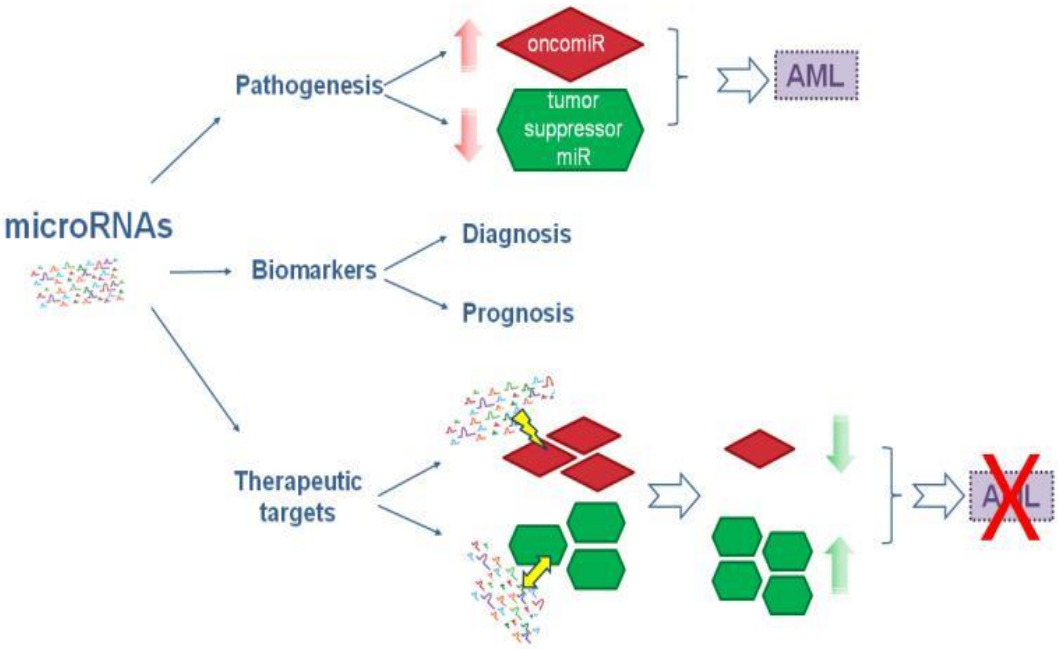
Human CD34 + leukemic cells were shown to repopulate the bone marrow(BM) of severe combined immunodeficient (SCID) mice, while CD34− leukemic blasts remained non-leukemogenic (Bonnet and Dick,1997) , and these CD34 + cells responsible for leukemia initiation and maintenance were termed LSC. Nowadays, they are documented as cells with enhanced capacities to selectively escape chemotherapy treatments (Bahr *et. al*.,2017).

It had been previously reported that CD34+ AML blasts are more resistant to apoptosis than their CD34- counterpart (van Stijn *et.*

al.,2003).Combined expression of both FLT3-ITD mutation and CD34 expression is an important prognostic and predictive factor for poor disease outcome in AML patients(Mona *et. al*.,2020).In addition , CD45dimCD34+ CD38− CD133+ cells in AML might potentially serve as Leukemia stem cells (LSCs).Moreover, the high CD45dimCD34+ CD38− CD133+ cell counts in AML patients served as a significantly poor risk factor for overall and event free survival. In addition, this cell population might represent a novel therapeutic target in AML(Heo *et. al*.,2020).

2.7: Micro RNAs

Are part of a recently described mechanism of post transcriptional modulation of gene expression, and they act by inhibiting translation and speeding up degradation of target messenger RNAs by an imperfect pairing with the 3'-untranslated region (3'-UTR) (O'Connell *et. al* .,2010 ; Elton *et. al*.,2012). MiRNAs play an important role in regulating normal hematopoiesis: myeloid differentiation, cell cycle, proliferation, apoptosis, and gene methylation (Pulikkan *et. al*.,2010 and Katzerke *et. al*.,2013).The expression of miRNAs is frequently deregulated in AML by different mechanisms, like: (i) copy number alterations; (ii) epigenetic changes; (iii) miRNA location in proximity of oncogenic genomic region due to chromosomal translocation or overexpression of protein-coding gene; (iv) aberrant targeting of miRNA promoter regions by altered transcription factors or oncoproteins; and finally (v) deregulated miRNA processing (Wallace *et. al.*,2017). The role of miRNAs in AML is noted in a schematic diagram in figure (2.3).



Figure(2.3): Schematic diagram of microRNAs role in AML. MicroRNAs are involved in pathogenesis and are considered as biomarkers and therapeutic targets in AML. Red arrows indicate overexpression of oncomiR and down expression of tumor suppressor miR which cause AML; lightning and two-headed arrow indicate targeting and restore, respectively; green arrows indicate decreased oncomiR or increased tumor suppressor miR levels that block AML; red cross indicates AML block.(Trino *et. al.*,2018).

Over the years, various methods have been developed to detect and quantify miRNAs, with major common approaches recently reviewed (Pritchard *et. al*, 2012). Quantitative reverse transcription polymerase chain reaction (RT – PCR)-based methods are frequently used to validate the expression of individual miRNAs (Lu *et. al.*, 2005).Certain studies have examined possible roles for miRNAs in the upstream control of KIT (Felli *et. al*, 2005;

Liu *et. al*, 2010; Gao *et. al*, 2011a,b), for example, MIR29B has been found to participate in a control network involving the interacting transcription factors SP1 (specificity protein 1) and nuclear factor kappa-light-chain-enhancer of activated B cells (NF j B) as well as histone deacetylases, which leads to KIT Proto-Oncogene, Receptor Tyrosine Kinase (KIT) overexpression (Liu *et. al.*, 2010).

2.8: Association of microRNAs with AML

MicroRNAs (miRNAs) are small, non-coding RNAs found throughout the eukaryotes that control the expression of a number of genes involved in commitment and differentiation of hematopoietic stem cells and tumor genesis. Widespread dysregulation of miRNAs have been found in hematological malignancies, including human acute myeloid leukemia (AML). A comprehensive understanding of the role of miRNAs within the complex regulatory networks that are disrupted in malignant AML cells is a prerequisite for the development of therapeutic strategies employing miRNA modulators. Liao *et. al.*,(2017) review the roles of emerging miRNAs and its regulatory networks in AML pathogenesis, prognosis, and miRNA-directed therapies. MiRNA expression profiles are a highly effective diagnostic technique, as demonstrated in a blind study on 22 different tumor types that allowed for tumor classification with an accuracy of 90% or higher when statistically analyzed (Rosenfeld *et. al.*,2008). The previous studies about the marker genes in AML have reported that miRNA was one of the most commonly used biomarkers in adult AML patients (Marcucci *et. al.*,2011). MiRNA is a small non-coding

RNA, which is prevalent in humans, animals, plants and some viruses and is highly conserved. Various developmental and physiological processes are adjusted by them, including cell division, immune response and cell apoptosis (Bissels *et. al.*,2012). Through analyzing the expression of miRNA in tumor cells, it could be easily found that some miRNAs were abnormally expressed in a variety of cancer cells. Since , miRNA was found, a large number of miRNAs were thought to be carcinogenic factors, while others had the function of tumor suppressors. Studies about the function of miRNA in AML have gradually increased in recent decades, and numerous researchers illustrated that miRNAs were crucial in the development of AML. For example, the absence of miR-145 and miR-146a could lead to long-term blood diseases in mice, and the introduction of these two miRNAs into AML cell lines could significantly induce cell death and slow the growth of cancer cells (Bissels *et. al.*,2012). Different subtypes of AML have also been associated with different miRNAs expression profiles, so multiple studies have shown that unsupervised clustering using miRNAs expression strongly correlates with known morphological and cytogenic subgroups (Debernardi *et. al.*,2007 ; Li *et. al.*,2008).

The relationship between the expression of a specific miRNAs (or group of it) and AML subtype has important implications for prognosis and treatment of AML(Blum *et. al.*,2010 , Whitman *et. al.*,2010), because , MiR expression is deregulated in many types of cancers, including leukemia's. In acute myeloid leukemia (AML), the expression of specific

miRNAs has been linked with both prognostically and cytogenetically defined subgroups .Certain studies have shown that deregulation of miRNA expression is not simply a consequence of AML but a potential contributor to leukemogenesis, and this commentary will focus on select findings that describe the different mechanistic roles for miRNAs in the development of leukemia (Hyde and Liu ,2010).The miRNA expression patterns were correlated with molecular abnormalities like t(11q23), trisomy 8 and FLT3-ITD mutations, also the specific miRNA alterations were correlated with prognosis, so that patients with a high expression of miR-191 and miR-199a had worse overall and event-free survival. A different signature was also generated and validated(Blum *et. al*.,2010).

Acute myeloid leukemia patients carrying NPM1 mutations show a downregulation of miR-204 (among other specific alterations), meantime, the investigators also showed that miR-204 targets HOXA10 and MEIS1, suggesting the HOX upregulation seen in these patients may be secondary to miRNA alterations. Some evidence linking ALL1 fusion protein to miRNA alterations through targeting DROSHA has also been postulated(Whitman *et. al*.,2010) .Meantime, miRNAs control several critical cellular processes and have emerged as a fundamental epigenetic regulatory mechanism. This is true in normal and abnormal cellular states, including cancer. In the past few years there has been a significant expansion of the understanding of the role of these small molecules in the pathogenesis of leukemia, therefore, further studies are needed

to define specific processes and molecular interaction to harness this knowledge to improve patient care (Yendamuri and Calin,2009).

The circulation of miRNAs in body fluids occurs either through direct secretion or through protective micro-structures that are secreted into the specific body fluid (Zen and Zhang,2012), and certain studies revealed that micro-vesicles or exosomes are the preferred source of transport for circulatory miRNAs (Hunter *et. al*.,2008;Kosaka *et. al*.,2010) , also, miRNAs have been well studied in various cancers including leukemia's (Bhatti *et. al*.,2009 ; Nana-Sinkam and Croce.,2010). Among various symptoms and manifestations identified in association with AML, one of the most common characteristics involved in ~ 50% of AML patients is a group of cytogenetic abnormalities, which is considered to be contributing to the disease heterogeneity and with prognostic significance (Mr´ozek *et. al*.,2004) . Other AML patients without detectable chromosomal abnormalities may display mutations or dysregulations in specific genes, a signature ubiquitously found in cancers (Mr´ozek *et. al*.,2007; Renneville *et. al*.,2008).

MicroRNA signatures in AML have been sought, and many groups of researchers performed large scale profiling of its expression in different populations of AML patients, and when compared the AML patient samples to acute lymphoblastic leukemia (ALL), both groups with similar chromosomal alterations, 27 miRNAs were reported to be different between the two groups (Mi *et. al*.,2007) Importantly, miR-146a was inversely correlated to overall survival in both AML and ALL

(Wang *et. al*.,2010). MiRNAs control several critical cellular processes and have emerged as a fundamental epigenetic regulatory mechanism. This is true in normal and abnormal cellular states, including cancer. Furthermore, miRNAs are adept biomarkers as they tend to be resilient in varying physical conditions, including wide ranges of temperature and pH, and can withstand degradation within formalin- fixed paraffin-embedded tissues (Nakajima *et. al*.,2007; Mitchell, *et. al*.,2008).

2.9: Therapy of AML

Treatment of AML is divided into induction phase and consolidation phase, so the induction phase of AML treatment seeks to clear the blood of blasts and reduce the number of blasts in the bone marrow. Currently, the induction phase involves high dose induction chemotherapy with cytarabine and an anthracycline (Iland *et. al* .,2015 , McCarthy and Walsh ,2017), then the consolidation chemotherapy is administered to destroy residual leukemic cells after the patient has recovered from induction (Ferrara and Schiffer,2013) . Stress cells including cancer cells express stress proteins which are recognized by the immune system through immunosurveillance, this leads to the elimination of these stress cells. Nevertheless, cancer cells are able to escape this immunosurveillance through various mechanisms including the shedding of the stress proteins (Chan *et. al*.,2014 ; Morvan andLanier,2016). Unlike chemotherapy and radiotherapy, immunotherapy is more specific in its activity and therefore associated with low toxicity (Gangadhar and Vonderheide,2014) , and this specificity is the core of immunotherapy that

eliminates cancer cells without harming normal cells. Immunotherapeutic agents specifically inhibit cancer cell proliferation, recruit effector cells to eliminate the cancer cells or induce apoptosis in the cancer cells (Gotwals *et. al*.,2017).

2.9.1: Chemotherapy, the common treatment option for AML .

Chemotherapy is the use of anticancer drugs for the treatment of cancer condition such as AML. It is the standard treatment option because chemotherapeutic drugs are readily available and affordable. More common routes of drug administration include intravenous, intrathechal or subcutaneous, also administration can be done orally (Vago *et. al*.,2016). Since , these drugs have high bioavailability and thus, are able to spread throughout the body, making it useful for the treatment of cancers such as AML. Chemotherapy of AML is usually done in two phases induction and consolidation (Lee *et. al*.,2016). The level of intensity of this treatment depends on the age and health state of the patient. Younger patients usually go through a more intensive chemotherapy compared to older patients who are mostly above the age of 60 (Fiedler *et. al*.,2015). Common drugs that are usually used in the induction phase are cytarabine, anthracycline, and cladribine (Pluta *et. al*.,2017).

2.9.2 :Immunotherapy as alternative treatment for AML

Immunotherapy also known as targeted therapy is arguably the most effective intervention for AML, and this type of cancers can only progress if they are able to escape the immunosurveillance of the immune system (Marcus *et. al*.,2014) Stress cells including these cancer cells express stress proteins that stimulates the immune system into action. These stress proteins are specific and therefore attract the specific immune cells that are able to recognize these stress proteins and therefore recruited to the site of cancer to exert their effector function (Kitamura *et. al*.,2015).

2.9.3: Antibodies

AML cells express a variety of surface antigens including CD33, CD123, CD47, and CD64 that serve as targets for monoclonal antibody therapy(Busfield *et. al*.,2014;Buckley and Walter,2015) , and these antibodies are usually designed to identify these specific antigens to help in the destruction of the cancer cells. Thus, the antibody mediates destruction of the cancer cells by recruiting appropriate immune cells, blocking particular signaling pathway relevant to cancer cell growth by binding to the related receptor or ligand, or delivering attached chemotherapeutic agents to the cancer cells. Since, a biomarker CD33 is expressed by about 80% of AML cells, therefore this makes CD33 the most suitable antigen for targeted therapy in AML(Pizzitola *et. al*.,2014 ; Zeijlemaker *et. al*.,2014).

Identifying other suitable antigens on AML for targeted immunotherapy is a challenge because most of the potential antigens are found on both AML cells and healthy myeloid precursors which can easily result in off tumor target effect in AML treatment, leading to prolonged thrombocytopenia (Wu *et. al*.,2016).

3.Materials and Methods

3.1: Materials

3.1.1 :Equipment and Instruments:

The equipment and instruments used in the current study have been listed in the table (table 3.1).

Table (3.1): Equipment's and instruments that used in this study with their companies and countries .

Equipment	Company	Country
Blood collection EDTA tube	AFCO	Jordan
Cylinder (100 ml)	AMSCO	Germany
ELISA reader system	Mindray	China
ELISA washer	Mindray	China
Eppendroff tubes	Eppendrof	Korea
Exispin vortex centrifuge	Bioneer	Korea
Gloves	Broche	Malaysia
High speed Cold Centrifuge	Eppendorf	Germany
Incubator	Memmert	Germany

Micropipettes (different volumes)	Eppendorf	Germany
Miniopticon Real Time PCR	Bio-Rad	USA
Nano drop	Thermo Scientific	UK
Oven	Memmert	UK
Plain test tube	AFCO-DISPO	Jordan
Rack	Sterellin Ltd.	UK
Refrigerator	Concord	Lebanon
Serum Gel Tube	AFCO-DISPO	Jordan
Sterile syringes	Sterile EO.	China
T100 Thermal cycler PCR	BioRad	USA
Tips	Sterellin Ltd.	UK
Vortex	CYAN	Belgium

3.1.2: Chemicals and Biological Materials:

The chemicals and biological materials that used in current study were listed in table (3.2).

Table (3.2): Chemicals and biological materials that used in this study .

Chemicals	Company	Country
Absolute Ethanol	Chem	Belgium
Chloroform	Chem	Belgium
DEPC water	Bioneer	Korea
Isopropanol	Chem	Belgium
nuclease free water	Bioneer	Korea

3.1.3:Kits

3.1.3.1:Molecular test Kit:

The molecular kits that used in present study were listed in table (3.3).

Table (3.3): The kits for miRNA-203 ,miRNA-143 and miRNA-495 that used in current study .

Kit	Company	Country
TransZol Up	**iNtRON**	**Korea**
TransZol Up 100ml		
RNA Dissolving Solution		
DNase I enzyme kit	**Promega**	**USA**

DNase I enzyme		
10x buffer		
Free nuclease water		
Stop reaction		
GoScript™ Reverse Transcriptase kit	**Promega**	**USA**
GoScript™ Reverse Transcriptase		
GoScript™ 5X Reaction Buffer		
PCR Nucleotide Mix		
Recombinant RNasin® Ribonuclease Inhibitor		
Nuclease-Free Water		
Oligo (dT)15 Primer		
Random Primers		
MgCl2(25mM)		
GoTaq® qPCR Master Mix	**Promega**	**USA**
GoTaq® qPCR Master Mix, 2X		
CXR Reference Dye, 30µM		
Nuclease-Free Water		

3.1.3.2: Enzyme Linked Immunosorbent Assay(ELISA Kit)

The company and country of ELISA kit that used to estimate the level of CD34 was shown in table (3.4) and the kit component for CD 34 were listed in table (3.5).

Table (3.4): ELISA kit for CD34 that used in present study .

Kit	Company	Country
Human Cluster of Differentiation , CD34 ELISA Kit	Biotech	Korea

Table(3.5): Kit components for CD34 .

Reagent	Quantity
Pre-coated ELISA plate	8 well x 12 strips x1
Standard Solution	0.5 ml x1
Standard Diluent	3ml x 1
Streptavidin – HRP	6ml x 1
Stop Solution	6ml x 1
Substrate Solution A	6ml x 1
Substrate Solution B	6ml x 1

WashBuffer Concentrated (25x)	20ml x 1
Biotinylated Human CD34 Antibody	1ml x1
Stop Solution	1vial 10 ml

3.1.4:Primers

The miRNA based stem loop RT-qPCR (SYBER Green dye) were designed by using (The Sanger Center miRNA database Registry) to selected miRNA sequence and using miRNA Primer Design Tool. These primers were provided by (Macrogen company, Korea) as illustrated in table(3.6):

Table(3.6): MiRNAs primers

Primer	Sequence 5′ - 3′	Reference
miR-203 qPCR primer	F : GCGGTGAAATGTTTAGGAC R:GTCGTATCCAGTGCAGGGTCCGAGGT ATTCGCACTGGATACGACCTAGTG	(Liu *et.al.*,2015)
MiR-143 qPCR primer	F: TGTAGTTTTCGGAGTTAGTGTCGCGC R: CCTACGATCGAAAACGACGCGAACG	(Yu *et.al.*,2017)

MiR-495 qPCR primer	F:GTCGTATCCAGTGCAGGGTCCGAGG R:TATTCGCACTGGATACGACCTGTCC	(Zhang *et.al.*,2020)
U6	F: GITTTGTAGTTITGGAGTTAGTGTTGTGT R: CTCAACCTACAATCAMMAACAACACAAA CA	(Liu *et.al.*,2015)

3.2: Methods

3.2.1:Study Population, Design, Setting and Data Collection Time

The study subjects comprised of 115 AML patients(38male and 77female) ,and their ages ranged between 18 and 66 years .These patients were suffered from Acute myeloid leukemia and there samples were collected from the Baghdad medical city hospital during the period from (December) 2020 to (September) 2021 under the supervision of specialized hematopathologist , and according to the medical ethics of the hospital and consent form taken from all patients and volunteers group. Also , a questionnaire was taken from the patients and case sheets including: number, age, sex, subtypes of AML (M0. M1, M2 ,M3,M4 , M5 ,M6 .M7) and duration of disease. Then , 60 patients were selected based on quantity and natural color of their samples (28male and 32

female) , in addition to the 30 samples from healthy subjects as control group (11 male and 19 female) and this group matched with the patients group.

3.2.2 : Collection of blood samples

Blood sample were collected by venipuncture from these groups (five millimeter of venous blood) were drawing by disposable syringe under aseptic technique 2 ml was placed into EDTA tubes and the remaining (3 ml) pushed slowly into disposable gel containing tubes.

Blood in the EDTA tubes was used to determine CBC . while blood in the gel containing tubes was allowed to clot at room temperature for 15 minutes and then centrifuged at 2,000 x g for 10 minutes , after that serum was obtained (Barbara and Anna,2012) and stored into two parts:

- 500µl of serum has been collected in Eppendrof tube contain 500 µml of Trizol then stored at -20 to be used for miRNA-203 and miRNA-143miRNA-495 qPCR.

- Five hundred µl of serum has been collected in Eppendrof tube then stored at -20 tobe used for CD34 ELISA assay .

3.3 : Study Design

The study design was illustrated in figure (3.1).

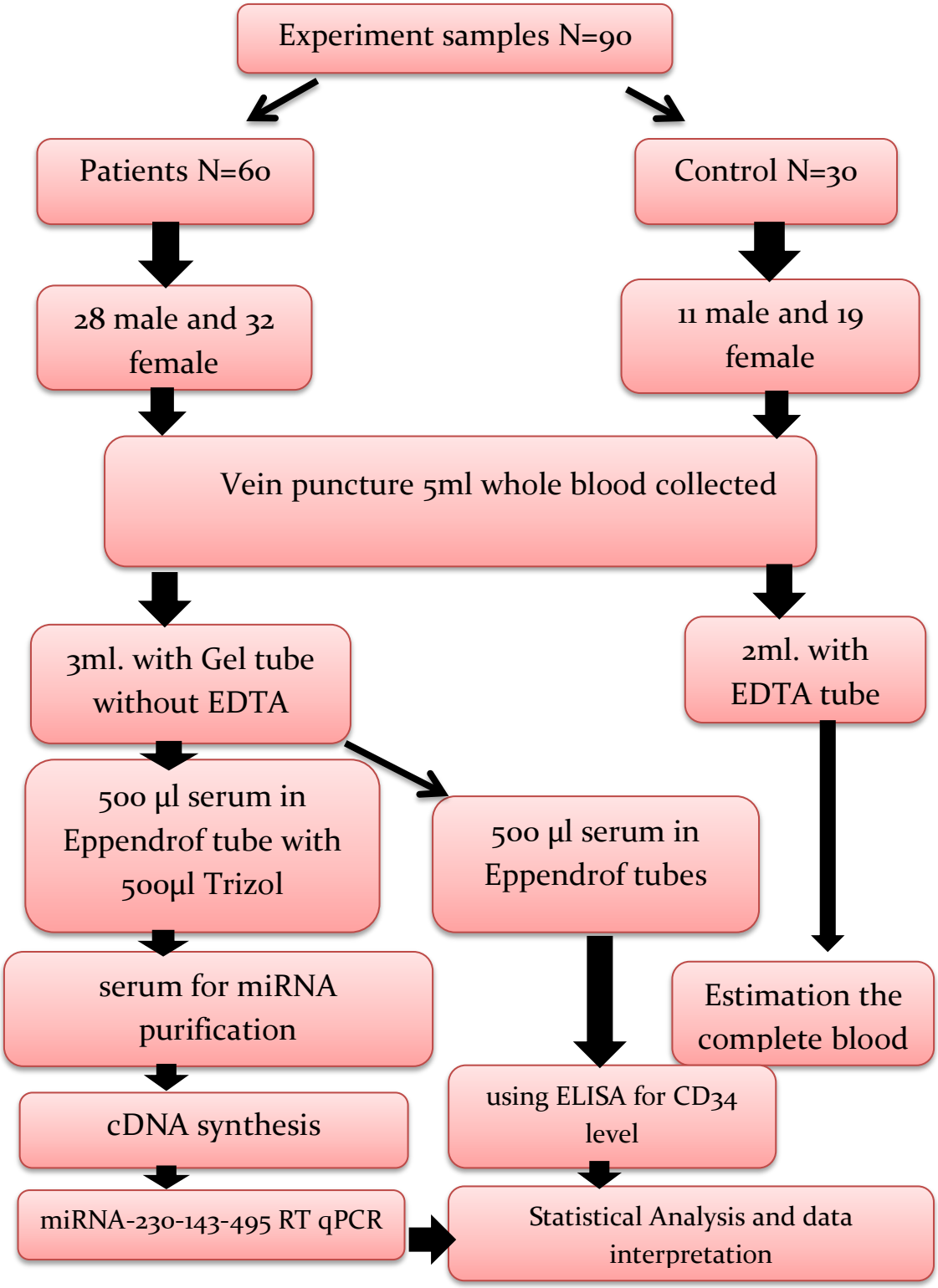


Figure (3.1) : Study Design .

3.4: Laboratory Assays :

The current study required two pathways of laboratory tests , including molecular detection of miRNA-203, miRNA-143 and miRNA-495 by using RT- PCR technique and estimating the serum levels of CD34 by using ELISA technique.

3.4.1: Molecular study

3.4.1.1:Total RNA extraction

Total RNA were extracted from serum samples by using (Tranzol UPreagent kit) and done according to the manual procedure of company instructions as following steps:

- Five hundred µl of serum samples or blood samples were placed in 1.5

Micro centrifuge tube, then centrifuged at 8000xg for 2 minutes at 2-8C° , after that 1ml of TRanzol UP reagent was added to each tubes.

- Pipetting up and down until no visible precipitates are present in the lysate.
    - Incubate at room temperature for 5 minutes.
    - Add 200 µl of chloroform per ml TransZol Up , then shake the tube vigorously by hand for 30 seconds and incubate at room temperature for 3 minutes.

- Centrifuge the sample at 10,000xg for 15 minutes at 2-8°C. The mixture separates into a lower pink organic phase, an interphase, and a colorless upper aqueous phase which contains the RNA. The volume of the aqueous upper phase is approximately 50 % volume of TransZol Up reagent .
- Transfer the colorless, upper phase containing the RNA to a fresh RNase - free tube , then add 500 µl of isopropanol for per ml TransZol Up used . Mix thoroughly by inverting tube and incubate at room temperature for 10 minutes .
- Centrifuge the sample at 10,000xg for 10 minutes at 2-8°C , then discard the supernatant and the colloidal precipitate can be seen at the wall and the bottom of the tube .
- Add 1 ml of 75 % ethanol (prepared with DEPC - treated water), vortexing vigorously (add at least 1 ml of 75 % ethanol for 1 ml of TransZol Up used).
- Centrifuge the sample at 7,500xg for 5 minutes at 2-8°.
- Discard the supernatant. Air - dry the RNA pellet (for about 5 minutes).
- RNA pellet is dissolved in 50-100 µl of dissolving solution .
- Incubate at 55-60°C for 10 minute. For long-term storage, store the purified RNA at -70 °C.

3.4.1.2 : Estimation RNA yield and quality

The extracted genomic RNA was checked by using Nanodrop spectrophotometer (THERMO. USA) that check RNA concentration and estimation of RNA purity through reading the absorbance in at 260 /280nm as following steps:

- After opening up the Nanodrop software, chosen the appropriate application (Nucleic acid, RNA).
- A dry wipe was taken and cleaned the measurement pedestals several times, then carefully pipet 2µl of ddH2O onto the surface of the lower measurement pedestal.
- The sampling arm was lowered and clicking OK to blank the Nanodrop, then cleaning off the pedestals.
- Finally , the pedestals are cleaned and pipet 1µl of RNA sample for measurement.

3.4.1.3:DNase I Treatment

The extracted RNA were treated with DNase I enzyme to remove the trace amounts of genomic DNA from the eluted total RNA by using samplesof DNase I enzyme kit and done according to method described by Promega company, USA instructions , as shown in table (3.7) :

Table(3.7): Reaction protocol of DNase I enzyme

Mix	Volume
Total RNA 100ng/ul	10ul
DNase I enzyme	1ul
10X buffer	4ul
DEPC water	Up to 20ul

Then , the mixture was incubated at 37C° for 30 minutes , after that 1µl stop reaction was added and incubated at 65C° for 10 minutes for inactivation of DNase enzyme action.

3.4.1.4: cDNA synthesis

cDNA synthesis for miRNA was done using GoScript™ Reverse Transcriptase Kit(Table, 3.8) and performed according to the manual procedure of company instructions as following:

- Mix and briefly centrifuge each component before use. Combine the following:

Table (3.8): Reaction protocol of GoScript™ Reverse Transcriptase:

Component	Volume
Experimental RNA (up to 5µg/reaction)	4µL
Primer [Oligo(dT)15 (0.5µg/reaction) and/or Random Primer (0.5µg/reaction) or gene-specific primer (10–20pmol/reaction)]	1µL
Nuclease-Free Water	0 µL
Final volume	5ul

- Heat in a 70°C heat block for 5 minutes. Immediately chill in ice water for at least 5 minutes, then centrifuge 10 seconds in a micro-centrifuge , after that store on ice until reverse transcription mix is added.
- Prepare the reverse transcription reaction mix, 15µl for each cDNA reaction. Combine on ice (Table, 3.9), as following steps :

Table (3.9): Reverse Transcriptase preparation for each reaction:

Component	Volume
GoScript™ 5X Reaction Buffer	4.0µL
MgCl2 (final concentration 1.5–5.0mM)1	1.2-6.4 µL
PCR Nucleotide Mix (final concentration 0.5mM each dNTP)2	1.0 µL
Recombinant RNasin® Ribonuclease Inhibitor (optional)	20 units
GoScript™ Reverse Transcriptase	1.0 µL
Nuclease-Free Water (to a final volume of 15µl)	X µL
Final volume	15ul

- Combine 15µl of reverse transcription mix with 5µl of RNA and

Primer mix.

- Anneal in a heat block at 25°C for 5 minutes.
- Extend in a heat block at for up to one hour. Reactions can be stopped at this point for analysis of the cDNA or may be frozen for long-term storage.
- Inactivate Reverse Transcriptase: Before proceeding with qPCR,

inactivate the reverse transcriptase in a heat block at 70°C for 15 minutes.

The RNA converted into cDNA in thermocycler under the following thermo cycler conditions (Table , 3.10):

Table (3.10): PCR Thermo cycler program:

Step	Temperature	Time
Anneal	25 °C	5 minutes
Extend	42°C	1 hour
inactivation Reverse Transcriptase	70 °C	15 minutes

3.4.1.5: Quantitative Real-Time PCR (qPCR)

The quantitative Real-Time PCR used in quantification of miRNA-203,miRNA-143 and miRNA-495 expression analysis that normalized by housekeeping gene (U6) in serum and blood patients and control samples by using Real-Time PCR technique and this method was carried out according to method described by Magdalena *et. al.*, (2019) which include the following steps:

A. qPCR master mix preparation

qPCR master mix was prepared by using GoTaq® qPCR Master Mix Kit that according on SYBER Green dye detection of gene amplification in Real-Time PCR system (Table , 3.11) as following :

Table (3.11): qPCR master mix preparation for each reaction:

qPCR master mix	Volume
GoTaq® qPCR Master Mix	10 µL
cDNA template (20ng)	5µL
Forward primer(10pmol)	1µL
Reverse primer (10pmol)	1µL
Supplemental CXR Reference Dye	1µL
Nuclease-Free Water	Up to 20ul
Final volume	20ul

After that, qPCR master mix component that mentioned above placed in qPCR premix standard plate tubes that contain the other PCR (RT-PCR syber green) components, then the plate mixed by Exispin vortex centrifuge for 3 minutes , after that placed in Miniopticon Real-Time PCR system.

B. qPCR Thermocycler conditions

The qPCR plate was loaded and the thermo cycler protocol was followed (Table , 3.12):

Table (3.12): qPCR Thermocycler conditions:

qPCR step	Temperature	Time	Repeat cycle
GoTaq® Hot Start Polymerase activation	95 °C	5min	1
Denaturation	95 °C	20 sec	45
Annealing\Extension Detection(scan)	60 °C	30 sec	

3.4.1.6: Data analysis of qPCR

The data results of qPCR for miRNA and housekeeping gene were analyzed by the relative quantification gene expression levels (fold change) by using The ΔCT Method Using a Reference that described by (Livak and Schmittgen, 2001) as following equations:

First, normalize the CT of the target gene to that of the reference (ref) gene, for both the test sample and the control sample:

$\Delta CT(test) = CT(target, test) - CT(ref, test)$

$\Delta CT(control) = CT(target, control) - CT(ref, control)$

Second, normalize the ΔCT of the test sample to the ΔCT of the control:

$\Delta\Delta CT = \Delta CT(test) - \Delta CT(control)$

Finally, calculate the expression ratio:

$2^{-\Delta\Delta CT}$ = Normalized expression ratio

3.4.2 : Immunological study

3.4.2.1: Estimation the levels of CD34 by ELISA assay

A. Assay Principle

The reagents preparation and assay procedure were carried out according to manufacturer's description of the Biotech / company(Korea) .

The kit is an Enzyme-Linked Immunosorbent Assay (ELISA). The plate has been pre-coated with Human CD34 antibody. CD34 present in the sample is added and binds to antibodies coated on the wells , then biotinylated Human CD34 Antibody is added and binds to CD34 in the sample. After that , Streptavidin-HRP is added and binds to the Biotinylated CD34 antibody. The incubation unbound Streptavidin-HRP is washed away during a washing step , then the substrate solution is added and color develops in proportion to the amount of Human CD34. The reaction is terminated by addition of acidic stop solution and absorbance is measured at 450 nm.

B. Reagent Preparation

- All reagents should be brought to room temperature before use.
- Standard reconstitute the 120µl of the standard (16ng/ml) with 120µl of standard diluent to generate a 8ng/ml standard stock solution. Allow the standard to sit for 15 mins with gentle agitation prior to making dilutions. Prepare duplicate standard points by

serially diluting the standard stock solution (8ng/ml) 1:2 with standard diluent to produce 4 ng/ml, 2 ng/ml, l ng/ml and 0.5ng/ml solutions. Standard diluent serves as the zero standard(0 ng/ml) , and any remaining solution should be frozen at -20°C and used within one month.

- Wash Buffer , diluent 20ml of Wash Buffer concentrate 25x into deionized or distilled water to yield 500 ml of 1x Wash Buffer , and if crystals have formed in the concentrate ,mix gently until the crystals have completely dissolved .

C. Assay Procedure

- All reagents, standard solutions, and specimens were prepared as instructed, and all reagents were brought to room temperature before use. The assay is performed at room temperature
- The number of strips required for the assay has been determined .the strips were inserted in the frames for use , unused strips were stored at 2-8°C.
- Fifty µl standard was added to standard well. Note: Don't add biotinylated antibody to standard well because the standard solution contains biotinylated antibody.
- Forty µl sample was added to sample wells and then 10µl anti-CD34 antibody was added to sample wells, then 50µl streptavidin-HRP was added to sample wells and standard wells (Not blank control well). Mixed well. The plate covered with a sealer and Incubated 60 minutes at 37°C.

- The sealer was removed and the plate was washed 5 times with wash buffer. Soak wells with 300ul wash buffer for 30 seconds to 1 minute for each wash. For automated washing, aspirate or decant each well and wash 5 times with wash buffer. Blot the plate onto paper towels or other absorbent material.
- Fifty µl substrate solution A was added to each well and then added 50µl substrate solution B to each well , then the plate covered with a new sealer was incubated for 10 minutes at 37°C in the dark.
- Fifty µl Stop Solution was added to each well, the blue color changed into yellow immediately.
- The optical density (OD value) of each well determined immediately using a microplate reader set to 450 nm within 10 minutes after adding the stop solution. The standard curve for CD34 biomarker was illustrated in figure (3.2).

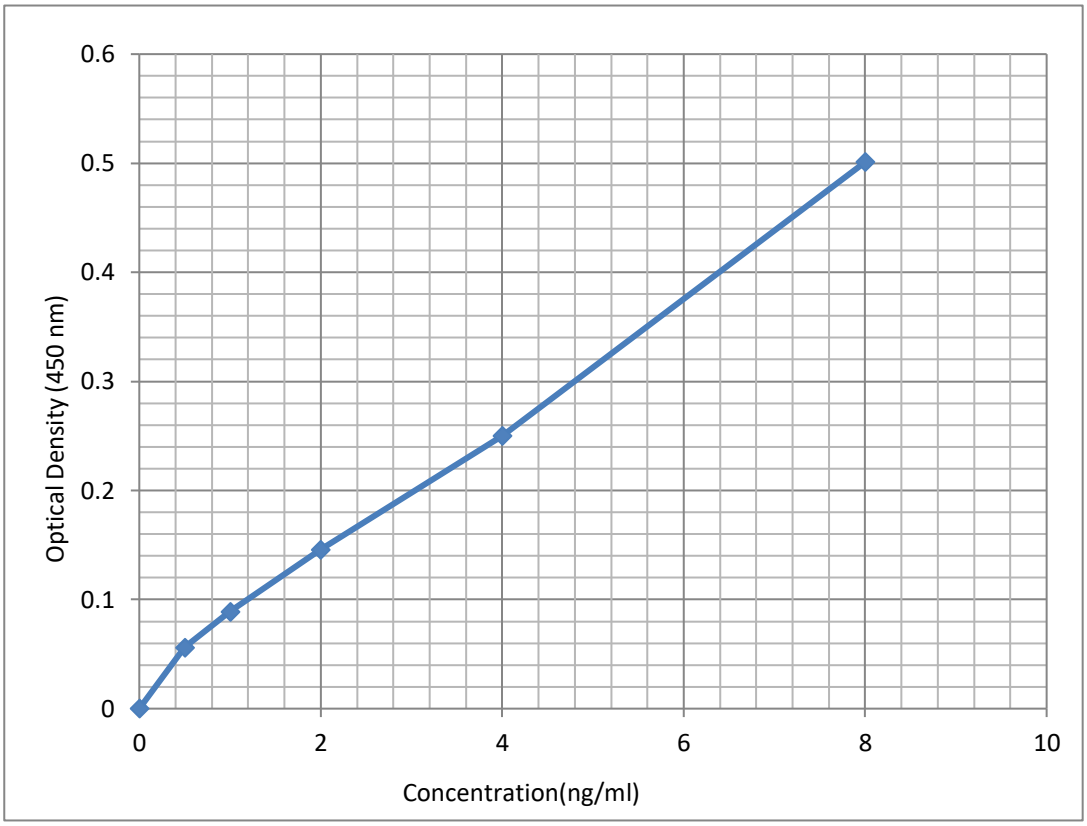


Figure (3.2): Standard curve for determine the level of CD34.

D. Calculation of Results

Construct a standard curve by plotting the average OD for each standard on the vertical (Y) axis against the concentration on the horizontal (X) axis and draw a best fit curve through the points on the graph. These calculations can be best performed with computer-based curve-fitting software and the best fit line can be determined by regression analysis. The standard curve was done as shown in figure (3.2).

3.5 :Statistical Analysis

Data were collected, summarized, presented and analyzed Statistical analysis was carried out using statistical package for social science(SPSS version 27). Categorical variables were presented as frequencies and percentages. Continuous variables were presented as (Means ± SE). Student t-test was used to compare means between two groups. ANOVA test was used to compare means between three groups or more. A *p*-value of ≤ 0.05 was considered as significant .

4. Results and Discussion

4.1: Distribution of patients with acute myeloid leukemia

Table(4.1) showed that distribution of patients with acute myeloid leukemia(AML) age and gender , in which the female patients percentage was 58.3% (35 out of 60), while it was 41.7% (25 out of 60) in male patients .

Table (4.1): Distribution of patients with acute myeloid leukemia according to gender .

Gender	Number	Percentage
Male	25	41.7%
Female	35	58.3%
Total	60	100%

AML remains a rare but fatal malignancy, The current results found that the disease was more recurrent in females than in males according to the collecting samples , and there is a possible reason for this, which is the occurrence of some genetic mutations in female ,which leads to the presence of disease genes in the newborn, then develop to the younger female that causes leukemia (Newell and Cook, 2021). In addition, Suresh *et. al.*, (2006) showed that myeloid cells in females and several pathophysiological mechanisms such as exposure to common environmental risk factors and repeated infections in female which might result in the development of this disease .

In the current study the age of patients distribution between 18-66 years, that may be related to the disease genes and some molecular features, that was most pronounced in the AML patients (Lindsley *et. al.*,2015). The age distribution by sex of leukemia patients, which is characterized by demography, indicates an important influence of age composition, and this can make age-matching the optimal condition for comparison (Engen *et. al.*,2020). Furthermore, Wang *et. al.*, (2019) found that there is an excess of females with AML compared to males, and the disease was also

concentrated among younger individuals more than adults, and the occurrence of many mutations in many genes whose representation is excess in females compared to males in the groups of the study .

4.2: Distribution of patients with acute myeloid leukemia according to subtypes

Figure (4.1) revealed that distribution of patients according to subtypes including M0, M1, M2, M3, M4, M5, M6 and M7. Majority of patients presented with subtype M3 (N=23, 38.3%) of total patients , while M4 subtype represent 20.0%(N=12) of total patients, but M2 and M5 represent 15%(N=9), in addition M1 and M7 subtypes represents 6.7%(N=4) and 5%(N=3) respectively of total patients and there was no patients presented with subtypes M0 and M6 (0.0%).

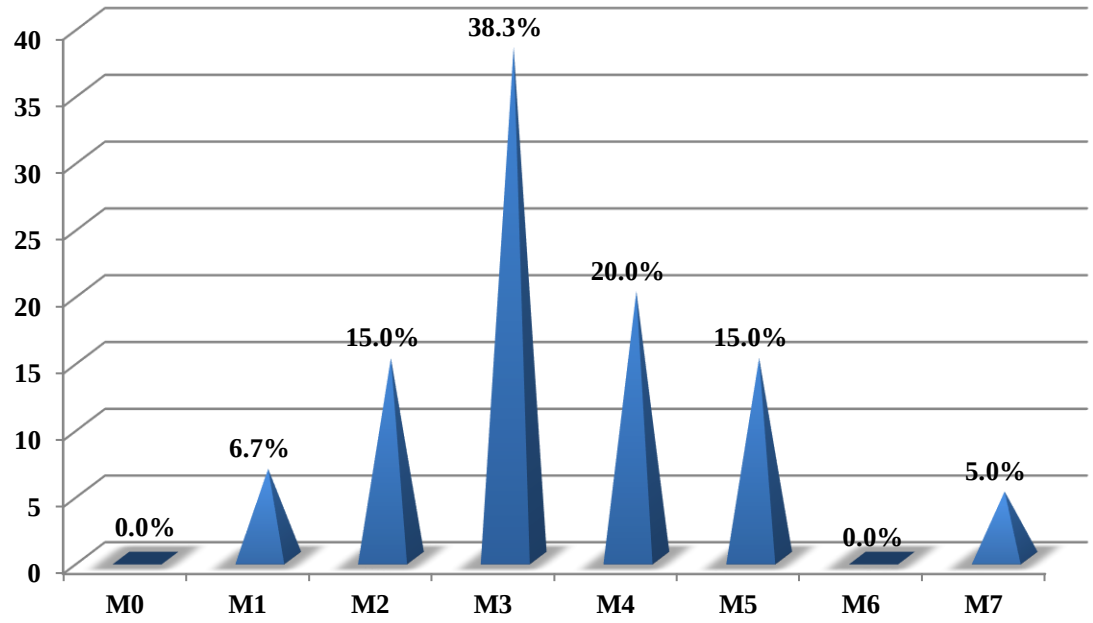


Figure (4.1): Distribution of patients with acute myeloid leukemia according to the subtypes (N=60)

The present results showed a large distribution between types of AML this was consistent with the results of Schoch *et. al*., (2003) that mention the differences between subtypes of AML which resulted from the epidemiological and

pathogenetic heterogeneity in AML patients such as diseases and environmental exposures that appear to be increase the risk of disease to the individual by AML subspecies. In addition, the risk of AML is significantly increased in patients with other hematopoietic disorders, including myelodysplastic syndromes (MDS), some myeloproliferative neoplasms (MPNs), and aplastic anemia (Sritana *et. al*.,2008).

In the current study(Figure,4.1) the M3 subtype (38%) is more than the other subtypes of AML , while the M0 and M6 (0%) subtypes was less, and these results are consistent with Naghmi and Khalid (2013) whom found the same subtypes are highly incidence in contrast to other subtypes. Also , in present study the most common subtype was M3 at (38%) followed by M4 at (20%) ,then equal number of M2 and M5 at (15%). In contrast to a study done in Japan on adult patients of AML, the most frequent subtype was M2 followed by M3 and M4 (Kuriyama *et. al*.,2001) .

4.3 : Distribution of patients with acute myeloid leukemia according to the age .

The present data (Table ,4.2) revealed that the mean age of patients was 37.52 ± 13.62 years with older patients was 66 years and younger patients was 18 years , while table (4.3) and figure(4.2) showed the mean differences of age (years) according to study groups (patients and AHC groups) , and there were no significant differences between means of age in studied groups.

Table (4.2): Distribution of patients with acute myeloid leukemia according to the age .

Age	Minimum	Maximum	M ± S.E
Male and Female	18	66	37.52 ± 13.62

Table (4.3): The mean differences of age according to the study groups (N=90) .

Study variable	Study groups	N	Mean ± SE	P-value
Age (years)	Patients	60	37.52 ± 1.76	0.549
	AHC	30	35.80 ± 1.97	

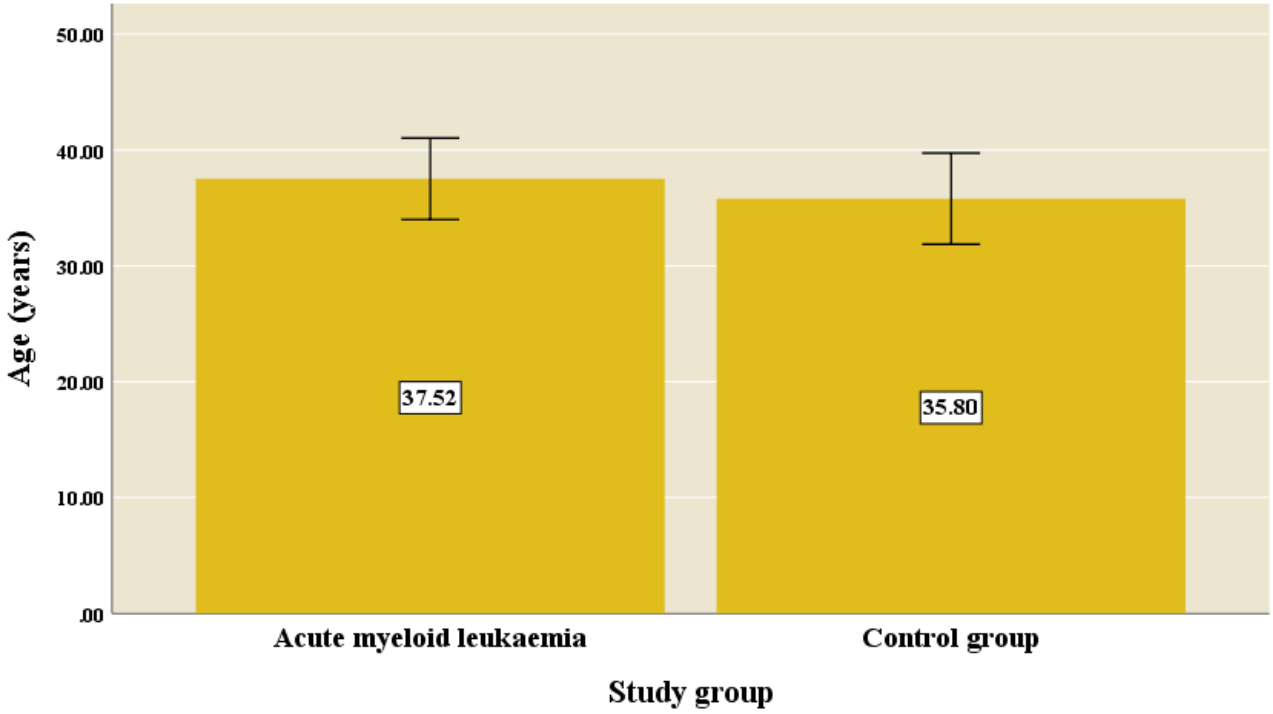


Figure (4.2): The mean differences of age (years) according to the study groups (N=90)

The use of growth factors to promote hematopoietic recovery has yield consistent reductions in treatment related morbidity or mortality . In addition , drug resistance by inhibiting drug efflux mechanisms or increasing sensitivity to cytotoxic agents, these strategies may be shown significantly effect on the age distribution in AML outcome (Sonneveld *et. al.*,2000).

The present study was undertaken to analyze different aspects of AML patients , in which the results found the more prevalent was in adults at mean of age (37.52) years than younger age, this results are consistent with Lowenber, (2000) who found

a high incidence of AML with ≥ 30 age in contrast to other ages , as well as the disease increased in this age category because the cytogenetic abnormalities that showed in many patients with AML , who mention by Grimwade *et. al*., (2001) that observed the cytogenetic abnormalities were associated with a lower age rate (30%) compared with patients with intermediate findings (75%).

One of the most important reasons that lead to an average life expectancy of acute leukemia, which appeared in current results, can be attributed to the different ages of patients admitted in hospitals, as most of the samples that collected were in the ages 20-40 years compared to the young or old ages who receive treatment in other hospitals or in other places in the country, these results was agreement with Rodrigues *et. al*., (2003), who mentioned that 66% of adult patients with AML admitted to Hospital São Paulo were over 30 years of age, as compared to the 34 % rate reported in other international series (McMullin and Mackenzie, 2001). The rapid diagnosis of AML and health care or early treatment was also from the reasons that distributed disease in age periods more than 20 in contrast to another periods (Goldstone *et. al*.,2000) ,therefore these finding was consistent with the present results that showed highly incidence in age 20-40 years.

4.4.: Acute Myeloid Leukemia patients and blood parameters

4.4.1: Complete blood count in patients and control .

The results in table (4.4) showed the mean differences of complete blood picture including , (WBC ($\times 10^3$/µl), Blast (%), Neutrophils (%), Lymphocytes (%),RBC ($\times 10^6$/µl), Hb (g/dl), HCT(%) , MCV (FL), MCH (pg), MCHC (g/dl) and PLT ($\times 10^3$/µl) according to study groups. There were significant differences between means of WBC , Blast , Neutrophils, Lymphocytes, RBCs, Hb, HCT,MCHC and PLTs between patients and AHC groups

Table (4.4) : The means of complete blood count in patients and AHC groups .

Complete blood picture	Study groups	N	Mean ± SE	P-value
WBC ($\times 10^3$/µl)	patients	60	5.67 ± 0.65	**0.011***
	AHC	30	7.60 ± 0.37	
Blast (%)	patients	60	62.63 ± 2.99	**<0.001***
	AHC	30	0.00 ± 0.00	
Neutrophils (%)	patients	60	17.35 ± 2.50	**<0.001***
	AHC	30	70.14 ± 1.56	
Lymphocytes (%)	patients	60	20.30 ± 1.47	**<0.001***
	AHC	30	30.19 ± 1.51	
RBCs ($\times 10^6$/µl)	patients	60	2.92 ± 0.10	**<0.001***
	AHC	30	4.76 ± 0.12	
Hb (g/dl)	patients	60	8.12 ± 0.20	**<0.001***
	AHC	30	13.09 ± 0.21	
HCT (%)	patients	60	24.51 ± 0.72	**<0.001***
	AHC	30	41.01 ± 0.75	
MCV (FL)	patients	60	84.44 ± 1.22	0.156
	AHC	30	87.17 ± 1.13	
MCH (pg)	patients	60	28.09 ± 0.43	0.649
	AHC	30	27.85 ± 0.34	
MCHC (g/dl)	patients	60	32.81 ± 0.22	**0.039***
	AHC	30	32.06 ± 0.24	
PLTs ($\times 10^3$/µl)	patients	60	86.48 ± 11.45	**<0.001***
	AHC	30	264.67 ± 12.72	

(*): means significant(< 0.001)

The complete blood count was used as a tool for diagnosis of AML , in addition the results showed a significant differences in WBCs, Blast , Neutrophils, Lymphocytes, R.B.Cs, Hb, HCT, MCHC and PLTs according to the statistical analysis, so that these parameters reflected their association with AML patients, that increasing with the severity of disease , therefore these results was agreed with Sree *et. al*.,(2013) who diagnosis AML depending on the complete blood film examination, gives definite and positive results in diagnosing of disease. AML is a heterogeneous disorder consisting of clonal expansion of myeloblasts, in addition , the blood parameters plays an important role in their pathogenicity , so the blood components (lymphocytes, WBCs, Blast , PLTs, Hb, etc.) are strongly associated with disease (Prasad *et. al*.,2021). In the current study the mean values of W.B.Cs , Hb and platelets was $5.67 \pm 0.65 \times 10^3$(cell/µl) , 8.12 ± 0.20(g/dl) , and $86.48 \pm 11.45 \times 10^3$(cell/µl) respectively, these results were similar to that reported by Weinberg *et. al.,* (2009) who mention the same value in patients with AML .

Table(4.4) showed that the relation between complete blood count (CBC) and AML patients and their important in diagnosis. Certain studies indicated that diagnosis of AML revolves around CBC, given the clinical situations, in which the CBC test simply allows to diagnosis of disease in a timely manner. With regard to the correct interpretation of CBC, in addition a number of very important data should be considered including , W.B.C.s, Blast , Neutrophils , lymphocytes, R.B.Cs, PLTs, Hb, MCV, MCH, and MCHC , also these studies revealed four out of five patients with AML have anaemia, so the diagnosis should not be excluded just because the patients does not have anaemia. Meantime, one in five patients with AML has no thrombocytopenia so having a normal platelet count does not exclude important this test for diagnosis(David,2014; Hunger,2015).

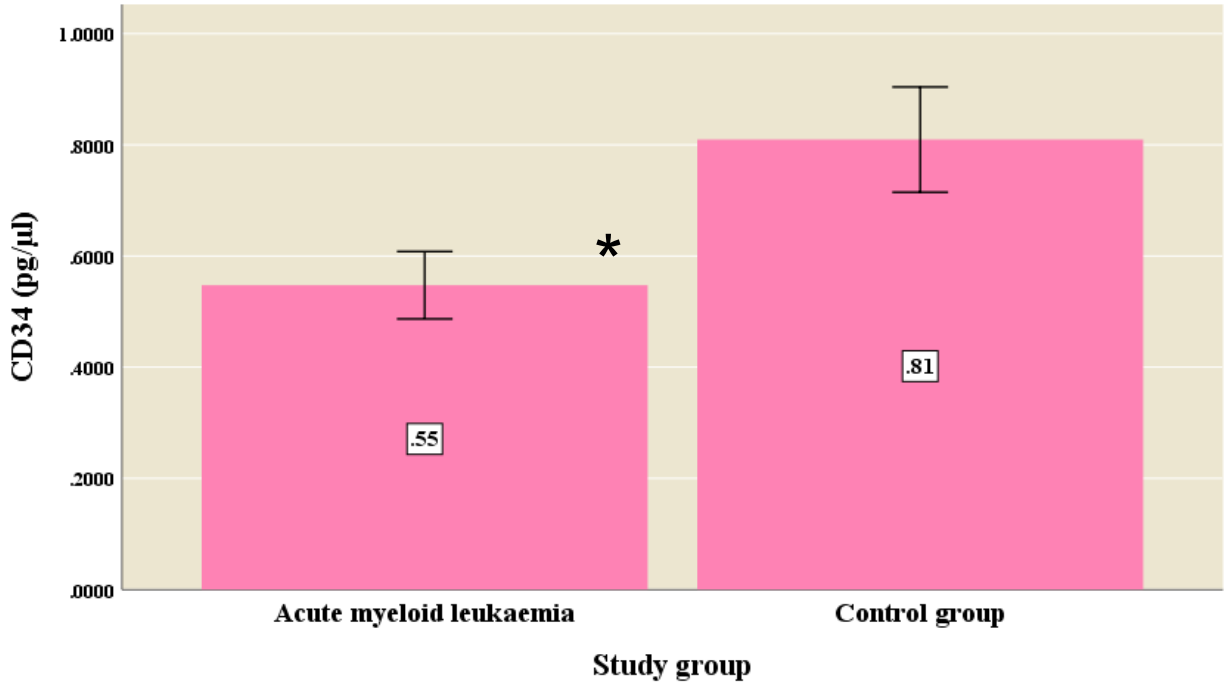
4.4.2. : Relationship between CD34 and miRNAs expression in AML patients.

Table (4.5) , and figures(4.3),(4.4),(4.5), and(4.6) showed the levels of study biomarkers , including CD34 (pg/µl), miRNA-203 (ng/µl), miRNA-143 (ng/µl) and miRNA-495 (ng/µl) in patients and AHC groups . Where all biomarkers shows a significant differences in relation with control at p-value (< 0.001).

Table (4.5) : The mean levels of study biomarkers in patients and AHC groups .

Biomarkers	Groups	N	Mean ± SE	P-value
CD34 (pg/µl)	Patients	57	0.55 ± 0.03	**<0.001***
	AHC	30	0.81 ± 0.05	
miRNA-203 (ng/µl)	Patients	57	0.17 ± 0.03	**<0.001***
	AHC	30	12.02 ± 2.32	
miRNA-143 (ng/µl)	Patients	57	0.15 ± 0.02	**<0.001***
	AHC	30	2.49 ± 0.22	
miRNA-495 (ng/µl)	Patients	57	0.04 ± 0.006	**<0.001***
	AHC	30	0.87 ± 0.15	

(*): means significant(< 0.001)



Figure(4.3) : The mean levels of CD34 (pg/µl) in patients and AHC groups(N.90) . (*): means significant

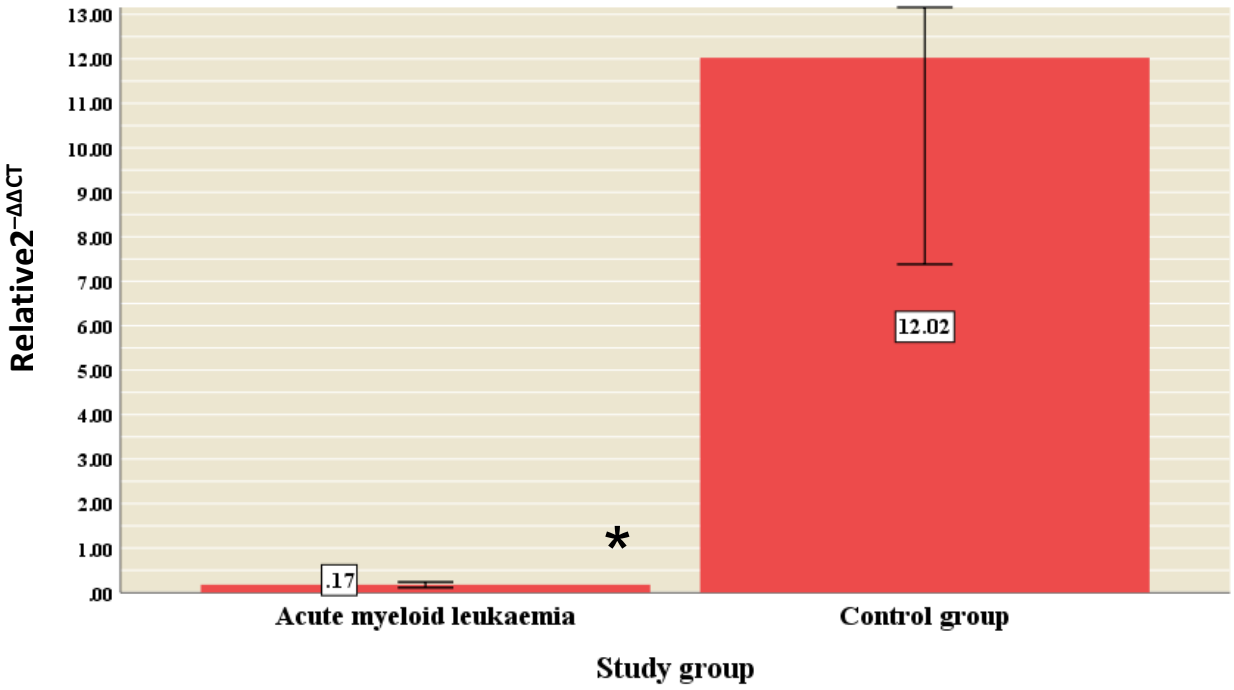


Figure (4-4): The mean levels of miRNA-203 expression (ng/µl) in patients and AHC groups(N.90) . (*): means significant

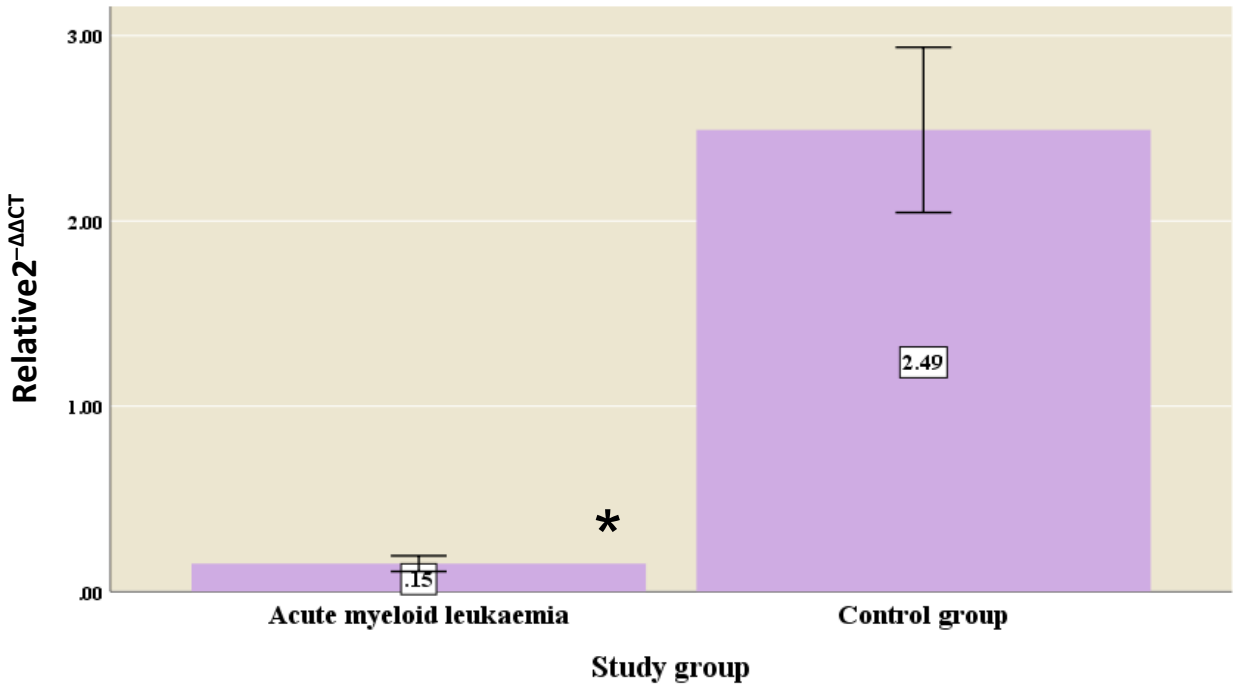
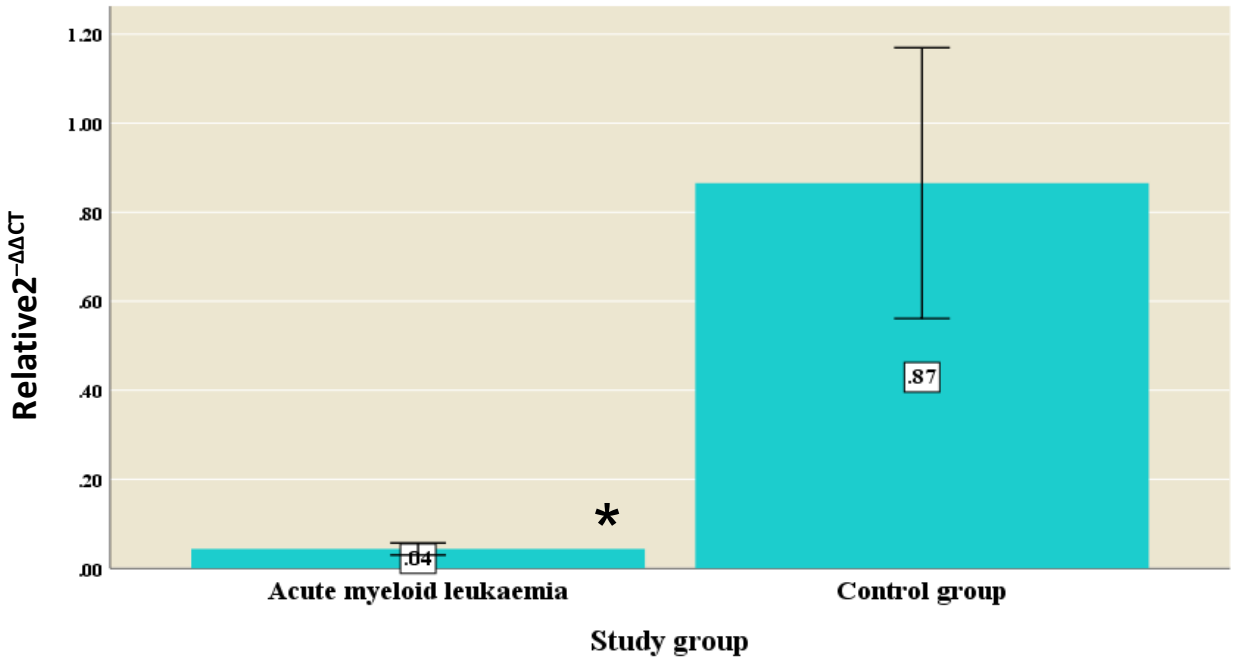


Figure (4.5): The mean levels of miRNA-143 expression (ng/µl) in patients and AHC groups(N.90) . (*): means significant



Figure(4.6):The mean levels of miRNA-495 expression (ng/µl) in patients and AHC groups(N.90) . (*): means significant

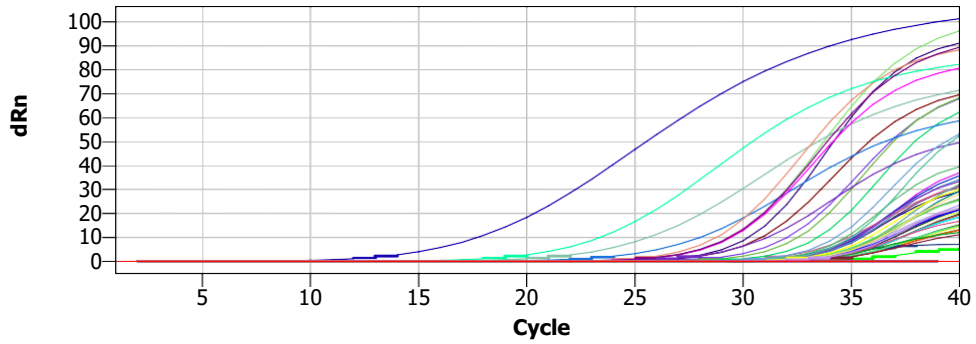
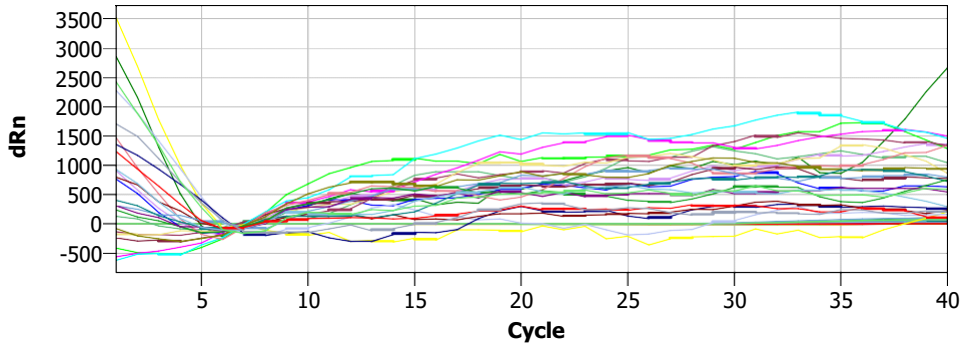


Figure (4.7):RT-qPCR amplification plot of miRNA-203



Figure(4.8):RT-qPCR amplification plot of miRNA-143

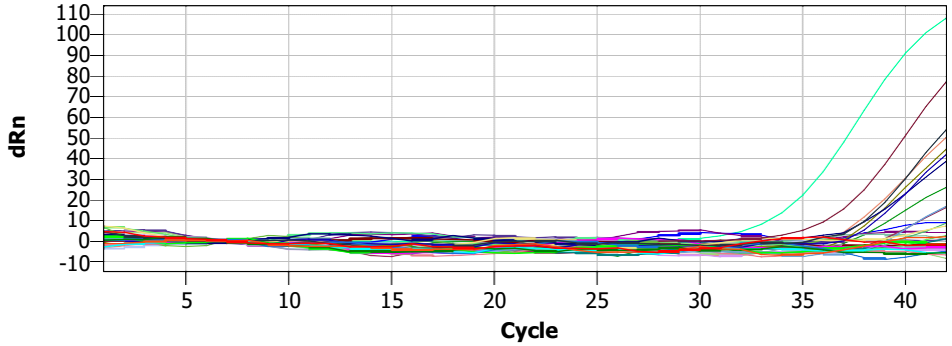


Figure (4.9):RT-qPCR amplification plot of miRNA-495

In the current study there was a highly significant differences between AML patients and AHC groups in the biomarker CD34 at p-value (< 0.001), these highly correlation was clarified by several studies on patients and the role of CD34 proteins in disease events, which indicated that such biomarker in AML syndromes play a crucial role in the behavior of the disease because it potentially contains the neoplastic precursors with clonogenic capability, in addition their role in the expression of apoptosis- and MDR-related proteins (Parker and Mufti,2001; Pecci *et. al*.,2003). The statistical analysis for the results on patients showed that those with positive CD34 had significantly higher severity of disease compared to CD34 other types of receptor . These results are consistent with data of other studies that mentioned CD34 have important role in pathogenicity and mortality in patients with AML (Amer *et. al*.,2019).

Table(4.5) showed that the prognostic significance of CD34 expression in patients and its relationship to other prognostic factors, especially miRNA-203 , miRNA-143, and miRNA-495 ,so that these biomarkers were significant associated with CD34 ,while the results by Zeijlemaker *et. al*., (2015) showed less correlation between them. MiRNAs, are class of regulatory found to be dysregulated in human cancers (Croce *et. al*.,2005) , and mature miRNAs act as negative gene regulators and have been shown to function both as tumor suppressors and oncogenes (Zhang *et. al*.,2007). In present study , it was found a highly relation between miRNA and AML patients , and these results are consistent with the data of Chen *et. al*.,(2014) that indicated the role of miR-143 in myeloid differentiation and AML. In this study, the results found a strong induction of miR-143, miRNA-203 and miRNA-495 expression in patients, which could be supported by publications of Donahue *et. al*., (2009) and Batliner *et. al*., (2012), furthermore, they found that the expression of miR-143 reached the highest levels in severe cases of the disease.

Figures (4.3),(4.4),(4.5) and (4.6) showed a highly miRNA-143 expression and significantly correlates with the survival of AML patients and is associated with good

prognostic factors , also these data show high miRNA-143 expression as a favorable prognostic factor in AML and substantiate a general role for miR-143 in prognosis, which is supported by data in solid cancers from Krakowsky *et. al*., (2018).

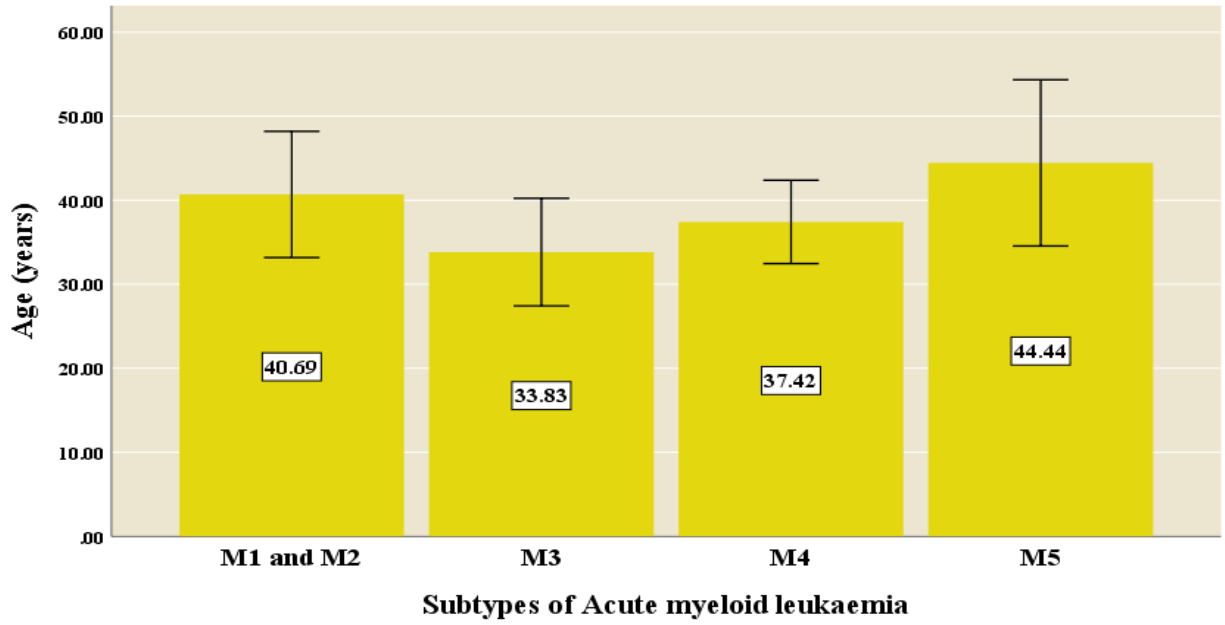
4.5: Association of acute myeloid leukemia subtypes with blood parameters

4.5.1: Distribution of Acute myeloid leukemia subtypes according to the age .

The data in table (4.6) and figure (4.10) shows the mean differences of age (years) according to subtypes of AML , including M1 , M2, M3, M4 and M5. The results revealed no significant differences between means of age according to the subtypes of AML .

Table(4.6): The mean differences of age according to the subtypes of AML (N=57) .

Study variable	Subtypes of AML	N	Mean ± SE	P-value
Age (years)	M1 and M2	13	40.69 ± 3.76	0.214
	M3	23	33.83 ± 3.20	
	M4	12	37.42 ± 2.48	
	M5	9	44.44 ± 4.95	



Figure(4.10):The mean differences of age (years)according to subtypes of AML (N=57) .

In the current study it was found an increase number of patients in M3 subtype and at mean value 33.83 ± 3.20, while the high mean 44.44 ± 4.95 was shown in M5 subtype with low number of patients (10) , in addition, the highest age of patients with AML was at 20-40 years , so that these correlation between age > 30 years and AML subtypes or abnormal mutation and other parameters such as cytogenetics, could be depending on it as prognostic parameter for diagnosis (Haferlach *et al*.,2003) .The age of AML patients presentation in current results showed increase between 20-40 years especially with the M3 subtype , and these result was consistent with Estey (2014) that showing age and AML subtypes mainly affected in severity and prognostic of disease .

AML is closely related to age, as most researchers has focused on the relationship between age and disease due to the concept of age-related clonal hematopoiesis , Most of the patients were found in ages equal to 30 years due to the increase of receptors in the cells that get the disease and the AML increases with increasing age until the age of 60 years (Jaiswal *et. al*.,2014) , so that these finding was consistent with present results where the highest incidence of patients was

between the age group 20- 40 years. There are clinical differences according to age and gender in patients with AML and it would like to clarify that the focus of the disease among this age periods referred to previously may be because the ages are less than 20 years and they may undergo less diagnostic procedures, such as morphological sub- classification and genetic evaluation , this explanation was consistent with the results of Sorror *et. al*., (2014).

4.5.2. : Distribution of AML subtypes according to the blood picture .

Table (4.7) showed the mean differences of complete blood picture including WBC ($\times 10^3$cell/µl), Lymphocytes (%),RBCs ($\times 10^6$cell/µl), Hb (g/dl), HCT(%) , MCV (FL), MCH (pg), MCHC (g/dl) and PLT ($\times 10^3$cell/µl) according to subtypes of AML (M1 and M2, M3, M4 and M5). The lymphocytes showed that significant differences at value (0.003), while the other parameters don't shows any significant differences.

Table(4.7) : The mean of complete blood picture according to subtypes of AML (N=57) .

Complete blood picture	Subtypes of AML	N	Mean ± SE	P-value
WBC ($\times 10^3$cell/µl)	M1 and M2	13	4.75 ± 1.37	0.268
	M3	23	6.85 ± 0.90	
	M4	12	3.89 ± 1.50	
	M5	9	7.25 ± 2.17	
Lymphocytes (%)	M1 and M2	13	43.96 ± 9.57	**0.003***
	M3	23	22.37 ± 3.97	
	M4	12	56.83 ± 6.93	
	M5	9	44.48 ± 10.14	
RBC ($\times 10^6$cell/µl)	M1 and M2	13	3.15 ± 0.37	0.215
	M3	23	3.06 ± 0.13	
	M4	12	2.53 ± 0.13	
	M5	9	2.86 ± 0.19	

Hb (g/dl)	M1 and M2	13	7.97 ± 0.58	0.062
	M3	23	8.73 ± 0.27	
	M4	12	7.24 ± 0.32	
	M5	9	7.98 ± 0.50	
HCT (%)	M1 and M2	13	25.38 ± 2.17	0.066
	M3	23	26.47 ± 1.06	
	M4	12	21.93 ± 1.06	
	M5	9	22.18 ± 1.28	
MCV (FL)	M1 and M2	13	81.27 ± 1.50	0.513
	M3	23	83.94 ± 2.54	
	M4	12	87.13 ± 1.55	
	M5	9	84.36 ± 3.69	
MCH (pg)	M1 and M2	13	25.88 ± 0.73	0.073
	M3	23	28.39 ± 0.84	
	M4	12	29.00 ± 0.59	
	M5	9	28.37 ± 0.85	
MCHC (g/dl)	M1 and M2	13	31.66 ± 0.36	0.056
	M3	23	33.15 ± 0.44	
	M4	12	33.28 ± 0.44	
	M5	9	32.61 ± 0.33	
PLTs ($\times 10^3$/µl)	M1 and M2	13	80.46 ± 27.89	0.068
	M3	23	126.55 ± 21.11	
	M4	12	59.59 ± 15.70	
	M5	9	52.13 ± 13.59	

(*): means significant(0.003)

The results of current study(Table, 4.7) revealed a significant differences between lymphocytes and AML subtypes at (0.003) , this is may be the lymphocytes plays an important role in tumor induction pathogenesis , while another blood parameters are less related to AML (Sidney *et. al*.,2014). The mean of WBCs count ($7.25 \pm 2.17 \times 10^3$ cell/µl) was strongly correlated with AML subtypes , so the lower

WBCs count was correlated with the lower severity of AML . This results agrees with study of Greenwood *et. al*., (2006) and Oliveira *et. al*., (2010). The higher mean value of Hb and platelets was closely correlated with higher relapse rates of AML subtypes in comparison to the lower mean value of it.

The increase in the incidence of AML subtypes and its relationship to these parameters, especially Hb, platelets, MCV, MCH and MCHC, indicates that this disease causes many blood disorders such as decrease the amount and content of haemoglobin that decrease the oxygen level in the blood , formation of clots inside the capillaries, and a higher normal red cell volume indicates that the red cells are larger than normal (macrocytosis), These pathological changes in the blood parameters can be used in diagnosing the disease, monitoring the progression of the disease, and thus controlling the exacerbation of the disease (Jahic *et. al*.,2017).

In present study, most patients showed highly mean value with M3 subtype in contrast to another AML subtypes , in addition to the mean value of total leukocyte count (TLC), hemoglobin, platelets count, MCV, and MCHC , which is indicates that there was a significant correlation between AML-subtypes expression and these parameters , and this was matching with the results of certain studies(Schuurhuis *et. al*., 2010; Zhu *et. al*., 2013).

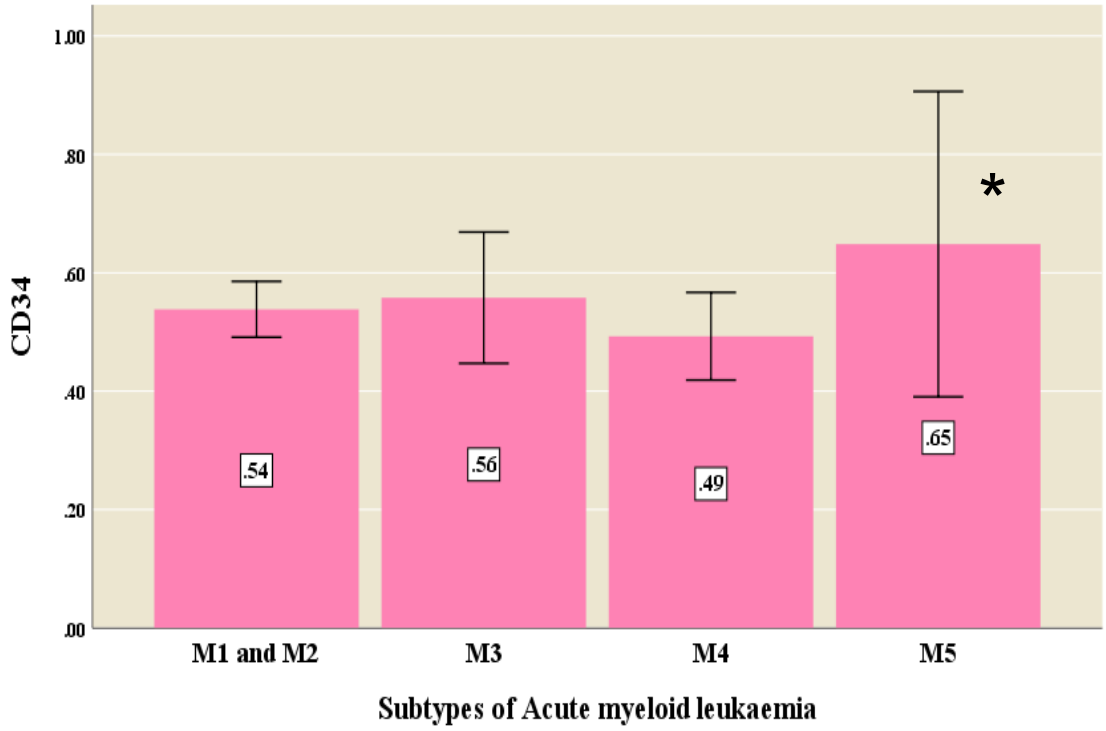
4.5.3:The relationship between AML subtypes and biomarkers (CD34,miRNA).

Table (4.8) , and figures (4.11),(4.12),(4.13) and (4.14) showed that the mean levels of biomarkers including CD34 (pg/µl), miRNA-203 (ng/µl), miRNA-143 (ng/µl) and miRNA-495 (ng/µl) according to AML subtypes (M1 and M2, M3, M4 and M5). There were significant differences between levels of miRNA-203 (ng/µl), miRNA-143 (ng/µl) and miRNA-495 (ng/µl) according to the subtypes of AML.

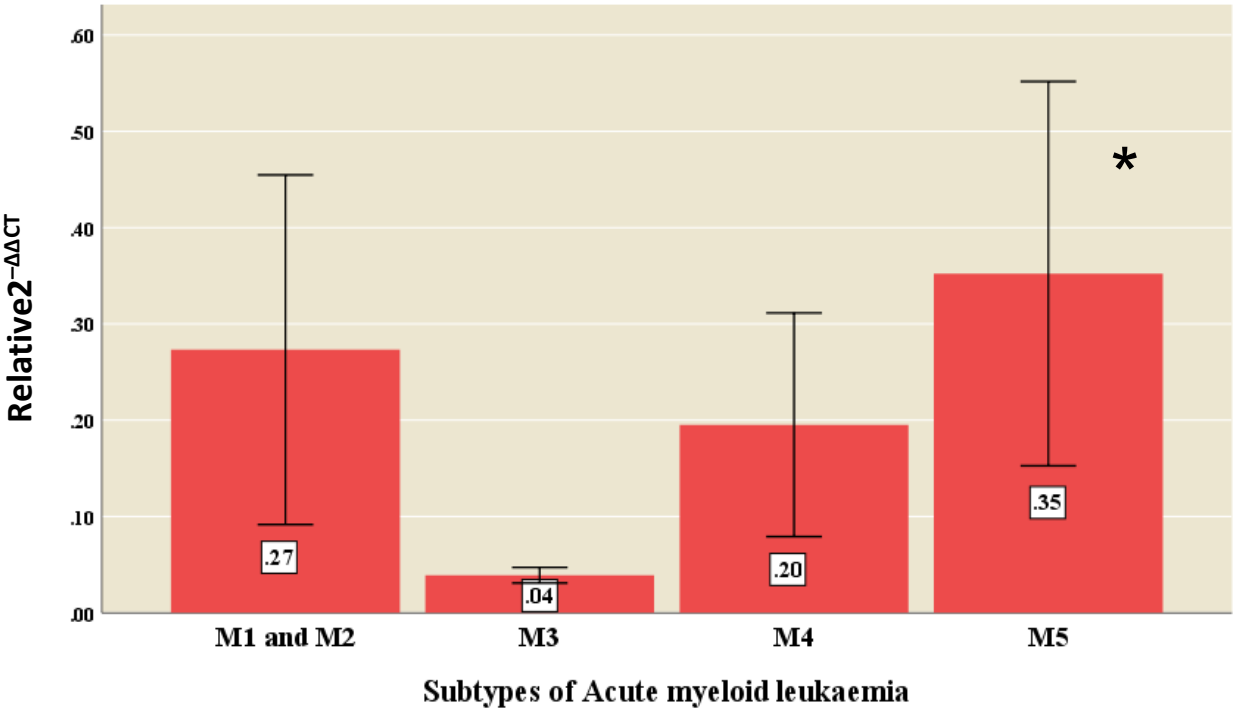
Table (4.8):The mean levels of biomarkers CD34 and miRNAs according to the AML subtypes (N=57) .

Study variables	subtypes of AML	N	Mean ± SE	P-value
CD34 (pg/µl)	M1 and M2	13	0.54 ± 0.02	0.525
	M3	23	0.56 ± 0.06	
	M4	12	0.49 ± 0.04	
	M5	9	0.65 ± 0.13	
miRNA-203 (ng/µl)	M1 and M2	13	0.27 ± 0.09	**0.001***
	M3	23	0.04 ± 0.004	
	M4	12	0.20 ± 0.06	
	M5	9	0.35 ± 0.09	
miRNA-143 (ng/µl)	M1 and M2	13	0.16 ± 0.04	**0.023***
	M3	23	0.08 ± 0.03	
	M4	12	0.24 ± 0.06	
	M5	9	0.22 ± 0.04	
miRNA-495 (ng/µl)	M1 and M2	13	0.07 ± 0.02	**<0.001***
	M3	23	0.01 ± 0.003	
	M4	12	0.04 ± 0.007	
	M5	9	0.08 ± 0.03	

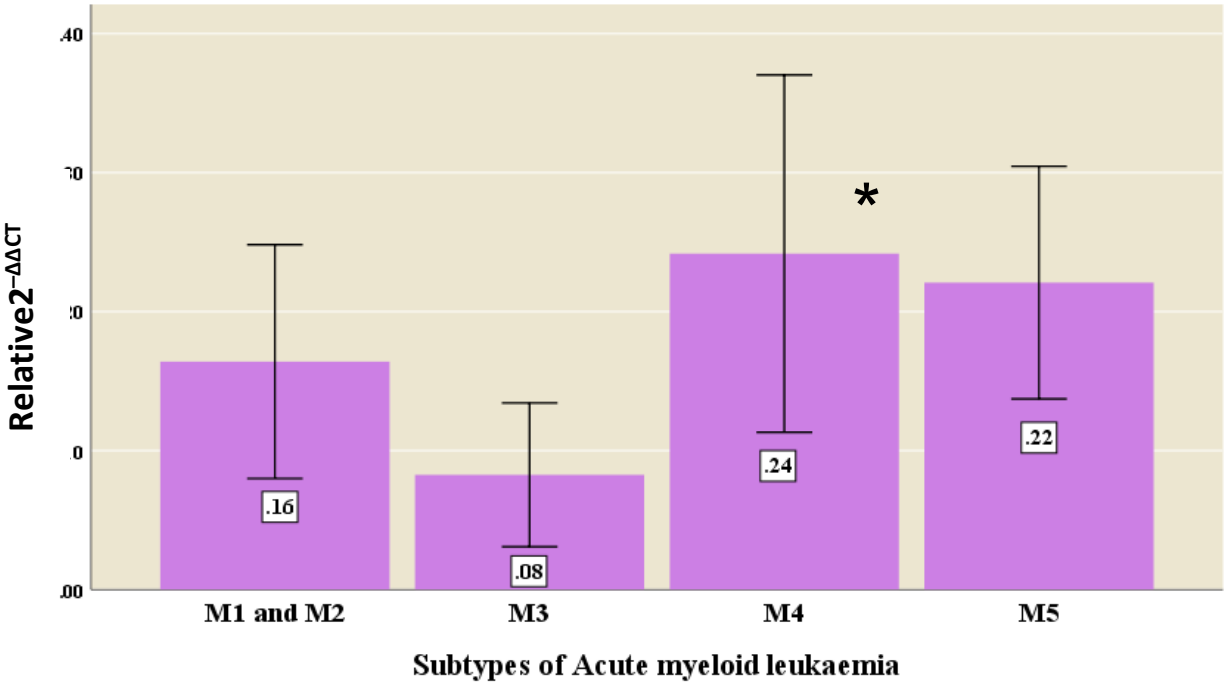
(*): means significant at(< 0.005)



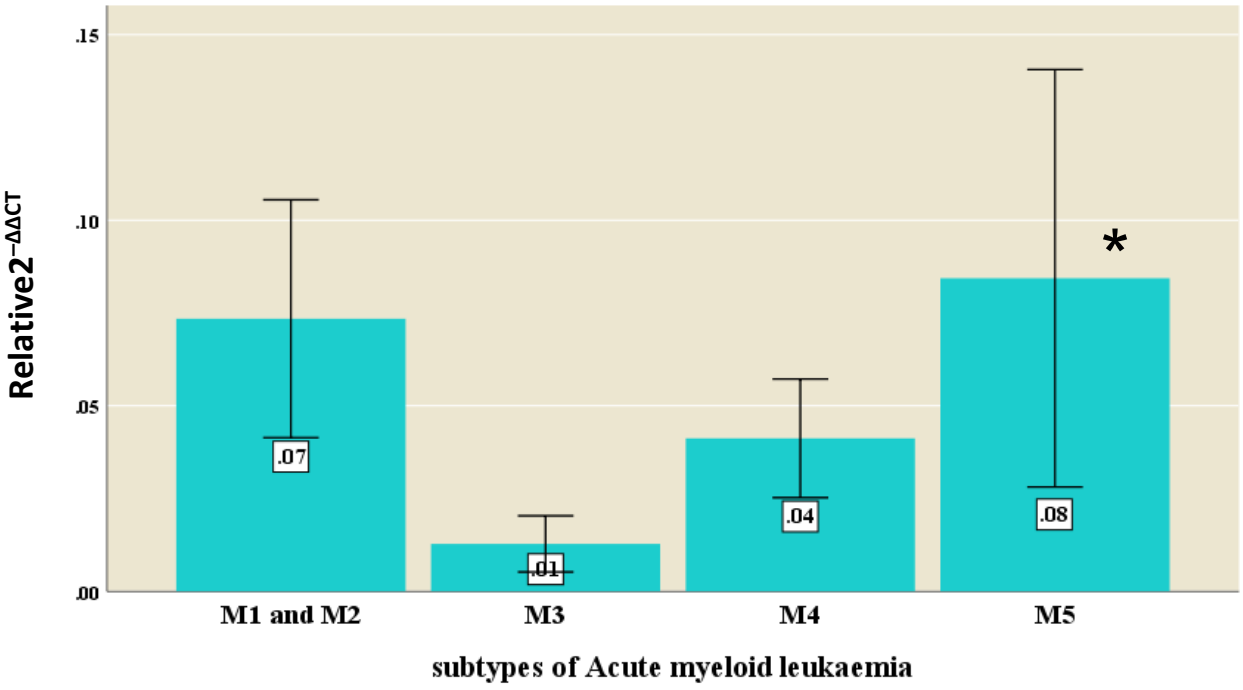
Figure(4.11):The mean levels of CD34(pg/µl) according to subtypes of AML (N=57) (*): means significant.



Figure(4.12):The mean levels of miRNA-203 (ng/µl) according to subtypes of AML. (*): means significant



Figure(4.13): The mean levels of miRNA-143 (ng/µl) according to subtypes of AML(*): means significant .



Figure(4.14):The mean levels of miRNA-495 (ng/µl) according to subtypes of AML. (*): means significant

The differences in the AML subtypes with the biomarkers under study in total sample, does not reflect the subtypes of AML patients in the another region , because the study did not include larger number of patients. The results confirmed that the correlation between AML subtypes and biomarkers (CD34 ,miRNA) as tool for monitoring the severity of disease and shorter overall survival, which is consistent with the results of Appelbaumm *et. al*., (2006) . Different studies reported about the important role of miRNA expression that distinguish between AML and acute lymphoblastic leukemia (ALL) (Wang *et al*.,2010)

In the current study, it was observed that the high levels of miR-495, miR-203, expression , was compatible with Dixon-McIver *et. al*., (2008) and Jongen-Lavrencic *et. al*., (2008) who noticed increased in certain types of miRNA with AML .

The biomarker CD34 is expressed on the surface of immature hematopoietic progenitor cells and is involved in cellular adhesion and mediates resistance to apoptosis. Differential CD34 expression in the blasts of acute leukemia patients has been reported to be a significant prognostic indicator, and the high percentages of CD34 in some types and low in another types of AML have been shown to correlate with good prognosis in AML patients in several studies (Rahul *et al*.,2014) , and these results are consistent with current data that found the expression of CD34 with M3 subtype are more expression from others.

In present study, it was found that all patients were CD34 high ($>$ 50% level) ,these meaning that the important of this biomarker in detection the severity of AML . These results were similar to a study of AML patients, as the value of CD34 was higher in some subtypes of AML . The differences between these subtypes were statistically significant ($P < 0.001$) when analysed by a two-tailed (T) test according to mean ± SE (Oyan *et. al*.,2005).

4.6 :Correlation between biomarkers with study variable parameters among patients with AML.

4.6.1 :The correlation between CD34 and study variables

Table (4.9) showed the correlation between the patients and CD34 according to the miRNA types and complete blood count, where the miRNA types don't shows significant differences , while the blood parameters shows significant differences for W.B.Cs at p-value (≥0.005).Also , the figure (4.15) showed the correlation between the patients and CD34 according to the complete blood count, where the blood parameters revealed a significant differences at R=0.359 and P-value =0.005.

Table (4.9): The correlation between CD34 (%)and study parameters among patients with AML (N=60) .

Study parameters	CD34 (%)		
	N	r	P-value
miRNA-203 (ng/µl)	60	0.027	0.837
miRNA-143 (ng/µl)	60	-0.052	0.692
miRNA-495 (ng/µl)	60	0.112	0.396
WBC ($\times10^3$/µl)	60	**0.359**	**0.005***
Blast (%)	60	0.071	0.59
Neutrophils (%)	60	-0.126	0.336
Lymphocytes (%)	60	0.043	0.746
RBCs ($\times10^6$/µl)	60	-0.028	0.832
Hb (g/dl)	60	-0.105	0.426
HCT (%)	60	-0.171	0.191
MCV (FL)	60	-0.129	0.327
MCH (pg)	60	-0.067	0.611
MCHC (g/dl)	60	0.078	0.552
PLTs ($\times10^3$/µl)	60	-0.066	0.617

(*): means significant at P-value =0.005.

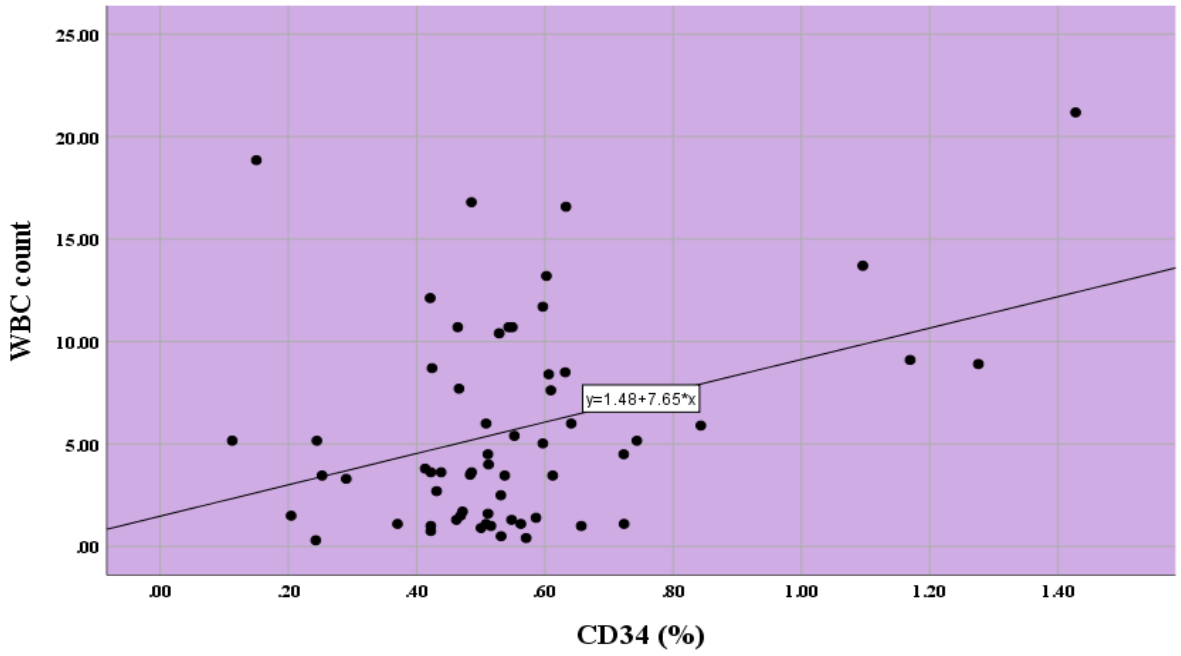


Figure (4.15): The correlation between CD34 (%)and WBC count (×10^3/µl) among patients with AML (N=60, r=0.359, P=0.005*).

Regarding the haematological parameters, CD34 was observed more correlation with WBC count at R = 0.395 and p-value at (0.05), comparable to the study by Muhsin and Al-Mudallal (2018) that showed the CD34 was significantly higher expressed in cases of AML, while the present work did not reveal any significant differences in CD34 expressions with mi-RNA types and blood parameters .

The results of the current study was found that elevated in the CD34 gene expression has variable effects on the blood parameters, as noted in figure (4.15) , in which the W.B.Cs count showed highly distribution on the scale of correlation at p-value ≥0.05 .These findings was consistent with Wang *et. al.*, (2010) who mentioned that the difference in the relationship between CD34 gene expression and haematological parameters . It can be explained by direct interaction between CD34 positive tumor cells and myeloid cells, which may be attributed to elevated expression of CD34 on tumour-associated myeloid cells, which lead to a significant change in the percentage of hematopoietic cells .

The present results showed that CD34 was highly expressed in WBCs, and this is consistent with the results of Iriyama *et. al*., (2013) who found that WBC count was higher in CD56 positive cases , but does not reach the significant level. Moreover, CD34 was significantly observed in cases with low at another parameters like miRNA types and some blood parameters . These results were comparable to that published by Hsiao et. al., (2002) , and this variations in hematological parameters may be explained as CD34 expression is associated with an abnormal overexpression in AML cells which lead to the changing of blood parameters (Gattenloehner *et. al*.,2007;Akagi *et. al*.,2009) .

The relationship between CD34 and miRNA types revealed no significant differences in AML patients, as the p-value for miRNA-203 ,miRNA-143 and miRNA-495 at 0.837, 0.692 and 0.396 respectively , as shown in table(4.9). These results was explain the correlation between CD-34 and miRNA types is little in contrast to blood parameters, and it was consistent with the study of Raspadori *et. al*., (2011).

4.6.2: The correlation between miRNA-203 type and other study variables .

The results in table (4.10) revealed that the correlation between miRNA-203 type and another miRNA types, CD34, and complete blood count, where the miRNA-143 and miRNA-495 types shows significant differences at p value<0.001 and (<0.001) respectively ,while the blood parameters (Hb, HCT and PLTs) shows significant differences for at value 0.007,0.026 and 0.018 respectively ,in which the statistical analysis was makes at p-value (≥0.005). Furthermore , figure (4.16) showed the correlation between the miRNA-203 and miRNA-143 types in patients, where the correlation shows significant differences at R=0.506, P<0.001.

Table(4.10): The correlation between miRNA-203 (ng/µl) and study parameters among patients with AML (N=60).

Study variables	miRNA-203 (ng/µl)		
	N	**r**	**P-value**
CD 34 (%)	60	0.027	0.837
miRNA-143 (ng/µl)	60	**0.506**	**<0.001***
miRNA-495 (ng/µl)	60	**0.461**	**<0.001***
WBC ($\times 10^3$/µl)	60	0.001	0.991
Blast (%)	60	-0.131	0.319
Neutrophil (%)	60	0.246	0.058
Lymphocyte (%)	60	-0.15	0.253
RBC ($\times 10^6$/µl)	60	-0.238	0.067
Hb (g/dl)	60	**-0.342**	**0.007***
HCT (%)	60	**-0.287**	**0.026***
MCV (FL)	60	0.027	0.838
MCH (pg)	60	-0.083	0.526
MCHC (g/dl)	60	-0.113	0.39
PLT ($\times 10^3$/µl)	60	**-0.304**	**0.018***

(*): means significant at p-value (≥0.005)

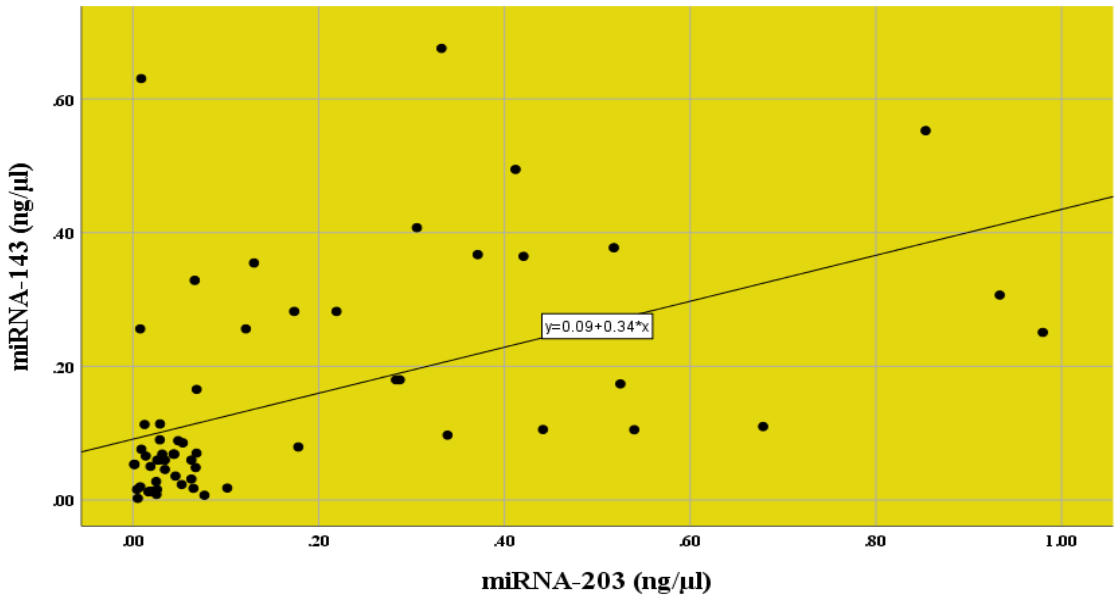
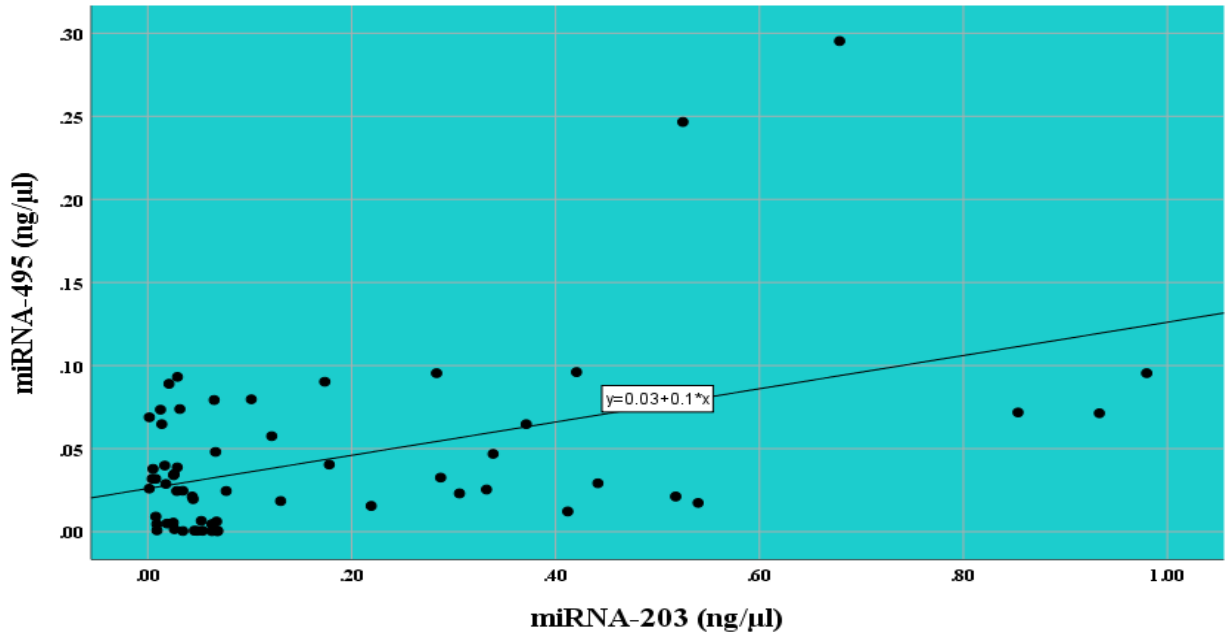


Figure (4.16): The correlation between miRNA-203 (ng/µl) and miRNA-143 (ng/µl) among patients with AML (N=60, r=0.506, P<0.001*)

The results of current study (figure, 4.17) showed the correlation between the miRNA-203 and miRNA-495 types in patients revealed a significant differences at R=0.461 and P<0.001.Also , figure (4.18) showed the correlation between the miRNA-203 and blood parameters for Hb, where the correlation shows significant differences at R=-0.342 and P=0.007.In addition , figure (4.19) showed a significant differences at R=-0.287 and P=0.026 in correlation between the miRNA-203 and blood parameters for HCT (Figure,4.19). As well as, there was a significant differences at R=-0.304 and P=0.018 in the correlation between miRNA-203 and PLTs , as shown in figure (4.20).



Figure(4.17): The correlation between miRNA-203 (ng/µl) and miRNA-495 (ng/µl) among patients with AML (N=60, r=0.461, P<0.001*)

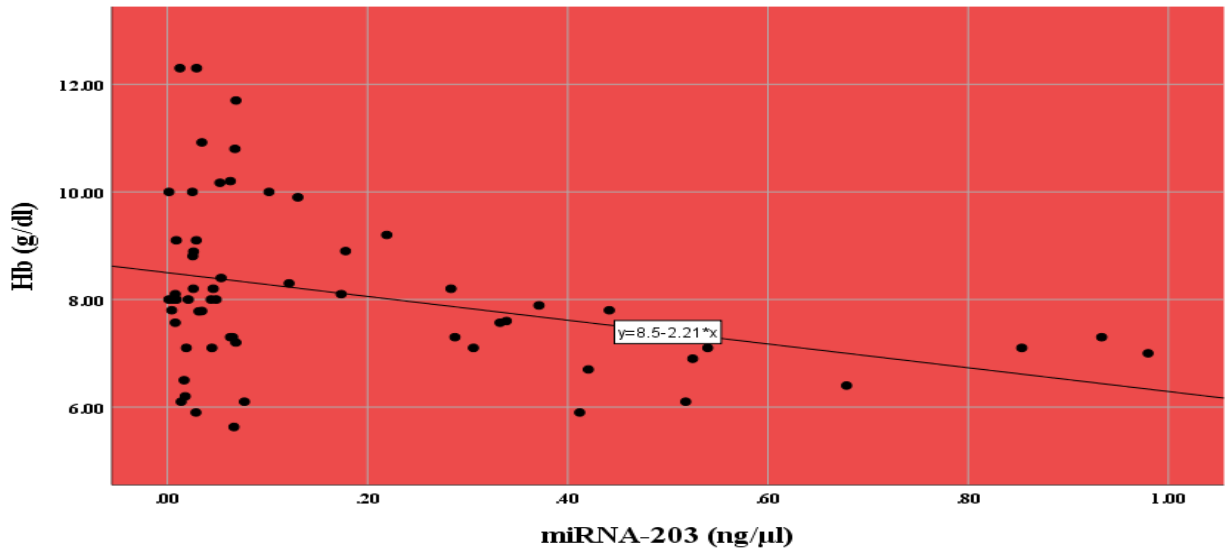


Figure (4.18): The correlation between miRNA-203 (ng/µl) and Hb (g/dl) among patients with AML (N=60, r=-0.342, P=0.007*)

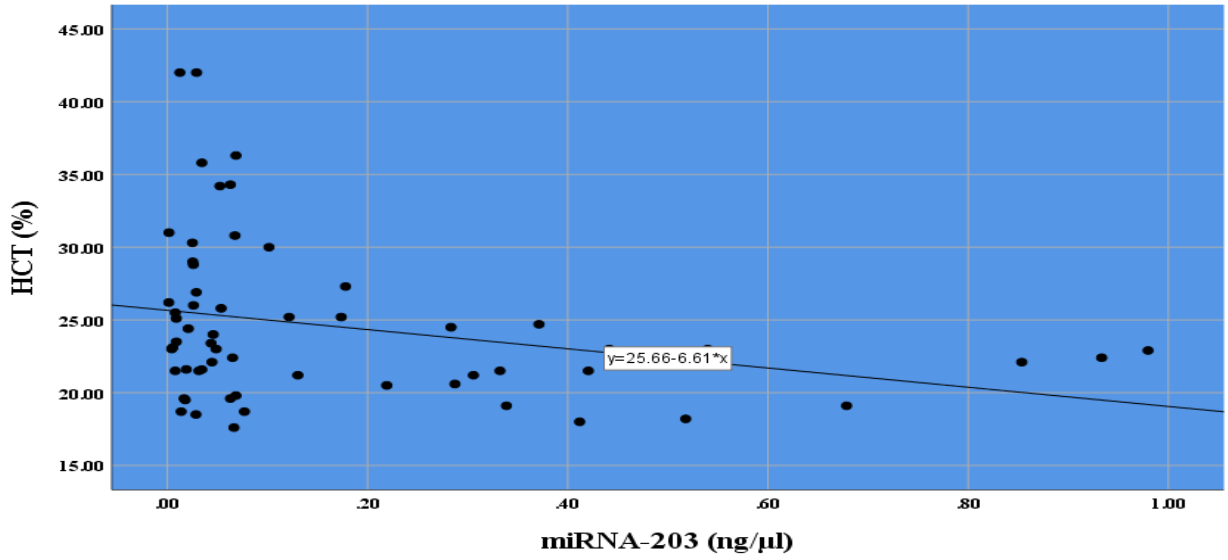
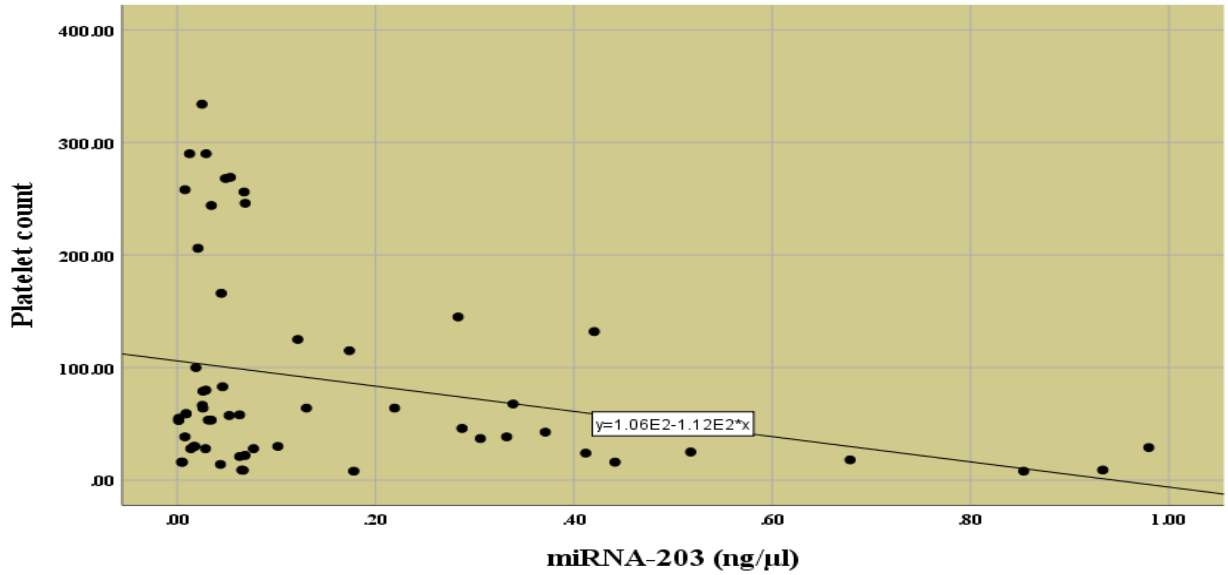


Figure (4.19): The correlation between miRNA-203 (ng/µl) and HCT (%) among patients with AML (N=60, r=-0.287, P=0.026*)



Figure(4.20):The correlation between miRNA-203 (ng/µl) and PLT count (*10^3/µl) among patients with AML (N=60, r=-0.304, P=0.018*).

The confirmatory examination currently used to diagnose cancer is the histological examination of tissues, which is performed by surgical excision, as this examination is expensive and may causes risk to the patients in addition take more time , therefore, there is need to find inexpensive , rapid alternative techniques and it

is not dangerous for the patients to diagnose a disease, including the use of biological fluids such as blood or miRNA types as a source of vital signs (Lu *et. al*.,2005). This confirms the current results that showed the correlation between clinical blood parameters and miRNA types in AML patients , then use them as important and confirmatory parameters to determine the severity of the disease.

The data showed significant differences between the miRNA-203 and miRNA-143 , miRNA-495 at p-value <0.001 and <0.001 respectively . These findings reflect the strong correlation between miRNA types and AML patients because the miRNA have a number of intrinsic characteristics that make them attractive as biomarkers such as highly expression profiles differ between cancer types according to diagnosis and the developmental stage of the tumor, with a greater resolution than traditional gene expression analysis (Lawrie *et. al*.,2007) ,in addition miRNA-203 remarkably stable and therefore can be robustly measured not only in biological fluids but also from routinely prepared formalin-fixed paraffin-embedded (FFPE) (Gupta *et. al*.,2019). Finally, miR-203 is a potential biomarker for diagnosis for many types of cancer like cervical cancer , esophageal squamous cell carcinoma, gastric cancer and colorectal cancer (Ye *et. al*.,2017;Wang *et. al*.,2018; Hasanzadeh *et. al*.,2019).

Table (4.10) showed a significant correlation between miRNA-203 type and hematological parameters, especially Hb, HCT, and PLTs at 0.007, 0.026 and 0.018 respectively in AML patients, especially those with high concentration of miRNA types . In addition, serum miR-203 has been closely associated with cytogenetic and was a potential diagnostic marker from AML (Chu *et. al*.,2016).

The current results found a strong correlation between miRNA-203 type and miRNA-143 , miRNA-495 types in patients , therefore it can be used as prognostic factor for monitoring the morbidity and mortality of disease .These results was compatible with Greither *et. al*., (2010) that demonstrated high miRNA-203

expression was an independent indicator of survival rate in patients with pancreatic ductal adenocarcinomas, indicating that miRNA-203 might be have an important role in carcinogenesis (Miao *et. al*.,2014).

Figures(4.18),(4.19) and (4.20) showed the distribution of blood parameters on scale of correlation especially in the concentration 8-10 (g/dl) for the Hb , (20-30%) for HCT and (≥100) for platelets that was explain by Qiong *e.t al*., (2017) who mentioned the miRNA-203 has major role in gene regulation and expression of some blood parameters in patients with AMLs, so that the miRNA-203 and their regulatory signaling pathways must be placed alongside traditional protein-coding oncogenes and tumor suppressors if need to understanding of the complex mechanisms of malignant AML transformation.

4.6.3: The correlation between miRNA-143 type and study variables .

Table (4.11) showed the correlation between and miRNA-143 type with other parameters of the study in patients ,including CD34 ,complete blood count and miRNA-203, miRNA-495 types, where the miRNA-203 shows significant differences at (<0.001) ,in which the blood parameters don't shows any significant differences with the miRNA-143 type , the statistical analysis was makes at p-value (≥0.005).

Table (4.11): The correlation between miRNA-143 (ng/µl) and study parameters among patients with AML (N=60)

Study parameters	miRNA-143 (ng/µl)		
	N	**r**	**P-value**
CD 34 (%)	50	-0.052	0.692
miRNA-203 (ng/µl)	50	**0.506**	**<0.001***
miRNA-495 (ng/µl)	50	0.064	0.627
WBC ($\times 10^3$/µl)	50	-0.137	0.297
Blast (%)	50	-0.210	0.108
Neutrophil (%)	50	0.310	0.016
Lymphocyte (%)	50	-0.124	0.344
RBC ($\times 10^6$/µl)	50	-0.143	0.275
Hb (g/dl)	50	-0.176	0.179
HCT (%)	50	-0.242	0.062
MCV (FL)	50	-0.067	0.611
MCH (pg)	50	0.028	0.834
MCHC (g/dl)	50	0.155	0.238
PLTs ($\times 10^3$/µl)	50	-0.197	0.132

(*): means significant at p-value (≥0.005).

The important role of miRNA types in the prognostic of AML has rendered them as promising targets in diagnosis and treatment this disease (Schotte *et. al.*,2012; Mosakhani *et. al.*,2013). In the present study, the data showed the correlation between the miR-143 and miRNA-203 and miRNA-495 with the CD34 , and blood parameters which regarded as a positive biomarker for detection the severity and risk of AML disease.

The findings of current results showed the significant correlation between miRNA-143 and miRNA-203 at p-value (<0.001). As miR-143 and miRNA-203 have been previously reported to play important roles in acute myeloid leukemia patients especially at initial diagnosis and at relapse stages (Hartmann *et. al.*,2018), in

addition the high miR-143 expression is associated with a higher probability of survival in leukemia and acute lymphadenopathy patients (Yang *et. al*.,2008).

In AML, the miRNAs types can act as oncogens ,as well as the miRNAs derived from tumor cells, endothelial cells, and cells of the surrounding micro environment help in regulate tumor angiogenesis, acting as pro-angiomiR and causes increased or decreased in the blood parameters (Annese, 2020). This supports the current results that show association between the three RNAs types in acute myelogenous leukemia patients, which was the reason for their use as a diagnostic tool for the disease.

4.6.4 : The correlation between miRNA-495 type and study variables .

The correlation between miRNA-495 type and other parameters of the patients with AML and according to the CD34 ,complete blood count and miRNA-203, miRNA-143 types, where the miRNA-203 shows significant differences at (<0.001) ,while the blood parameters don't shows any significant differences , the statistical analysis was makes at p-value (≥0.005), as shown in table (4.4).

Table (4.12): The correlation between miRNA-495 (ng/µl) and study parameters among patients with AML (N=60)

Study variables	miRNA-495 (ng/µl)(		
	N	**r**	**P-value**
CD 34 (%)	60	0.112	0.396
miRNA-203 (ng/µl)	60	**0.461**	**<0.001***
miRNA-143 (ng/µl)	60	0.064	0.627
WBCs ($\times 10^3$/µl)	60	-0.134	0.306
Blast (%)	60	0.017	0.898
Neutrophils (%)	60	0.089	0.5
Lymphocytes (%)	60	-0.198	0.13
RBCs ($\times 10^6$/µl)	60	-0.057	0.667
Hb (g/dl)	60	-0.171	0.191
HCT (%)	60	-0.072	0.583
MCV (FL)	60	-0.014	0.914
MCH (pg)	60	-0.118	0.371
MCHC (g/dl)	60	-0.193	0.14
PLTs ($\times 10^3$/µl)	60	-0.134	0.306

(*): means significan at p-value (≥0.005)

MiRNAs are now widely regarded as playing a critical role in AML pathogenesis, and specific miRNA expression profiles can help classify subtype, determine prognosis, and predict response to treatment in AML, but the use of miRNAs as biomarkers is not yet routine practice (Jared and Ryan,2017).

The present study analyzed the clinical significance of the miR‑495 expression levels in AML, and these results was consistent with the results reported in previous studies, which revealed the miR‑203 expression in peripheral blood of patients with AML was strongly correlated with the expression on miRNA-143 and miRNA-495 (Jiang *et. al*.,2012;Zhao *et al*.,2018).

The present results showed that there is a very close association between RNA-203 and RNA- 143 at a p-value (<0.001),as shown in table (4.4), where previous studies indicated that there is a very large role for these species in disease control, especially those who suffer from high gene expression miR-495 (Yan *et. al*.,2017) , as well as in other studies it has been reported that miR-495 can affect a variety of biological behaviors of cancer cells such as increasing or preventing the spread cancer cells (Huang *et. al*.,2015).Moreover , Mao *et. al*., (2016) noted that miRNA-495 has an important role in the diagnosis of AML , in addition miRNA-372 and miR-495 are major factors influencing prognosis of cancer patients ,whereas Wang *et. al*., (2015) verified that miR-495 act as prognostic factor for AML. These findings are agreement with current study . Also , this biomarker was affected on many parameters such as age, WBCs count, immature percentage cells in peripheral blood, chromosome typing, and survival of AML patients (Björkholm *et. al*.,2011;Green *et. al*.,2013).

Certain patients with AML suffering from genetic abnormality in chromosome t (8; 21) (q22; q22.1) mutations (Gu *et. al*.,2013) ; therefore, more studies about the correlation between miRNA-143, miRNA-203, and miRNA-495 should be makes for monitoring the morbidity and mortality that correlated with these findings ,in addition, different AML subtypes have different treatment plans, therefore this need to further analyze the clinical significance of miRNA‑372,miRNA-143,miRNA-203 and miR‑495 expression in AML subtypes (Michallet *et. al*.,2012).

Conclusions:

1. There was association between acute myeloid leukemia (AML) and age ,gender and subtypes of AML.
2. Complete blood picture (WBC, Blast , Neutrophil , Lymphocyte, RBCs, Hb, HCT, MCHC, and PLT) can also be used in prognosis of AML patients.
3. CD34 as a prognostic marker might be easily adopted in clinical practice to rapidly identify patients with AML.
4. The serum levels of miR-203, miRNA-143, and miRNA-495 are associated with aggressive clinical features and poor survival in AML patients and might be a promising biomarkers for the prognosis and diagnosis of AML.
5. The serum levels of miR-203, miRNA-143, miRNA-495 , and Lymphocytes (%) depend on the AML subtypes .

Recommendations:

1. Further Study with large samples of AML patients is required to confirm the results of current study .
2. Studying the relationship between chemotherapy and expression of CD34 and miRNA.
3. Studying the relationship between HLA and gene expression of miRNA.
4. miRNA could be new markers for diagnosis and prognosis of AML and as target therapy for future .

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الخلاصة

يعتبر سرطان ابيضاض الدم النخاعيني الحاد هو أكثر سرطانات الدم شيوعا" بين الافراد البالغين ويشكل حوالي 80‰ من كل الحالات ، وهو يتمثل بتوسع نسيلة خلايا الدم النخاعينية الغير ناضجة في الدم المحيطي ونقي العظم الاحمر .

تم في هذه الدراسة تحديد المعايير التالية : العد الكلي لخلايا الدم (CBC)، تقدير مستوى عنقود التمايز بواسطة المقايسة الامتصاصية المناعية للأنزيم المرتبط (ELISA) ، تقدير كمية ونوعية الحمض النووي الرايبوزي الدقيق miRNA ؛ والتقدير الكمي لتعبير الجينات miRNA-203 و miRNA-143 و miRNA-495 بواسطة تفاعل البلمرة المتسلسل الكمي (qPCR) في دم مرضى سرطان ابيضاض الدم النخاعيني الحاد (AML) ومجموعة الافراد الاصحاء.

أجريت هذه الدراسة خلال الفترة بين كانون الأول(2020) و أيلول(2021) في جامعة بابل ، كلية العلوم ، قسم علوم الحياة. تم في هذه الدراسة جمع عينات الدم من 115 مريضًا من مرضى سرطان الدم النخاعيني الحاد بمعدل 38 ذكرًا و 77 أنثى وقد تراوحت أعمارهم بين 18الى 66 عامًا. بعد ذلك . تم اختيار 60 مريضًا بناءً على الكمية واللون الطبيعي لعيناتهم (28 ذكرًا و 32 أنثى) ، بالإضافة إلى ذلك تم جمع 30 عينة من الافراد الأصحاء كمجموعة سيطرة (11 ذكرًا و 19 أنثى) ، وهذه المجموعة كانت متطابقة مع مجموعة المرضى.

لقد بينت الخصائص الديموغرافية لمرضى ابيضاض الدم النخاعي الحاد (AML) ان معدل العمر كان للمرضى هو 37.52 سنة ، والمرضى الأكبر سنًا كان 66 سنة والمرضى الأصغر سنًا كان 18 سنة ، وكانت هناك فروق معنوية طفيفة بين متوسط العمر في المجموعة المدروسة (المرضى ومجموعة الافراد الأصحاء). في حين كانت نسبة المرضى الإناث أعلى (58.3٪) من نسبة المرضى الذكور (41.7٪). كذلك كان غالبية المرضى من النوع الفرعي M3 (N = 23، 38.3 ٪) من إجمالي المرضى ، في حين أن النوع الفرعي M4 يمثل 20 ٪ (N = 12) من إجمالي المرضى ، لكن النوعين الفرعيين M2 و M5 يمثلان 15 ٪ (N = 9) من الإجمالي الكلي للمرضى، بالإضافة إلى ذلك مثل النوعين الفرعيين M1 , M7 6.7٪(N = 4)) و 5٪ (N =

3) على التوالي من إجمالي المرضى ، ولم يكن هناك مرضى مصابون بالأنواع الفرعية الاخرى (M0 و M6).

أظهرت نتائج الدراسة الحالية وجود فروق معنوية ($P<0.05$) بين مجموعتي الافراد الاصحاء ومرضى ابيضاض الدم النخاعي الحاد (AML) في بعض المعايير الدموية ، اذ كان متوسط عدد خلايا الدم البيض WBC في مجموعة الافراد الاصحاء 7.60×10^3خلية / ملم3 ، بينما انخفض العدد في مجموعة المرضى بشكل ملحوظ إلى 5.67×10^3خلية / ملم3 ، كذلك كان متوسط نسبة الخلايا النخاعينية غير الناضجة Blast (٪) في مجموعة المرضى هو 62.63 ، بينما انخفضت نسبة تلك الخلايا في مجموعة الإفراد الاصحاء بشكل كبير إلى 0.0 . ولقد بلغت نسبة الخلايا العدلة neutrophils في مجموعة المرضى 17.35 ، بينما ازدادت تلك النسبة معنويا" في مجموعة الافراد الاصحاء الى 70.14، كذلك بينت الدراسة ان معدل النسبة المئوية للخلايا اللمفية lymphocytes في مجموعة المرضى قد بلغ20.30 ، بينما ازدادت تلك النسبة معنويا" في مجموعة الافراد الاصحاء اذ بلغت 30.19 . لقد بلغ متوسط عدد كريات الدم الحمر في مجموعة المرضى 2.92×10^6 خلية / ملم3، بينما أزداد تعدادها معنويا" في مجموعة السيطرة إلى 4.76×10^6خلية / ملم3 ، بالإضافة الى ذلك بلغ متوسط تركيز الهيموغلوبين Hemoglobin (Hb) في مجموعة المرضى 8.12 (غم / ديسيلتر) ، بينما زاد تركيزه معنويا" في مجموعة الافراد الاصحاء إلى 13.09 (غم / ديسيلتر) . كذلك بينت الدراسة ان النسبة المئوية لمكداس الدم(HCT) Hematocrit (٪) في مجموعة المرضى 24.51 ، بينما ازدادت النسبة معنويا" في مجموعة الافراد الأصحاء إلى 41.01. وبلغ معدل تركيز الهيموغلوبين الكريي Mean corpuscular hemoglobin concentration (MCHC) في مجموعة المرضى 32.81 (غم / ديسيلتر) ، بينما انخفض ذلك التركيز مجموعة الافراد الاصحاء بشكل كبير إلى 32.06 (غم / ديسيلتر) ، علاوة على ذلك كان متوسط عدد الصفيحات الدموية Platelets(Plt) في مجموعة المرضى 86.48×10^3 (خلية /ملم^3) ، بينما ازداد العدد معنويا" في مجموعة الافراد الاصحاء الى 264.67×10^3 (خلية /ملم^3) .

من جهة اخرى بينت نتائج الدراسة عدم وجود فروق معنوية ($P<0.05$) بين مجموعة المرضى ومجموعة الافراد الاصحاء في المعايير الدموية . اذ بلغ معدل حجم الكريات (MCV Mean corpuscular volume (FL في مجموعة المرضى 84.44 ، بينما ازداد ذلك المعدل

في مجموعة الافراد الاصحاء إلى 87.17، كذلك كان معدل الهيموغلوبين الكريي Mean corpuscular hemoglobin (MCH)في مجموعة المرضى قد بلغ 28.09 (بيكوغرام) بينما انخفض `معدل الحجم في مجموعة الافراد الاصحاء إلى 27.85 (بيكوغرام).

أظهرت جميع المؤشرات الحيوية في هذه الدراسة والتي تضمنت عنقود التمايزCD34 (pg /ul) ، miRNA-203 (نانوغرام / ميكرولتر) ، miRNA-143 (نانوغرام / ميكرولتر) و miRNA-495 (نانوغرام / ميكرولتر) في المرضى ومجموعة الافراد الاصحاء وجود اختلافات معنوية في العلاقة مع مرضى ابيضاض الدم النخاعيني الحاد تحت مستوى معنوية (P<0.001). كانت مستويات CD34 المصلية في مجموعة المرضى 0.55 (بيكوغرام / ميكرولتر) ، بينما ازداد التركيزمعنويا" في مجموعة الافراد الاصحاء إلى 0.81 (بيكوغرام / ميكرولتر) ، بينما كانت مستويات الواسم الحيوي miRNA-203 في مجموعة المرضى 0.17 (نانوغرام / ميكرولتر) ، في حين زاد تركيزها معنويا" في مجموعة الافراد الاصحاء اذ بلغ 12.02 (نانوغرام / ميكرولتر) . اضافة الى ذلك كانت مستويات miRNA-143 في مجموعة المرضى 0.15 (نانوغرام / ميكرولتر) ، في حين زاد تركيزها معنويا" في مجموعة الافراد الاصحاء إلى 2.49 (نانوغرام / ميكرولتر) . علاوة على ذلك ، كانت مستويات miRNA-495 في مجموعة المرضى 0.04 (نانوغرام / ميكرولتر) ، في حين زاد تركيزها في مجموعة الافراد الاصحاء إلى 0.87 (نانوغرام / ميكرولتر).

اظهرت نتائج الدراسة الحالية عدم وجود فروقات ذات دلالة إحصائية بين الأنواع الفرعية من مرض ابيضاض الدم النخاعيني الحاد حسب العمر (p=0.214)، في حين كانت هنالك هنالك فروقات ذات دلالة إحصائية بين الأنواع الفرعية من المرض بالنسبة للخلايا الليمفاوية (٪) ، الخلايا النخاعينية غير الناضجة (٪) وكذلك العدلات (٪) . بالإضافة الى ذلك بينت نتائج الدراسة وجود فروقات معنوية طفيفة (P <0.05) بين الأنواع الفرعية من المرض والمعايير الدموية المتمثلة بالعد الكامل لخلايا الدم CBC ، عد خلايا الدم البيض WBC، عد كريات الدم الحمر ، تركيز الهيموغلوبين Hb ، معدل حجم الهيموغلوبين الكريي HCT ، معدل حجم الكريي MCV، معدل الهيموغلوبين الكريي MCH، معدل تركيز الهيموغلوبين الكريي MCHC و عدد الصفيحات الدموية PLT.

بينت نتائج الدراسة الحالية وجود علاقة بين الأنواع الفرعية من المرض والواسمات الحيويةCD34 و miRNAs ، اذ تبين وجود فروقات معنوية طفيفة بين الأنواع الفرعية من المرض مع الواسم CD34 (p=0.525)، في حين وجدت فروقات ذات دلالة إحصائية بين الأنواع الفرعية من المرض مع الاحماض النووية الرايبوزية الدقيقة miRNAs وهي miRNA-203 ، miRNA-143 ، miRNA-49 .

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وزارة التعليم العالي والبحث العلمي

جامعة بابل / كلية العلوم

قسم علوم الحياة

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أطروحة مقدمة

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هند صالح محيميد الحمداني

(بكالوريوس /علوم حياة / احياء مجهرية/2011)

(ماجستير/علوم حياة / حيوان/ 2014)

بإشراف

أ. د. علاء جواد حسن

كلية العلوم/ جامعة بابل

أ. م .د. حسين ناجي عبد الله

كلية الطب/جامعة بابل

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