Introduction

Angiogenesis is the formation of new blood vessels from a preexisting network; with continuing expansion of vascular tree in response to an increase in tissue mass ***(Carmeliet, 2005).*** Vascular endothelial growth factor (VEGF) and its receptors have multiple biologic functions related to angiogenesis; hemopoiesis, tissue repair and inflammatory reactions. Three VEGF receptors (VEGFRs) have been identified: VEGFR-1(FLT -1), VEGFR-2 (KDR /flk-١) and VEGFR-٣(flt-٤) ***(Shibuya, ٢٠٠٦).***

Increased angiogenesis contributes to patho-physiology of solid tumors and other non-malignant diseases (rheumatoid arthritis, diabetes retinopathies, psoriasis, ect) ***(Shibuya, 2008).***

In addition recent studies show that angiogenesis and angiogenic factors play an important role in hematological malignancies ***(Li et al., 2008).***

Neovascularization is required for tumor growth and metastasis ***(Schuch et al., 2002).*** Increased angiogenesis and tumor vascularity have been reported in both solid tumors and hematologic neoplasms ***(Mangi and Newland 2000).*** It has been shown in animal models that the suppression of tumor angiogenesis inhibits tumor growth and metastasis. Several studies of tumor vascularity have indicated that

neovascularization is associated with tumor's clinical behavior and is a significant independent prognostic indicator of survival in prostatic, gastrointestinal, ovarian, and breast carcinomas. Increased vascularity also has been reported in patients with acute lymphocytic leukemia and acute myeloid leukemia (AML). Increased vascularity in neoplastic diseases appears to correlate with increased expression of VEGF and its receptors ***(Hu et al., 2004).***

VEGFR-1 and VEGFR-2 are expressed on vascular endothelial cells as well as on leukemic cells, while VEGFR-3 is expressed mainly on the lymphocytic endothelium. VEGF binds both VEGFR-1 and VEGFR-2 but not VEGFR-3 ***(Verstovsek et al., 2002).***

Recently, a naturally occurring soluble form of vascular endothelial growth factor recrptor-1 has been identified (sVEGFR-1). Soluble form of vascular endothelial growth factor recrptor-1 (sVEGFR-1) retains its high affinity binding to VEGF ***(Karmasheva, 2008).***

Soluble vascular endothelial growth factor recrptor-1 is likely to be a negative regulator of VEGF availability by sequestrating the ligand, and by forming heterodimers with membrane -bound VEGF receptors, or it may prolong the different VEGF activities associated with this protein**.** Based on these findings, sVEGFR-1 may be involved in the

pathophysiology of acute leukemia and its interaction with VEGF may influence the clinical course and prognosis of the disease ***(Wierzbowska et al., 2003).***

Aim of the Work

The aim of this study is to measure the levels of soluble vascular endothelial growth factor receptor- 1 (sVEGFR-1) in patients with acute leukemia and healthy individuals to correlate between its level with known prognostic factors.

Acute Leukemia

Definition:

Acute leukemias are characterized by uncontrolled proliferation of hematopoietic precursor cells, with loss of maturation and differentiation. The malignant cells (blasts or minimally differentiated precursors) take over the bone marrow and suppress normal hematopoiesis .The acute leukemias can be divided into acute lymphoblastic leukemia (ALL) and acute myeloid leukemia (AML). Rare cases are undifferentiated or mixed ***(Lichtman and Liesveld, 2007).***

Epidemiology:

Although the incidence of acute leukemias accounts for less than 3% of all cancers, these diseases constitute the leading cause of death due to cancer in children and persons aged less than 39 years ***(Deschler and Lubbert, 2006).***

AML is the most common type of leukemia in adults, as it accounts for approximately 25% of all leukemias in adult in the Western world ***(Greenlee et al., 2001).*** It continuously shows 2 peaks in occurrence in early childhood and later adulthood, with an incidence of 3. 7 per ١٠٠, ٠٠٠ persons ***(Deschler and Lubbert,).*** It is slightly more common in males. Little difference in incidence is seen between individuals of African or European descent at any

age. A somewhat lower incidence is seen in persons of Asian descent ***(Lichtman and Liesveld, 2007).***

ALL is the most common malignancy diagnosed in patients under the age of 15 years, accounting for nearly one third of all pediatric malignancy and 76% of all leukemias in this age group. Only 20% of adult acute leukemias are ALL ***(Sakamoto et al., 2004)*** with overall incidence 1 to 1. 5 per 100, 000 persons ***(Jabbour et al., ٢٠٠٥).*** It was found that ALL occurs more frequently in whites, and affects males more often than females in all age groups ***(Jemal et al., 2004).***

Risk Factors:

For most types of leukemia, the risk factors and possible causes are not known (figure 1). Leukemia risk factors include:

- Exposure to very high levels of radiation.
- Exposure to certain chemicals such as benzene and formaldehyde.
- Smoking. Smoking cigarettes increases the risk of acute myelogenous leukemia.
- Receiving chemotherapy as alkylating agent.
- Down syndrome and other genetic conditions.
- Viruses - HTLV-1 (human T-lymphotropic virus) and HIV (human immunodeficiency virus).

- Certain blood disorders, such as myelodysplastic syndromes ***(Lichtman and Liesveld, 2007).***

Pathogenesis:

In general, leukemia occurs when some blood cells acquire mutations in their DNA. The mutations cause the cell to grow and divide more rapidly and to continue living when normal cells would die. Over time, these abnormal cells can crowd out healthy blood cells, causing the signs and symptoms of leukemia ***(Le Viseur et al., 2008).***

AML results from a series of somatic mutations in either a hematopoietic multipotential cell or, occasionally, a more differentiated, lineage restricted progenitor cell. Somatic mutation results from a chromosomal translocation in the majority of patients ***(Kelly et al., 2002).*** The translocation results in rearrangement of a critical region of a proto-oncogene. Fusion of portions of two genes usually does not prevent the processes of transcription and translation; thus, the fusion gene encodes a fusion protein that, because of its abnormal structure, disrupts a normal cell pathway and predisposes to a malignant transformation of the cell ***(Lichtman and Liesveld 2007).***

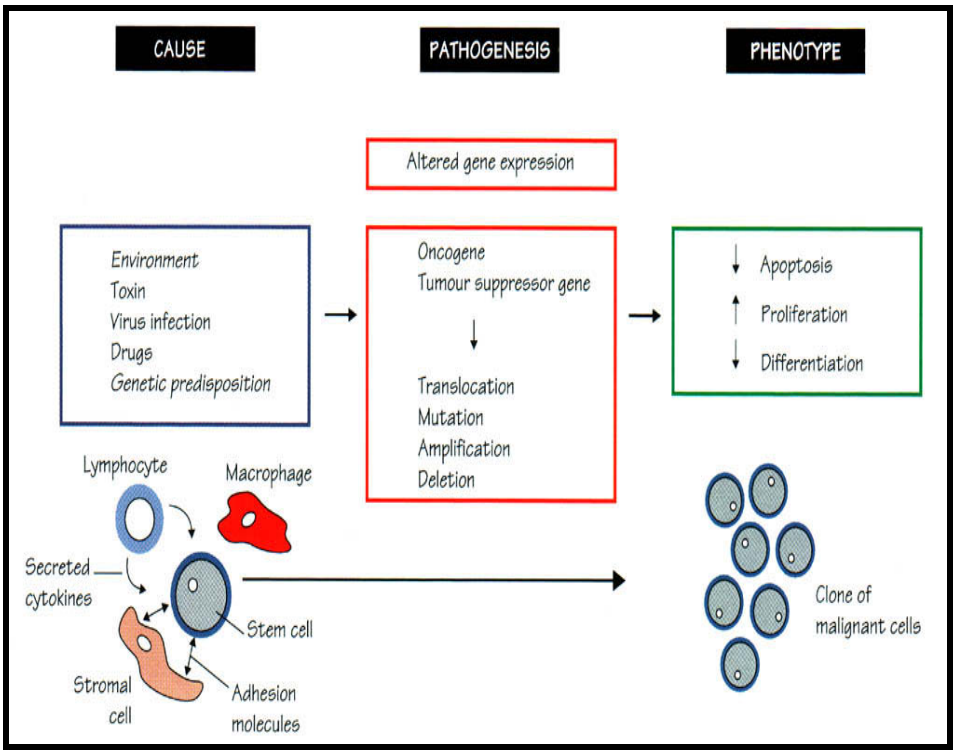

Fig (1): Risk factors and Pathogenesis of haematological malignancies ***(Mehta and Hoffbrand, 2000).***

Different types of AML cells exhibit a proliferative response to various hemopoietic growth factors mediated through specific growth factors receptors which are receptors for (CSF-G) Colony stimulating factor granulocyte, (CSF-M) Colony stimulating factor macrophage, (EPO) erythropoietin, IL3, IL4, IL5, IL6 & IL7. Alterations in growth factor or growth factor receptor functions may play an important role in the pathogenesis of AML ***(Rosenfeld and List, 2001).***

The progression of ALL is driven by successive mutations that alter cellular functions, including an enhanced ability of self-renewal, a subversion of control of normal proliferation, a block in differentiation, and an increased resistance to death signals (apoptosis**)** ***(Gilliland and Tallman, ٢٠٠٢).*** In most cases, the pathophysiology of transformed lymphoid cells reflects the altered expression of genes whose products contribute to the normal development of B cells and T cells. Several studies indicate that leukemic stem cells are present in certain types of acute lymphoblastic leukemia with sensitive cytogenetic analysis, most cases of ALL have cytogenetic aberrations (e. g., translocations, deletions, inversions) ***(Le Viseur et al., 2008)***.

Classifications:

Current classification schemes incorporate morphologic features, immunophenotype, molecular genetics, and clinical data to specifically categorize leukemias into various subtypes ***(Olsen et al., 2008).***

I- French-American-British (FAB) Classification:

The acute leukemias have been traditionally classified by the FAB (French-American-British) classification system. The FAB classification is based largely on morphology and a few cytochemical stains and has limited significance in terms of prediction of prognosis and choice of therapy, FAB classification of AML and ALL are shown in (table1s 1, 2).

Table (1): FAB classification of AML

FAB subtype	*Morphologic Feature*
M0 (AML- Minimally differentiated)	> 30% myeloblasts without azurophilic granules.
M1 (AML- without maturation)	>30% myeloblasts, few if any azurophilic granules; < 10 show maturation beyond blast stage. Auer rods may be present.
M2 (AML-with maturation)	>30% myeloblasts without azurophilic granules; promyelocytes or mature cells > 10%, monocytic cells < 20% Auer rods may be present.
M3 (Acute promyelocytic leukemia)	>30% hypergraular myeloblasts and promyelocytes with prominent granules and multiple Auer rods per cell.
M4 (Acute myelomonocytic leukemia)	Myeloblasts, monoblasts, and promyelocytes > 30% marrow cells monocytic cells > 20% M4 with eosinophilia (M4Eo) is a subtype characterized by abnormal eosinophils with specific eosinophilic granules and large basophilic granules.
M5a (Acute monoblastic leukemia without differentiation)	> 80% monocytic cells are large monoblasts with lacy nuclear chromatin and abundant cytoplasm.
M5b (Acute monoblastic leukemia with differentiation)	> 80% monocytic cells with monoblasts, promonocytes, monocyte, predominant blood monocytosis.
M6 (Acute erythroleukemia	Megaloblastic erythroid precursors (>50%), myeloblasts (> 30%).
M7(Megakaryobla-stic leukemia)	Megakaryoblasts "lymphoid" morphology (L1, L2, M1), cytoplasmic budding.

(Kinney and Lukens, 2001).

Table (2): FAB classification of ALL

	L1	*L2*	*L3*

Cell size	Small	Large, heterogeneous	Large, homogeneous
Amount of cytoplasm	Scanty	moderately abundant	Abundant
Nucleoli	Indistinct or not visible	Prominent	Present, may be prominent
Cytoplasmic vacuoles	Variable	Variable	Prominent

(Mckenna, 2000).

Each subtype of ALL is then further classified by determining the surface markers of the abnormal lymphocytes, called immunophenotyping. There are 2 main immunologic types: pre-B cell and pre-T cell. The mature B-cell ALL (L3) is now classified as Burkitt's lymphoma/leukemia. Subtyping helps determine the prognosis and most appropriate treatment in treating ALL ***(Pui, 2005).***

II- WHO classification of acute leukemias:

The society of Hematopathology and the European Association of Hematopathologists jointly developed a classification of hematologic neoplasms for the World Health Organization (WHO) ***(Jaffe et al., 2001).*** In the WHO classification the blast threshold for the diagnosis of AML is reduced from 30% to 20% BM blasts (i. e. most patients

previously diagnosed as Refractory anemia with excess blast in transformation (RAEB-t) will be classified as AML with multilineage dysplasia) and patients with clonal recurring abnormalities t(8;21) (q22;q22), inv(16) (q13q22), t(16;16) (p13;q22) or t(15;17) (q22;q12) should be considered to have AML regardless of the blast percentage ***(Provan et al., 2004).***

The 2001 World Health Organization (WHO) classification incorporated cytogenetic and molecular genetic findings and introduced important prognostic correlations (table 3) ***(Vardiman et al., 2002).***

The 2008 WHO classification expanded the number of entities with recurrent chromosomal translocations and included two provisional entities characterized by gene mutations, AML with cytoplasmic/mutated NPM1 and AML with CEBPA mutations ***(McKenney and Arber, 2009).***

Table (3): The 2001 WHO classification of acute myeloid leukemia

- *Acute myeloid leukemia with recurrent genetic abnormalities*
  - Acute myeloid leukemia with t(8;21)(q22;q22), (*AML1/ETO*)
  - Acute myeloid leukemia with abnormal bone marrow eosinophils and inv(16)(p13q22) or t(16;16)(p13;q22), (*CBFβ/MYH11*)
  - Acute promyelocytic leukemia with t(15;17)(q22;q12), (*PML/RARα*) and variants
  - Acute myeloid leukemia with 11q23 (*MLL*) abnormalities
- *Acute myeloid leukemia with multilineage dysplasia*
  - Following MDS or MDS/MPD
  - Without antecedent MDS or MDS/MPD, but with dysplasia in at least 50% of cells in 2 or more myeloid lineages
- *Acute myeloid leukemia and myelodysplastic syndromes, therapy related*
  - Alkylating agent/radiation–related type
  - Topoisomerase II inhibitor–related type (some may be lymphoid)
  - Others
- *Acute myeloid leukemia, not otherwise categorized*
  - Acute myeloid leukemia, minimally differentiated
  - Acute myeloid leukemia without maturation
  - Acute myeloid leukemia with maturation
  - Acute myelomonocytic leukemia
  - Acute monoblastic/acute monocytic leukemia
  - Acute erythroid leukemia (erythroid/myeloid and pure erythroleukemia)
  - Acute megakaryoblastic leukemia
  - Acute basophilic leukemia
  - Acute panmyelosis with myelofibrosis
  - Myeloid sarcoma

(Vardiman et al., 2002).

The 2008 WHO Classification of AML:

- ***AML with recurrent genetic abnormalities***
  - AML with t(8;21) (q22;q22); (RUNX1-RUNX1T1)
  - AML with inv(16)(p13. 1q22) or t(16;16) (p13. 1;q22)
  - Acute promyelocytic leukemia (APL) with t(15;17)(q22;q12); (PML-RARA)
  - AML with t(9;11)(p22;q23); (MLLT3-MLL)
  - AML with t(6;9)(p23;q34); (DEK-NUP214)
  - AML with inv(3)(q21q26. 2) or t(3;3)(q21;q26. 2); (RPN1-EVI1)
  - AML (megakaryoblastic) with t(1;22)(p13;q13); (RBM15-MKL1)
  - Provisional entity: AML with mutated NPM1
  - Provisional entity: AML with mutated CEBPA
- ***AML with MDS-related changes***
  - AML following a myelodysplastic syndrome
  - AML with multilineage dysplasia
  - AML with MDS-related cytogenetic abnormalities
- ***Therapy-related myeloid neoplasms***
- ***AML- not otherwise categorized***
  - AML with minimal differentiation
  - AML without maturation
  - AML with maturation

- Acute myelomonocytic leukemia
- Acute monoblastic/monocytic leukemia
- Acute erythroid leukemias
  - Pure erythroid leukemia
  - Erythroleukemia, erythroid/myeloid
- Acute megakaryoblastic leukemia
- Acute basophilic leukemia
- Acute panmyelosis with myelofibrosis

- ***Myeloid sarcoma***
- ***Myeloid proliferations related to Down syndrome***
  - Transient abnormal myelopoiesis
  - AML associated with Down syndrome

(McKenney and Arber, 2009).

WHO Classification of ALL:

١- Acute lymphoblastic leukemia

i. Precursor B acute lymphoblastic leukemia. Cytogenetic subtypes:
   - t(12;21)(p12, q22) TEL/AML-1
   - t(1;19) (q23;p13) PBX/E2A
   - t(9;22)(q34;q11) ABL/BCR