SERUM SELENIUM LEVEL, LIPID PEROXIDATION AND GLUTATHIONE PEROXIDASE ACTIVITIES IN PATIENTS WITH ACUTE LYMPHOBLASTIC LEUKEMIAS PRE-AND POST-INDUCTION CHEMOTHERAPY

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LIST OF ABBREVAIATION

ADP	**:**	Adenosine Di Phosphate
AIDS	**:**	Acquired Immunodeficiency syndrome
ALL	**:**	Acute Lymphoblastic Leukemia
AML	**:**	Acute Myeloid Leukemia
ATP	**:**	Adenosine Tri Phosphate
BM	**:**	Bone Marrow
CAT	**:**	Catalase
CD	**:**	Cluster of differentiation
COX	**:**	Cycloxygenase
CSF	**:**	Cerebrospinal Fluid
Cyt Ig	**:**	Cytoplasmic immunoglobulin
DTNB	**:**	5,5/-dithiobis-(2-nitrobenzoic acid)
DV	**:**	Daily Value
EDTA	**:**	Ethylene diamine tetra acetic acid
eNO	**:**	Endothelial nitric oxide
FAB	**:**	French -American - British
FAD	**:**	Flavin Adenine Dinucleotide
FDA	**:**	Food and Drug Administration
FR	**:**	Free Radical
GPX	**:**	Glutathione Peroxidase
GSH	**:**	Glutathione
GSHr	**:**	Glutathione reductase
GSSG	**:**	Glutathione disulfide
GST	**:**	Glutathione-S-transferase
H_2O_2	**:**	Hydrogen peroxide
H_2Se	**:**	Hydrogen selenide
HIV	**:**	Human Immunodeficiency Virus
HLA-DR	**:**	Histocompatibility antigens
HOCl	**:**	Hypochlorous acid
iNOS	**:**	Inducible nitric oxide synthase
kDa	**:**	Kilo Dalton

L1-L3	**:**	Categories of acute Lymphoblastic leukemia
$LO^{\cdot}$	**:**	Lipid alkoxyl radical
$LOO^{\cdot}$	**:**	Lipid peroxyl radical
LOX	**:**	Lipoxygenase
M0-M7	**:**	Categories of acute myeloid leukemia
MAbs	**:**	Mono Clonal Antibodies
MDA	**:**	Malondialdehyde
MIC classification	**:**	Morphology, Immunophenotyping and Cytogenetic classification
MPO	**:**	Myeloperoxidase
NADPH	**:**	Reduced NADP
NFκB	**:**	Nuclear Factor-kappa B
NK	**:**	Natural Killer cells
nNO	**:**	Neural tissue nitrogen oxide
Non Se-GPX	**:**	Se-independent Glutathione peroxidase
NOS	**:**	Nitrogen oxide synthase
NO_x	**:**	Oxides of nitrogen
NSE	**:**	Non-specific esterases
$ONOO^{\cdot}$	**:**	Peroxynitrite
OS	**:**	Oxidative Stress
8-OHdG	**:**	8-Hydroxy deoxyguanosine
PAS	**:**	Periodic Acid Schiff
PB	**:**	Peripheral Blood
PBS	**:**	Phosphate Buffer Saline
PUFA	**:**	Poly Unsaturated Fatty Acids
RDA	**:**	Recommended Dietary Allowance
RNS	**:**	Reactive Nitrogen Species
ROOH	**:**	Organic hydroperoxide
ROS	**:**	Reactive Oxygen Species
SBB	**:**	Sudan black B
SDS	**:**	Sodium Dodecyl Sulphate
Se	**:**	Selenium
Se IW	**:**	Selenoprotein W
Sec	**:**	Selenocysteine

Se-GPX	**:**	Se-dependent Glutathione peroxidase
Non Se-GPX	**:**	Se-independent Glutathione peroxidase
SeP	**:**	Selenoprotein P
SmIg	**:**	Surface membrane immunoglobulin
SOD	**:**	Super Oxide Dismutase
SPPS	**:**	Statistical Package for Social Sciences
TBA	**:**	Thiobarbituric Acid
TCA	**:**	Trichloroacetic acid
TdT	**:**	Terminal Deoxynucleotidly transferase
tGPX	**:**	Total Glutathione Peroxidase Activity
TMP	**:**	Tetramethoxypropane
TNF-α	**:**	Tumor Necrosis Factor-α
tRNA	**:**	Transfer RNA

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INTRODUCTION

I-LEUKEMIA

Acute leukemias are a heterogeneous group of neoplasms affecting uncommitted or partially committed hematopoietic stem cell [1]. They could be defined as malignant proliferation of hematopoietic progenitor cells involving primarily the peripheral blood (PB) and the bone marrow (BM) leading to increased numbers of blast cells at the expense of normal elements[2].They have been recognized as a genetic disease, resulting from a series of acquired or inherited mutations in the structure of certain genes[3]. These mutations are passed from the original transformed progenitor cells to its clonal descendants. Most of the genetic aberrations fall into genetic classes of functional dysregulation that subvert normal hematopoietic developmental programs by circumvention of cell cycle controls, inhibition of differentiation and resistance to therapeutic apoptosis in leukemic blasts [4].

The disease can be originated from lymphoid cells of different lineage, giving rise to B- or T- cell leukemias or sometimes mixed-lineage leukemia. It is a curable disease with an expected long term survival rate of at least 70%, when treated with modern therapeutic regimen. In general, ALL standard treatment protocols consist of induction and maintenance remission with chemotherapeutic drugs [5].

The diagnosis of acute leukemia usually follows the presentation of a patient with clinical features suggestive of this disease, with relevant laboratory evaluation being performed. In some patients, diagnosis of acute leukemia can be made from a blood count and blood film but in others bone marrow aspiration is essential for diagnosis, for further classification and to provide optimal material for cytogenetic analysis. Cases with hypocellualr or fibrotic BM, only trephine biopsy permits a definitive diagnosis [6].

Classification

A uniform classification system for acute leukemias was developed by an international group of investigators in 1976 known as French - American -British (FAB) classification, this system is based on the morphologic appearance of bone marrow and blood leukemic blasts in Romanovsky -stained smears, supplemented when necessary with cytochemical stains [7]. Selective cytochemical stains improve the accuracy and reproducibility of lineage assessment and acute myeloid leukemia (AML) subclassification. Myeloperoxidase (MPO), Sudan black B (SBB) and Non-specific esterases (NSE) are useful in this regard [8,9].

Table (1): A summary of FAB classification of acute leukemia [4]

Categories of AML	Categories of ALL
M0, AML with minimal evidence of myeloid differentiation	L1, ALL with fairly small uniform lymphoblasts
M1, AML without differentiation	L2, ALL with more pleomorphic lymphoblasts
M2, AML with differentiation	L3, ALL with basophilic vacuolated lymphoblasts
M3, AML and M3 variant, acute promyelocytic leukemia and its hypogranular/microgranular variant	
M4, AML acute myelomonocytic leukemia	
M5, acute monoblastic (M5a) or monocytic (M5b) leukemia	
M6, acute leukemia with at least 50% erythroblasts in the bone marrow	
M7, acute megakaryoblastic leukemia	

Cytochemistry:

- The MPO reaction: is positive in cells of granulocytic series and weakly positive in monocytes, while lymphocytes and erythriod precursors are negative.
- SBB stains: the pattern of staining closely parallels MPO, but SBB positivity is usually stronger than MPO.
- NSE activity: is found in monocytes.
- A periodic acid schiff (PAS): ALL lymphoblasts often have prominent PAS staining in the form of coarse granules or blocks. Myeloblasts may be positive or negative. The pattern of staining is faint and diffuse.
- Acid phosphatase: is not useful in diagnosis of acute leukemia [10].

The definition of acute leukemia and the distinction between AML and ALL according to the FAB system are based on 2 criteria:

1- Percentage of blasts in the bone marrow is > 30% of all nucleated cells.
2- Three percentage or more (≥ 3%) of blasts show a positive reaction for MPO or SBB in the bone marrow [11].

When facilities are limited, acute leukemia can be classified on the basis of cytology and cytochemistry, as initially proposed by the FAB group. This means that cases of AML with minimal evidence of myeloid differentiation could be misclassified as ALL. If cytology and cytochemistry are supplemented by immunophenotyping in all cases that are not obviously myeloid, the diagnosis will be further refined. Also cases of acute biphenotypic leukemia will remain unrecognized without extensive use of immunophenotyping [12].

The optimal approach to the classification of acute leukemia is based on a combination of morphology, immunophenotyping and cytogenetic analysis (MIC classification), or with the addition of molecular genetic analysis (MIC M classification). This approach has been adopted by the World Health Organization (WHO classification) [13]. Thus WHO classification incorporates and interrelates morphology, cytogenetic, molecular genetics and immunologic markers in an attempt to construct a classification that is universally applicable and prognostically valid [14].

The definition of acute leukemia according to WHO classification include lowering of the threshold for the percentage of blast cells to 20% in the blood or bone marrow [12].

Immunophenotyping

The impact of immunophenotyping by flow cytometry in the diagnosis and management of acute leukemia has expanded rapidly. This advance can be attributed mainly to the production of several hundred monoclonal antibodies (MAbs) labeled with flurochromes to a variety of antigens expressed by hematopoietic cells [15].

The panel used includes at least one highly sensitive marker (CD19 for B-lineage cells, CD7 for T- lineage cells, and CD 13 or CD33 for myeloid cells) and one marker that are highly specific cytoplasmic CD79a for B-lineage cells, cytoplasmic CD3 for T-lineage cells, and cytoplasmic myeloperoxidase for myeloid cells [16].

Cytogenetic study:

Genetic analysis is a diagnostic tool in acute leukemia. The results contribute to the confirmation of the diagnosis, but, more important, the karyotype of the leukemic blasts gives important information concerning the prognosis of the disease [17].

Molecular genetic analysis has an advantage over conventional cytogenetic analysis that it can yield results when cytogenetic analysis has failed or when only normal metaphases are seen [18].

Acute Lymphoblastic Leukemia (ALL)

ALL is a malignant disorder that originates in a single B- or T-lymphocyte progenitor. The proliferation and accumulation of blast cells in the marrow results in suppression of hematopoiesis and, therefore, anemia, thrombocytopenia and neutropenia. Extramedullary accumulation of lymphoblasts may occur in various sites especially the meninges, gonads, liver, spleen or lymph nodes [19].

ALL is the most common malignancy diagnosed in patients under the age of 15 years accounting one quarter of all cases, 76% of all leukemias are in this age group [20].

Clinical Features:

Clinical presentation is variable reflecting marrow failure and extramedullary spread.

About one half of patients present with fever which occurs either from cytokines released from leukemic cells or from infection regardless of its origin.

Fatigue and lethargy are frequent manifestations of anemia. More than one quarter of patients may present with limp, bone pain, arthralgia or refusal to walk owing to leukemic infiltration of the periosteum, bone or joint or expansion of marrow cavity by leukemic cells [21-23].

Laboratory Features:

Anemia, neutropenia and thrombocytopenia are common findings in patients with newly diagnosed ALL, and their severity reflects the degree of marrow replacement by leukemic lymphoblasts.

Hyperleucocytosis (>100 × 10^9 / L) occur in 10-16 % of the patients [21].

Serum lactate dehydrogenase and serum uric acid are usually elevated, serum immunoglobulin levels are markedly decreased reflecting decreased number and impaired function of normal lymphocytes [24].

CSF (Cerebrospinal Fluid) examination is an essential diagnostic procedure since leukemia blast cells may be identified in CSF at diagnosis[25].

FAB classification of ALL:

Three subtypes of ALL are distinguished on the basis of cell size, nuclear shape, prominence of nucleoli and relative amount of cytoplasm.

L1 lymphoblasts → are small with high nucleocytoplasmic ratio.
L2 lymphoblasts → larger blasts with moderatory abundant cytoplasm.
L3 lymphoblasts → the largest with abundant intensely basophilic cytoplasm [25]

Table (2): A summary of FAB classification of acute lymphoblastic leukemia [10]

	L1	L2	L3
Cell Size	Small	Large	Large
Nuclear chromatin	Fine or clumped	Fine	Fine
Nuclear shape	Regular, may have a cleft or indentation	Irregular, may have cleft or indentation	Regular, oval to round
Nucleoli	Indistinct or not visible	One or more per cell large prominent	Once or more cell large prominent
Amount of cytoplasm	Scanty	Moderately abundant	Moderately abundant
Cytoplasmic basophilia	Slight	Slight	Prominent
Cytoplasmic vacuoles	Variable	Variable	Prominent

Immunologic classification of ALL:

Monoclonal antibodies allowed the subclassification of ALL into T and B lineage and each of these can be further subdivided according to the expansion of certain maturation antigen molecules [26].

Table (3): Immunophenotyping of B-ALL subtypes [27].

ALL-subtype	Tdt	HLA-DR	CD19	CD10	Cyt Ig	SmIg
Pro B or early B	+	+	+	–	–	–
Common ALL	+	+	+	+	–	–
Pre-B ALL	+	+	+	+	+	–
B-ALL	–	+	+	-/ +	–	+

Table (4): Immunophenotyping of T-ALL subtypes [27].

ALL_ subtypes	Tdt	HLA_DR	CD2	CD5	CD7	CD4	CD8	CD3
Pre-T ALL	+	+	–	–	+	–	–	–
Early T- ALL	+	–	+	+	+	–	–	–
Cortical T-ALL	+	–	+	+	+	+	+	–
Late T-ALL	+	–	+	+	+	+	+	+