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Original article

Evaluation of the Immunological and Hematological Parameters in Acute Leukemia Patients: A Multi-center Study in Libya

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Abstract

Acute leukemia (AL) is a cancer of the leukocytes. Classification of AL used blast morphology and cytochemical stains to categorize the diseases broadly into (ALL) and (AML). ALL is the most common type of leukemia in young children. This disease also affects adults, especially over the age of 65 years. The AML is the most common subtype of leukemia in adults and accounts for 15-20% of childhood leukemia. This study aimed to evaluate the changes in inflammatory markers and immune components during treatment of different acute leukemia subtypes in Libya. Furthermore, the effects on the hemostasis were also observed. The study was carried out on 105 acute leukemia Libyan patients their ages ranged from (2-88) years who were referred to the hematology clinic of different hospitals in Libya for the diagnosis and treatment. The mean age of AML patients was 31 years .49% of patients were males and 51% were females. 40% of patients were in M3 subtype. A+ was the most detected blood group (34%). There was a significant impact of blood group on basophils count (p=0.000), early diagnosis date on the monocytes count (p=0.03), and age on the MCHC (p=0.005). Also, there was a significant relationship between disease stages and PDW, ESR, and IG% (p=0.03, 0.012, and 0.035 respectively). CD33, CD14, and CD13 were the most detected receptors. In All group, the mean age was 15 years.59% were males and 41% were females. O+ was the most detected blood group (38%).53% of patients were B-All and 47% were T-All. There was a significant impact of date of diagnosis on the lymphocytes count (p=0.007), Blood group on the MCV (p=0.038). CD3, CD2, CD5 were the most detected receptors. Acute leukemia had a significant impact on the clinical outcome in compared to the healthy controls. Several clinical parameters such as patient's age, gender, date of diagnosis, and blood group had an impact on the disease process and prevalence and could be considered as risk factors. Additionally, these factors could be correlated with the laboratory parameters.

Keywords: *Acute Myeloid Leukemia, Acute Lymphocytic Leukemia, Inflammatory Makers, Laboratory Parameters, Evaluation.*

Introduction

Leukemia is a hematological malignancy arises from irregular division or the life span of a blood cell or its precursor. The progenitor cell starts to proliferate uncontrollably and forms a large cell population derived from a single cell in the absence of differentiation. A block in differentiation results in an accumulation of immature cells that fail to fully mature and subsequently die. Proliferating immature cells that are stopped in their differentiation and escape immune surveillance eventually dominate the bone marrow and invade other tissues and organs leading to death [1]. A typical feature of leukemia is that the cells accumulate in the bone marrow and blood. Leukemia is divided into acute and chronic types, which are further classified into lymphoid and myeloid types depending on the cell lineage represented by the leukemic clone [2]. In acute leukemia (acute lymphoblastic leukemia and acute myeloid leukemia), the malignant cells are typically immature blast cells that are unable to differentiate, while in the chronic lymphocytic leukemia the malignant cells are morphologically mature and in chronic myeloid leukemia, though derived from precursor cells, the differentiation of leukemic cells is almost normal in the first stage of the disease [3].

While the processes of proliferation and differentiation are central to the development of leukemia, apoptosis is also involved in the control of leukemia. Homeostasis is maintained by balancing cell proliferation with cell death and an imbalance in either may result in cancer [4]. Acute leukemia is a cancer of the white blood cells. There are two types of acute leukemia, acute Lymphoblastic Leukemia (ALL) and acute myeloid leukemia (AML). In 1971, the diagnosis of leukemia cells was based on their morphology [5]. AML is a clonal disease, which is characterized by an increase in the number of myeloid cells in the bone marrow and

an arrest in their maturation. This frequently results in a severe suppression of normal hematopoiesis (granulocytopenia, anemia and/or thrombocytopenia) [6]. ALL is the most common type of leukemia in young children. This disease also affects adults, especially over the age of 65 years [7]. AML develops in both adults and children. AML is the most common subtype of leukemia in adults and accounts for 15-20% of childhood leukemia [8]. AML is characterized by continued proliferation and suppressed differentiation of haemopoietic progenitors in the bone marrow with disease cells characterized by enhanced survival and self-renewal. Thus, accumulating numbers of immature haemopoietic progenitors replace the normal red blood cells, white blood cells and platelets [9]. The AML is divided into ten major French-American-British (FAB) subtypes (M0-M8) and the World Health Organization (WHO) systems that are include 15 subtypes [10]. Acute leukemia (AL) classifications used blast morphology and cytochemical stains to categorize the diseases broadly into ALL and AML [11]. The current WHO classification of tumors of hematopoietic and lymphoid tissues segregates ALs based on lineage as demonstrated by antigen expression into lymphoid or myeloid malignancies [12]. Within each lineage, distinct subtypes are defined based on clinical and morphologic features in conjunction with immunophenotyping by immunohistochemistry) and/or flow cytometry and an emphasis toward classification by molecular genetics [13]. Diagnosis of AML usually starts from a clinical suspicion. Clinical features leading to suspicion of acute leukemia include pallor, hepatomegaly, splenomegaly, lymphadenopathy, fever, petechiae, ecchymosis, and other hemorrhagic manifestations, bone pain, gum hypertrophy and skin infiltration.[14] Laboratory, Leukemia can be diagnosed initially by blood tests and then by bone marrow examination. The typical blood picture shows anemia and thrombocytopenia, with a moderate or marked increase in leukocyte, the majority of which are blast cells [15]. Marrow aspirate is usually hyper cellular and heavily infiltrated by leukemic blast cells, which largely replace the normal marrow. For the diagnosis of AML, the WHO classification requires that 20% or more bone marrow or peripheral blood cells are myeloblasts, and/ or monoblasts/ promonocytes and/ or megakaryoblasts [16].This was adjusted from 30%, which was the blast count required according to the FAB classification. In addition to Flow Cytometry that based on the ability to rapidly sort neoplastic populations and simultaneously perform multiple antigen analyses [17], and immunohistochemistry where the a variety of clinical and biologic parameters, including immunophenotype, has been examined for potential value in predicting treatment response and survival. Some reports have suggested a relationship between some antigens e.g. (CD7, CD9, CD11b, CD13, CD14, CD15, CD33, and CD34) and AML prognosis [18]. The etiology of leukemia is unknown; however, different environmental factors were considered as risk factors for leukemia which include: ionizing radiation, organic solvents, smoking, number of perinatal factors (Hyperemesis, Down's syndrome, and some types of medications [19-22]. There are different protocols for acute leukemia treatment such as: chemotherapy [15], radiotherapy [23], immunotherapies [24], and hematopoietic Stem Cell Transplantation. [25] This study aimed to evaluate the changes in inflammatory markers and immune components during treatment of different acute leukemia subtypes in Libya. Furthermore, the effects on the hemostasis were also observed.

Methods

Blood sample

The study was carried out on 105 acute leukemia Libyan patients their ages ranged from (2-88) years who were referred to the hematology clinic of different hospitals in Libya such as: Medical Tripoli center, National Cancer Institute, Sabratha, and National Cancer Institute, Misrata, for the diagnosis and treatment. The diagnosis for patients was made by the consultant medical staff at the clinic, based on international criteria FAB M0-M8 subtypes for the AML and the lymphocytes stages for the ALL. The selected patients were classified into two groups: the first group included 73 AML patients; the second group included 32ALL patients. The diagnosis based on bone marrow examination. The induction phase in AML was accomplished by Cytosine Arabinoside, 100 mg/m2continuous IV infusion (day 1-7) plus Daunorubicin, 60 mg/m2 IV for the first 5 days. Patients were diagnosed as having acute myelogenous leukemia, acute lymphoblastic leukemia, and sixty blood samples from healthy blood volunteers matched patients for age and gender used as control group. After informed consent, the blood samples were collected from the all patients and healthy controls according to the blood aspirated technique. The collected blood samples were immediately tested. The healthy control samples were investigated in Al-Farouk laboratory, Tripoli.

General Laboratory investigations

Patient's peripheral venous blood was collected into 10- ml heparin/EDTA vacationer tubes. Samples were tested according to the standard procedures. Whole blood was analyzed automatically for complete blood count, inflammatory markers such as: C-reactive protein (CRP) and Erythrocyte sedimentation rate (ESR) using different instruments**.**

Immunohistochemical Evaluation of Tumor Tissues by Envision Method

Preparation of Tissue Sections

Paraffin embedded sections of patient's bone marrow were cut into 5 μm thicknesses using a microtome. The sections were applied on Fisher brand positively charged slides and left overnight to dry at room temperature.

Assay Procedure

The immunohistochemical staining techniques is used for visualization of tissues antigens by sequential reaction, was used to detect the expression of CD13, CD33, CD14 (for AML), and CD3, CD2, CD5, CD10 (for ALL). The primary antibody (e.g. anti- CD13, CD33 ,CD14) reacts with its corresponding antigen (CD13, CD33 ,and CD14) in the tissue, and then a secondary antibody system (a polymer backbone to which multiple antibodies and enzyme molecules are conjugated), binds to the primary antibody ;When the conjugate is added, the polymer secondary antibody system will form a complex with the peroxidaseconjugated streptavidin, and by adding the substrate, which contains diaminobenzidine (DAB) in a chromogenic solution, a brown colored precipitate will form at the antigen site. After staining, the slide was examined by a light microscope at 40x, and after comparing the tested section with negative and positive control slides, the following score was adopted according to a laboratory protocol: Score 1 (+): The positive cells (stained) represented 10% of total cells, Score 2 (++): The positive cells (stained) represented more than 10% to 30% of total cells ,Score 3 (+++): The positive cells (stained) represented more than 30% to 50% of total cells, Score 4 (++++): The positive cells (stained) represented more than 50% of total cells and Score 0 (Negative): No stained cells.

Statistical analysis

Statistics (means, minimal, and maximal values) were used to describe patient baseline characteristics. Results were presented as mean values and / or% of cells or parameters and p-values. Data were analyzed using the Licensed IBM SPSS 23.0 for Windows statistical analysis packages. Chi – Square test was used to ascertain the significance relationship between two independent nominal variables. Student's t-test and ANOVA test were used to ascertain the significance of differences between mean values of two continuous variables. The differences in the indicators were considered statistically significant at $p \leq 0.05$.

Results

Characteristics of AML Patients

The age of the 73 AML patients who were included in this study ranged between (2-88) years old. The mean of patients group age was (31) years. Their disease period ranges from several months to ten years. 36 patients were male (49.3%) and 37 female patients (50.7%), the proportion of females is higher than males (1: 0.9). Results showed that 25cases out of 73 AML patients were A^+ with percentage of (34%) followed by O^+ (22%) whereas the lowest one was in A^-.

Characteristics of ALL Patients

The age of the 32 ALL patients ranged between (2-70) years. The mean of patients group age was (15) years. The disease period range from several months to five years. 19 patients were male(49.4%) and 13 female patients(40.6%) , the proportion of males is higher than females (1.5: 1). 12 cases out of 32 ALL patients were O^+ with percentage of (38%) followed by A^+ (28%) whereas the lowest one was in A^-.

Table 1. Incidence of AML& ALL according to age

Variable	AML (%)	ALL (%)
Age(years)		
<10	11	15.6
10-20	6.8	34.4
20-30	9.6	21.9
30-40	19,2	9.4
40-50	16,4	6.3
50-60	8,2	3.1
60-70	11	9.4
70-80	13,7	
< 80	4,2	

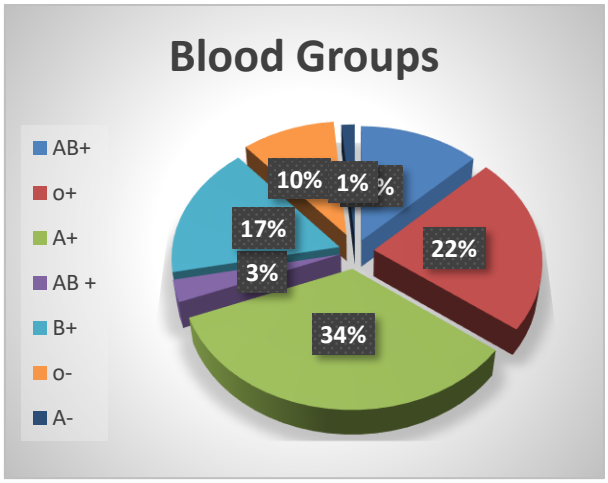

Figure 1. Showing the AML incidence according to blood groups

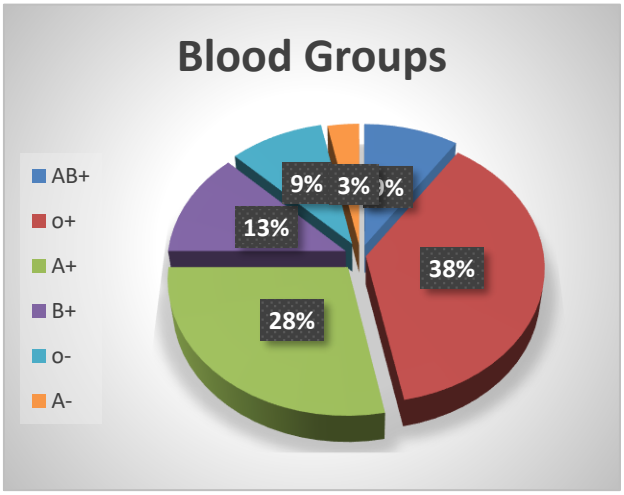

Figure 2. Showing the ALL incidence according to blood groups

The Subtypes Distribution of AML Patients

Results showed that 29 cases out of 73 AML patients were Acute Promyelocytic Leukemia (APL) subtype (M3) subtype with percentage of (39.70%) followed by (AML-M2) (13%) whereas the lowest one was in M6.

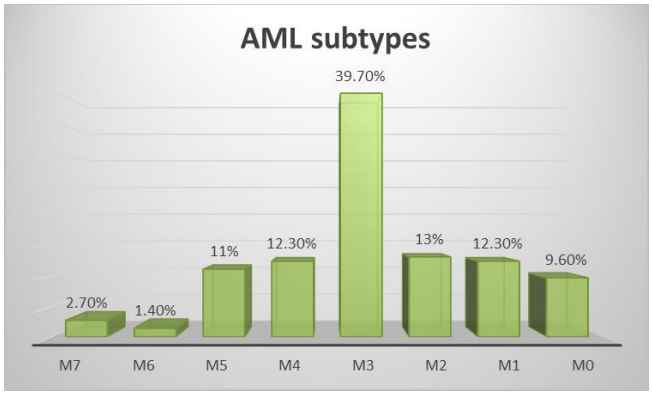

Figure 3. The Distribution of AML subtypes
M0: Minimally differentiated acute myeloblastic leukemia; **M1:** Acute myeloblastic leukemia without maturation; **M2:** Acute myeloblastic leukemia with granulocytic maturation; **M3:** Acute promyelocytic leukemia (APL); **M4:** Acute myelomonocytic leukemia; Myelomonocytic leukemia together with bone marrow eosinophilia; **M5:** Acute monocytic leukemia; **M6:** Acute erythroid leukemia; **M7:** Acute megakaryoblastic leukemia

Subtypes Distribution of ALL Patients
17 patients were B cell acute lymphocytic Leukemia (53%) and 15 patients were T cell acute lymphocytic Leukemia (47%).

Impact of AML related parameters on White blood cells as whole population
No significant relationship between WBC count and multiple factors such as: age, gender, blood group, AML subtypes and date of diagnosis were detected.

Table 2. AML related elements and WBC count

Factors during AML	P-value
Age	0.857
Gender	0.564
Blood group	0.339
Subtypes	0 .520
Date of diagnosis	0 .519

We also correlated between every cell type with the above mentioned factors, and found no significant effect on neutrophils, eosinophils, and lymphocytes. There was a significant correlation between basophils and blood groups (p=**0.000**), monocytes and date of diagnosis (p=**0.03**).

Impact of AML related parameters on red blood cells count and related indexes
Additionally, we correlated the incidence of AML with erythrocytes and their related hematological indices and only a significant impact of patient's age on the percentage of mean cell hemoglobin concentration (MCHC) was determined.

Table 3. Effect of factors during AML on RBC count & related indexes

	P-Value							
Factors during AML	**RBC**	**MCV**	**MCH**	**MCHC**	**HB**	**HCT**	**RDW-SD**	**RDW-CV**
Age	0.628	0.128	0.620	**0.005**	0.863	0.710	0.515	0.601
Gender	0.443	0.134	0.898	0.349	0.398	0.564	0.653	0.543
Blood group	0.392	0.139	0.149	0.096	0.789	0.903	0.233	0.428
Subtypes	0.806	0.714	0.501	0.143	0.619	0.772	0.484	0.819
Date of diagnosis	0.057	0.078	0.441	0.053	0.539	0.686	0.501	0.923

RBC: Red blood cells**; MCV:** Mean cell volume**; MCH:** Mean cell hemoglobin**; MCHC:** mean cell hemoglobin concentration**; HB:** Hemoglobin**; HCT:** Hematocrit**; RDW-SD:** Red cell distribution width-standard deviation**; RDW-CV:** Red cell distribution width-coefficient of variation**.**

Effect of AML on Platelets count and Platelets distribution width (PDW)
Significant relationships between the platelet's distribution width and the disease subtypes (P=**0.03**), and the date of disease diagnosis (P=**0.00**) were detected.

Table 4. Effect of factors during AML on platelets count &PDW

Factors during AML	P-Value	
	Platelets	**PDW**
Age	0.540	0.417
Gender	0.443	0.417
Blood group	0.838	0.542
Subtypes	0.748	**0.030**
Date of diagnosis	0.389	**0.000**

Impact of AML related elements on the inflammatory markers:
Upon investigation of the influence of several factors such as patient's age, gender, blood groups, disease subtyping, and date of diagnosis on the concentration of one of the acute phase proteins: C-reactive protein (CRP) and erythrocyte segmentation rate (ESR), we found a significant relationship between date of diagnosis and ESR (P=0.012). There was no effect on CRP concentration.

Table 5. Correlation between AML related factors and inflammatory markers

Factors during AML	P-Value	
	CRP	**ESR**
Age	0.203	0. 215
Gender	0.441	0.315
Blood group	0. 549	0.747
Subtypes	0.737	0.931
Date of diagnosis	0. 168	**0.012**

Impact of AML related elements on the percentage of immature granulocytes (IG):
AML subtyping had a significant effect on the percentage of IG.

Table 6. Effect of AML subtyping on the % of IG

Factors during AML	P-Value
	IM %
Subtypes	**0.035**

Immunohistochemical evaluation of Bone Marrow Biopsy
Results indicated that CD13, CD33, and CD14 were positive in the majority of cases studied. CD13or CD33 or both may be seen in all subtypes of AML.

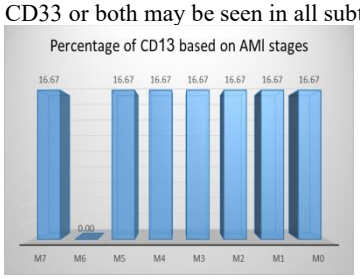

Figure 4. Percentages of CD13

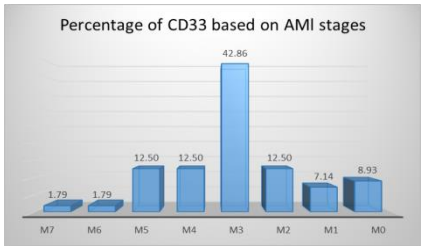

Figure 5. Percentages of CD33

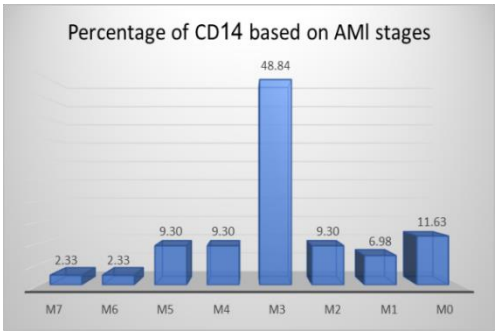

Figure 6. Percentages of CD14

Comparison between AML laboratory parameters and healthy controls:
Several laboratory parameters levels such as: white blood cells count, red blood cells count, hemoglobin, hematocrit, lymphocytes, platelets, mean cell hemoglobin, mean cell hemoglobin concentration, red cell distribution width-SD, red cell distribution width-CV, C-reactive protein, and erythrocyte sedimentation rate were influenced by the disease incidence.

Table 7. Comparison of AML laboratory results with that of healthy controls

Two independent samples T-test			
Variables	**Mean of Controls**	**Mean of AML**	**P-Value**
WBC	7.36	40.95	**0.010**
RBC	4.62	2.85	**0.000**
HB	13.22	8.77	**0.000**
HCT	40.10	26.24	**0.000**
Neutrophils	3.74	24.37	0.075
Eosinophils	0.19	0.19	0.983
Basophils	0.02	0.25	0.066
Lymphocytes	2.98	9.33	**0.027**
Platelets	256.71	113.73	**0.000**
MCV	86.79	101.93	0.206
MCH	28.72	30.93	**0.000**
MCHC	32.99	34.07	**0.011**
RDW-SD	43.64	54.54	**0.000**
RDW-CV	13.22	16.43	**0.000**
PDW	13.32	13.93	0.390
CRP	1.83	83.61	**0.000**
ESR	8.66	53.37	**0.000**

WBC: White blood cells; **RBC:** Red blood cells; **HB:** Hemoglobin; **HCT**: Hematocrit; **MCV:** Mean cell volume; **MCH**: Mean cell hemoglobin; **MCHC:** mean cell hemoglobin concentration; **RDW-SD:** Red cell distribution width-standard deviation; **RDW-CV**: Red cell distribution width-coefficient of variation; **PDW:** platelets distribution width; **CRP:** C-reactive protein; **ESR:** Erythrocyte sedimentation rate.

Impact of ALL related factors on laboratory parameters:
Upon correlation between ALL associated factors and different laboratory investigations such as complete blood count, CRP and ESR, There was only a significant relationship between percent of lymphocytes and the date of diagnosis and a significant impact of the blood groups on the mean cell volume (P=0.007, P=0.038 respectively).

Table 8. Impact of ALL related factors on laboratory parameters.

	P-Value
Factors during AML	
Date of diagnosis vs. Lymphocytes %	**0.007**
Blood group vs. Mean cell volume	**0.038**

Comparison between ALL laboratory parameters and healthy controls:
Several laboratory parameters levels such as: white blood cells count, red blood cells count, hemoglobin, hematocrit, basophils, lymphocytes, platelets, mean cell hemoglobin concentration, red cell distribution width-SD, C-reactive protein, and erythrocyte sedimentation rate had a role in the disease incidence**.**

Table 9. Comparison of ALL laboratory results with that of healthy controls

Two independent samples T-test			
Variables	**Mean of Controls**	**Mean of ALL**	**P-Value**
WBC	7.358	25.41	**0.001**
RBC	4.62	3.12	**0.000**
HB	13.22	8.99	**0.000**
HCT	40.10	26.03	**0.000**
Neutrophils	3.74	4.00	0.712
Eosinophils	0.19	0.16	0.650
Basophils	0.02	0.07	**0.001**
Lymphocytes	2.98	11.88	**0.002**
Platelets	256.71	123.64	**0.000**
MCV	86.79	86.71	0.954

MCH	28.72	29.75	0.062
MCHC	32.99	34.37	**0.000**
RDW-SD	43.64	51.62	**0.000**
RDW-CV	13.22	67.81	0.141
PDW	13.32	21.38	0.166
CRP	1.83	58.93	**0.000**
ESR	8.66	42.92	**0.000**

WBC: White blood cells; **RBC:** Red blood cells; **HB:** Hemoglobin; **HCT:** Hematocrit; **MCV:** Mean cell volume; **MCH**: Mean cell hemoglobin; **MCHC:** mean cell hemoglobin concentration; **RDW-SD:** Red cell distribution width-standard deviation; **RDW-CV**: Red cell distribution width-coefficient of variation; **PDW:** platelets distribution width; **CRP:** C-reactive protein; **ESR:** Erythrocyte sedimentation rate.

Immunohistochemical evaluation of Bone Marrow Biopsy

Results indicated that CD19, CD10, and CD 34 were the main receptors in B cell acute lymphocytic Leukemia, whereas CD5, CD3. And CD2 were the main receptors in T cell acute lymphocytic Leukemia.

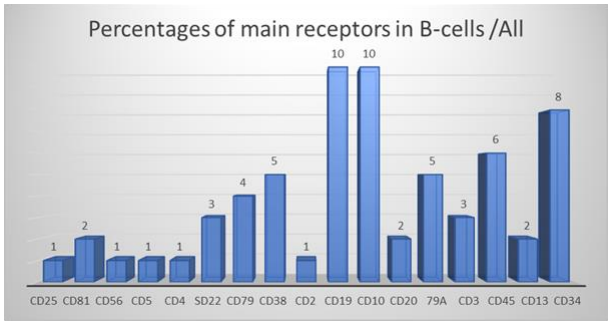

Figure 6. Percentages of main receptors in B- ALL

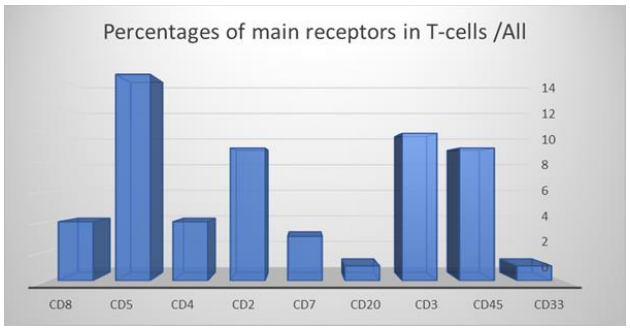

Figure 7. Percentages of main receptors in T- ALL

Discussion

The production of abnormal white blood cells identifies leukemia as a primary or secondary process. It can be classified as acute or chronic on the basis of speed of spread and myeloid or lymphocytic on the basis of cell of origin. The predominant subtypes are acute myeloid leukemia (AML) and acute lymphoblastic leukemia (ALL). Of the 105 cases examined within the framework of this study, there were 73 cases of AML and 32 cases of ALL. The occurrence of AML was found to be more common in our study. This is consistent with study conducted by Vinshith, F J, et al, and 2002.Their results showed that the occurrence of AML is more common than All [26].

The mean of patient's age was (31) years for AML**,** and the proportion of the total number of patients was close to each other (males and females**).** Other local studies showed higher mean age with 68 years [27]. Mirghofran and his co-worker found that the mean age in AML patients was 32.7 years, while Legrand found that 57 years is the mean age in AML patients. The differences could be due to the certain criteria used for the selection of the cases and the geographical factors [28,29]. ALL patients had an average age of (15) years. The highest percentage of the total number of patients was male at 59%, and these results are similar to the results of study conducted by [30]. Patient age is always important to consider when evaluating data in clinical studies. However, organ function measures could substitute for age [31]. Differences in age distribution in studies might also give as inclusion rates in the studied population, in addition to true differences between time periods and geographical regions as well as random variation [32].

Results showed that 29 cases out of 73 AML patients were acute Promyelocytic Leukemia (APL) (M3) subtype with percentage of (39.70%) followed by (M2) (13%) whereas the lowest one was in M6. Our result agreed with in New Delhi, who recorded that the percentage of M3 were (27.6 %) of total AML cases in his study.[33] while our result showed that M1and M4 were equally in the third position (12.30%), Arber et al, 2003 reported M2 accounting (28.6%) as the commonest subtype followed by M4 (26.7%) in USA [34]. Harakati et al, 1998, explained the M4 as a high incident (40%) followed by M5 (33%) in Saudi Arabia. .[35] Many of the differences in AML subtypes may be due to the subjectivity of morphologic diagnosis together with variable nature of acute myeloid leukemia subtypes, with no real demarcation. Some genetic factors may be responsible for a particular FAB subtype's distribution of AML in our population. The other reason for this discrepancy may be patients of different ethnic group and or geographical variation.

Previous study of Nagy et al, showed an increase rate of in blood O group in female acute leukemia patients, this is in contrast to the result of our study which reported an increase in the rate of blood group A+ in AML patients by 34% and then blood group O^+ by (22%) [36]. Out of 32 ALL patients were O^+ with percentage of (38%) followed by A^+ (28%) whereas the lowest one was in A^-. These findings were similar to those of Elzein HO, 2024 [37]. Additionally, the significant correlation between basophils and type of blood group has been determined [36]. Our results found that the absolute monocyte count had a role as an emerging independent predictor of better outcome in AML similar to the study conducted by Manar Ismail, 2019 [38]. Our data proved the predictive importance of the age of AML patients and the important role of MCHC in acute myeloid leukemia patients with ($P = 0.005$) indicated that even in the younger cohorts increasing age may be an additional unfavorable factor and this showed a compatible relationship Significant with results of Beverley G and Parker Katherine, 2003 [39].

This study investigated the relationship between platelets distribution width(PDW) and AML prognosis and found that PDW had an impact on the disease stages and could be useful as indicators of certain disease states. It seems possible that PDW can be used as surrogate markers for follow-up in patients with leukemia, and there is an association between PDW values and leukemia subtype ($P = 0.030$) and date of diagnosis ($P=0.000$) in contrast to the findings of the study by AlSuweedan et al., who found no significant difference in PDW in patients with AML versus ALL and therefore cannot be used as indicator to discriminate between the subtypes of leukemia in children [40,41]. The critical role of platelets indices in leukemia remains to be analyzed by different levels to confirm the possible clinically significant of its determination**.**

Statistical analysis did not show a statistically significant difference in CRP concentrations among AML patients according to the study elements, but were significantly higher in those with severe complications. These findings were similar to those of Vladimirova et al., 2013, that indicated that CRP was a marker of the severity of an infectious process in AML patients [42]. In contrast there was a statistically significant relationship between ESR and date of diagnosis ($P=0.012$), thus elevated inflammatory markers, including ESR, have been demonstrated in AML [43, 44].

Previous studies on tumor patients have detected that tumor cells are capable through different mechanisms to promote the growth of myeloid and thus the expansion of leukocytosis, which is a sign of tumor formation, as well inhibit the differentiation of myeloid cells and induce the natural change of these cells and the accumulation of immature myeloids. The identification of immature granulocytes (IG) provides a more critical indicator for the tumor-related myeloid proliferation compared with white blood cell counting and it recognize the early detection of tumor-related myeloid proliferation. Furthermore, our findings that AML subtypes had a significant effect on immature granulocytes percentages ($P= 0.035$) were consistent with that of CHE, et al, 2014 who indicated that the measurement of IG could provide important data for clinical disease diagnosis and therapy monitoring [45].

The diagnosis of AML is based on cell morphology, cytogenetic and molecular changes, cell markers and clinical data [46]. As a result, immunophenotyping has been integrated into the FAB classification in the diagnostic protocols needed to recognize AML-M0 [47] and M7 [48]. The tissue expression of CD13, CD33, and CD14 markers, the results concerning the prognostic value of surface antigens expression in AML, and the investigation on the percentage of these immunological parameters in AML showed a significant increase in them. CD13 distinguishes antigens on the surfaces of the granulocyte and monocytes into mature and immature, whereas CD33 is a transmembrane receptor expressed on cells of myeloid lineage [49]. It is usually considered myeloid-specific, but it can also be found on some lymphoid cells [50]. Results indicated that CD13& CD33 were positive in the majority of cases

studied. CD13or CD33, or both may be seen in all subtypes of AML using immunohistochemical examination and this agreed with other investigator Dybkaer, K, et al, in that the percentage of the CD13 marker in myeloid cells isolated from AML patients showed the significant increase in the level of these markers when compared with healthy people [51]. The CD33, CD13 were the most commonly expressed antigens in AML and had much higher specificity since it was rarely observed in ALL. The same writer referred to myeloid markers: CD13, CD33 expressed in the vast majority of AML samples at the rate of 86.7%, of 96.1% [52]. So Sanaat et al., and her group found, among different markers, the most positive markers are the following CD13 (81%), CD33 (84.9%) the myeloid lineage antigens [53].

CD13, CD33, or both may be seen in all subtypes of AML. However, by immunohistochemical examination. In previous investigations, presence of CD33 was considered a favorable prognostic factor [54]. The frequent expression of CD33 on both AML blasts and AML stem cells has made it a target antigen of choice for developing monoclonal antibodies mAb-based approaches in the past [55,56].

In the ALL small patient's cohort, 53% of patients were B-cell subtypes and47% T-cell acute lymphoblastic subtypes. The patients' number is small to draw definitive conclusions. Upon correlation between the ALL related factors and different laboratory parameters there was no impact on leukocytes, erythrocytes and platelets indices whereas a significant relationship between lymphocytes percent and date of diagnosis (p=0.007) was detected. Additionally, there was a strong correlation between the mean cell volume and type of blood group (P=0.038), these results were compatible with those of Terwilliger et al., 2017 and Nagy p, et al, 1981, in that age, blood group, and white blood cell count might be considered as risk factors of the ALL [36,57].

B-cell acute lymphoblastic leukemia composed of immature B cells is termed either precursor B-cell acute lymphoblastic leukemia (B-ALL) if mainly marrow –based with >25% replacement of bone marrow by lymphoblasts or precursor B-cell lymphoblastic lymphoma (B-LBL) if mainly tissue based with ≤ 25%bone marrow involvement by lymphoblasts. An important differential in the diagnosis of these types based on the presence or absence of different B-cell markers expressed during B-cell maturation stages. Our findings revealed the expression of multiple markers using immunophenotyping such as CD34, CD20, CD22, CD10, CD79a, and CD19. The discrimination between precursor T-cell acute lymphoblastic leukemia (T-ALL) and precursor T-cell lymphoblastic lymphoma (T-LBL) carries the same diagnostic criteria as above mentioned. The expression of CD34, CD2, CD3, CD5, CD7, CD4 and CD8 were detected while CD13, CD33, and CD14 were cross-expressed in lymphocytes and myeloid cells, which were similar to the results of Olsen et al, 2008 and AL-Gwaiz et al, 2008 [58,59].

The mean of white blood cell count was 40.5/ μL in AML and 25.41/ μL in ALL, the mean of hemoglobin level was 8.77 in AML, 8.99 in ALL and the mean of platelets count in AML was 113.73 /μL, 123.64 /μL in ALL The results were similar to the results of study conducted by Choudhury R, et al [60]. By investigation of different laboratory parameters of AML and ALL patients and comparing them by the levels of healthy controls, a significant difference in various parameters such as leukocytes, erythrocytes, platelets and inflammatory markers such as CRP and ESR were determined [60]. These parameters could come in focus as independent predictors of the clinical outcome of acute leukemia and disease monitoring. More investigation is required to study the role of some important cytokines such as IL-10, IL-12, and gamma interferon in bone marrow biopsy in larger groups of AML patients by using immunohistochemistry technique to determine the level of these cytokines and their role in disease prognostic, also the study of larger groups of AML and ALL patients and compare the differences in CD marker expression among their subtypes and so use more variable Mark as diagnostic marker is recommended.

Conclusion

In this retrospective study of acute leukemia patients, the results showed that different factors such as: age, gender, disease stages, type of ABO blood group and date of diagnosis had an impact on each other and also on the levels of various laboratory parameters which therefore, affect the incidence and severity of the disorder. To confirm these observations, several laboratory parameters levels such as: white blood cells count, red blood cells count, hemoglobin level, hematocrit, basophils, lymphocytes, platelets, mean cell hemoglobin concentration, red cell distribution, C-reactive protein, and erythrocyte sedimentation rate were compared with those of healthy individuals and found their influence on the disease incidence. Collectively, these important correlations could considered as predictive indicators that have

prognostic values and help in disease monitoring. Furthermore, early detection, prompt treatment, and regular follow-up could improve the clinical outcome.

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References

1. Mwirigi A, Dillon R, Raj K. Acute Leukaemia. Medicine.2017May; 45**(**5):280-286.
2. McKenna R. W. Multifaceted approach to the diagnosis and classification of acute leukemias. Clin Chem.2000Aug; 46(8): 1252-1259.
3. Stock W, Hoffman R. White Blood Cells 1: non-Malignant disorders. Lancet. 2000Apr; 355(9212):1351 – 1357.
4. Hoff brand A.V. and Moss P.A.H (2011). Essential Haematology.6 Th ed. Willey_Blackwell Publishing, Ltd. Oxford.
5. Szczepanski T, van der Velden HV, van Dongen JM. Classification systems for acute and chronic leukaemias. Best Pract Res Clin Haematol. 2003Dec; 16(4): 561-582.doi:10.1016/s1521-6926 (03)00086-0.
6. Khawaja A, Bjorkholm M, Gale RE, Levine LR, Jordan TC, Ehninger G, Bloomfield C, Estey E, Burnett A, Cornelissen JJ, Scheinberg DA, Bouscary D, Linch DC. Acute myeloid leukaemia. Nat Rev Dis Primers. 2016Mar; 10(**2**): 16010.
7. Ching-Hon P, Robisin L, Look TA. Acute lymphoblastic leukemia. Lancet. 2008Mar; 371(9617):1030-1043.
8. Redaelli A, Botteman, M.F, Stephens, M J., Brandt S, Pashos C. Economic burden of acute myeloid leukemia: a literature review. Cancer Treat Rev. 2004; 30(3): 237-247.
9. Smith M., Barnett M., Bassan R, Gatta G, Tondini C, Kern W. Adult acute myeloid leukemia. Crit Rev Oncol Hematol. 2004Jun; 50(3): 197-222.
10. Tenen D.G. Disruption of differentiation in human cancer: AML shows the way. Nat Rev Cancer. 2003;3(2):89-101**.**
11. Thiele J, Kvasnicka H, Vardiman. J. Bone marrow histopathology in the diagnosis of chronic myeloproliferative disorders: a forgotten pearl. Best Pract Res Clin Haematol.2006 Jun; 19**(**3): 413–37.
12. Jaffe E S, Harris NL, Stein H. and Vardiman JW. World Health Organization Classification of Tumours: Pathology and Genetics of Tumours of Hematopoietic and Lymphoid Tissues. IARC WHO Classification of Tumours Ann Oncol .2002 Mar; 13 (3): 490- 491**.**
13. Vardiman JW, Harris NL, and Brunning RD. The World Health organization (WHO) classification of the myeloid neoplasms. Blood.2002 Oct; 100**(7):**2292–2302.
14. Cornell RF, Palmer J. Adult acute leukemia. Dis Mon.2012 Apr; 58(4):219-238.
15. Hoffbrand AV, Moss P.A.H., Pettit J.E. (2006). Essential Hematology. 5th ed. Blackwell Publishing, Ltd. Oxford**.**
16. Jabbour E, Estey E, Kantarjian H. Adult acute myeloid leukemia. Mayo Clin Proc. 2006; 81(2):247-260.
17. Cherie H. Dunphy. Gene expression profiling data in lymphoma and leukemia: review of the literature and extrapolation of pertinent clinical applications. Arch Pathol Lab Med. 2006; 130(1):483–5**2.**
18. Lee S, Kim H, Kim S. Defining an Optimal Number of Immunophenotypic Markers for Lineage Assignment of Acute Leukemias Based on the EGIL Scoring System. Korean J Lab Med. 2006;26(6):393-399.
19. Descatha A, Jenabian A, Conso F, Ameille J. Occupational exposures and haematological malignancies: Overview on human recent data. Cancer Causes Control. 2005; 16(8):939-953**.**
20. Greer JP, Baer M R, Kinney MC (1999). Acute myelogenous leukemia. In Lee G.R, Forester J, Lukens J, Paraskevas F, Greer JP, Rodgers GM,. Wintrobe's. Clinical Hematology. 10th edition. Baltimore, MD: Williams and Wilkins; pp: 2272-2319.
21. Pogoda JM, Preston-Martin S, Nichols PW, Ross RK. Smoking and risk of acute myeloid Leukemia: results from a Los Angeles County Case Control study. Am. J. Epidemiol. 2002 Mar; 155(6):546- 553.
22. Roman E, Simpson J, Ansell P, Lightfoot T, Mitchell C, Eden TO. Perinatal and reproductive factors: a report on hematological malignancies from the UKCCS. Eur J Cancer.2005 Mar; 41 (5):749-59.doi:10.1016/j.ejca.2004.11.006.
23. Magnander K, Elmroth K .Biological consequences of formation and repair of complex DNA damage. Cancer Lett. 2012 Dec; 327(1- 2):90-6.
24. Barrett A J, Blanc K L. (2010). Immunotherapy prospects for acute myeloid leukemia. British Society for Immunology, Clinical and Experimental Immunology. 2010 Apr; 161(2): 223–232.
25. Reiss U, Bolotin E. New approaches to hematopoietic cell transplantation in oncology. Pediatr Clin N Am.2002 Dec; 49**(6):**1437-66**.**
26. VinSheth F. J., Sheth, J. J., Patel, A. I., Shah, A. D., Verhest, A. Usefulness of Cytogenetics in Leukemias. Indian journal of cancer. 2002 Oct; 39(4), 139-142.
27. Juliusson G, Antunovic P, Derolf A, Lehmann S, Mollgard L, Stockelberg D, et al. Age and acute myeloid leukemia: real world data on decision to treat and outcomes from the Swedish Acute leukemia Registry. Blood. 2009 Apr; 113(18):4179-4187. doi: 10.1182/blood-2008-07-172007.
28. Mirghofran Z, Shaseddin A, Zakrinia M. Immunophenotypic analysis in Iranian patients with acute myelogenous leukemia: correlation with leukemia subgroups. Iran J Med.1999; 24:40-44.
29. Legrand O, Perrot, J.Y, Baudard M, Cordier A, Lautier R, Simonin G, Zittoun R, Casadevall N, Marie J P. The immunophenotype of 177 adults with acute myeloid leukemia: Proposal of a prognostic score. Blood.2000 Aug; 96(3): 870–877.doi:10.1182/blood.V96.3.870.
30. Harani M S, Adil S N, Shaikh M U, Kakepoto G N, Khurshid M. Frequency of fab subtypes in acute myeloid leukemia patients at Aga Khan University Hospital Karachi. J Ayub Med Coll. 2005 Jan-Mar; 17(1): 26-29.
31. Walter R, Othus M, Borthakur G, Ravandi F, Cortes J, Pierce S, Appelbaum F, Kantarjian H A, Estey E H. Prediction of early death after induction therapy for newly diagnosed acute myeloid leukemia with pretreatment risk scores: a novel paradigm for treatment assignment. J Clin Oncol. 2011 Nov; 29(33):4417-24.

32. Regoes, R.R .Population genetics meets cancer genomics. Proc Nat Acad Sci U S A. 2010 Oct; 107(43):18241-18242.
33. Shah M A, Gupta A, Kaur S, Gousuddin M. A Retrospective study of acute promyelocytic Leukemia. IOSR Journal of Pharmacy. 2012 Dec; 2(6):52-59.
34. Arber D A, Stein A S, Carter N H, Ikle D, Forman S J, Slovak M L. Prognostic impact of acute myeloid leukemia classification. Importance of detection of recurring cytogenetic abnormalities and multilineage dysplasia on survival. Am J Clin Pathol. 2003 May; 119(5):672-680.
35. Harakati M, Al-Momen A, Ajarim D, Al-Moharib F, Al-Theyab A, Fawzy E, Al-Gwaiz L, Al-Khairy K S. Adult acute myeloblastic leukemia: Experience at King Khalid University Hospital. Ann Saudi Med. 1998;18(3):221-5.
36. Nagy P, Jako J, Kiss A, Tamas E, Telek B, Rak K. Sex-linked difference in blood group distribution among patients suffering from acute leukaemias. Br J Haematol. 1981 J; 48(3):507-8.
37. Elzein H O. Association of leukemia with ABO blood group distribution and discrepancy: A review article. Cureus. 2024 Mar; 16(3):e56812.
38. Ismail MA, Abdulateef N A. Absolute monocyte count is superior than absolute lymphocyte count at day 28 as an independent prognostic factor in acute myeloid leukemia. Indian J Hematol Blood Transfus. 2019 Jul; 35(1):100-10.
39. George-Gay B, Parker K. Understanding the complete blood count with differential. J Perianesth Nurs.2003 Apr; 18(2): 96-114.
40. Alsweedan S A, Al-Shurman A, Mahmoud, A. Diagnostic value of platelet indices in children with leukemia. J Pediatr Hematol. Oncol. 2008;30(12):953-955.
41. Al-Sweedan S, Matalka I. Platelet indices as quality markers for remission in patients with Leukemia. Journal of Hematology. 2012;1(2-3):54-57.
42. Valdimirova S G, Tarasova L N, Sokol'skaia O, Cherepanova V V.C-reactive protein as a marker of the severity of an infectious process in acute myeloid leukemia patients with neutropenia. Ter Arkh. 2013;85(11):34-40.
43. Fest J, Ruiter R, Mooijaart S, Ikram M, Eijck C, Stricker B. Erythrocyte sedimentation rate as an independent prognostic marker for mortality: a prospective population –based cohort study. J Intern Med. 2019 Mar; 285(3):341-348.
44. Malcolm L. Clinical utility of the erythrocyte sedimentation rate. Am Fam Physician. 1999;60(5):1443-1450.
45. Yi-Qun Che, Di Shen, Zhang S M, Qi J. Identification of immature granulocytes in cancer chemotherapy patients by cell counting vs. microscopic examination of blood. Molecualr and clinical oncology. 2014 Jan; 2:207-211.
46. Harris N, Jaffe E, Diebold J, Flandrin G, Mueller-Hermelink H, Vardiman J, et al. WHO classification of neoplastic diseases of the hematopoietic and lymphoid tissues reports of the clinical advisory committee meetingAirlie House, Virginia, November 1997. Ann Ocol. 1999 Dec; 10 (12):1419-1432.
47. Bennett J, Catovsky D, Daniel M, Flandrin G, Galton D, Gralnick H, Sultan C. Proposals for the recognition of minimally differentiated acute myeloid leukemia (AML-M0). Br J Hematol.1991 Jul; 78(3):325-329.doi:10.1111/j.1365-2141.1991.tb04444.x.
48. Jennings C, Foon K. Recent advances in flow cytometry: application to the diagnosis of hematologic malignancy. Blood. 1997 Oct; 90(8):2863-2892.
49. Garnache-Ottou, F, Chaperot L, Biichle S, Ferrand C, Remy-Martin J.P, Deconinck E, de Tailly, P.D, Bulabois B, Poulet J, Kuhlein E, Jacob M.C, Salaun V, Arock, M, Drenou B, Schillinger F, Seilles E, Tiberghien, P, Bensa J.C, Plumas J, Saas, P. Expression of the myeloid-associated marker CD33 is not an exclusive factor for leukemic plasmacytoid dendritic cells. Blood.2005 Feb; 105(3): 1256–64.doi:10.1182/blood-2004-06-2416.
50. Hernández-Caselles T, Martínez-Esparza M, Pérez-Oliva A.B, Quintanilla-Cecconi A, Garcia-Alonso A, Alvarez-Lopez D, Garcia-Penarrubia P. A study of CD33 (SIGLEC-3) antigen expression and function on activated human T and NK cells: two isoforms of CD33 are generated by alternative splicing. J. Leukoc. Biol.2006 Jan; 79 (1): 46–58.doi:10.1189/jlb.0205096.
51. Dybkaer K, Pedersen B, Pedersen F, Kristensen J. Identification of acute myeloid leukemia patients with diminished expression of CD13 myeloid transcripts by competitive reverse transcription polymerase chain reaction(RT-PCR). Leuk Res. 2000 Jun; 24(6):497-506.
52. Zheng J, Wang X, Hu Y, Yang J, Liu J, He Y, Gong Q, Yao J, Li X, Du W, Huang S. A Correlation study of immunophenotypic, cytogenetic, and clinical Features of 180 AML Patients in China. Cytometry B Clin Cytom. 2008 Jan; 74(1):25–29.
53. Sanaat Z, Amizadeh Y, Akbari, A M, Dolatkhah R. The Relationship between immunological markers, disease free survival and overall survival in acute myeloid leukemia in north-west of Iran. International Journal of Hematology Oncology and Stem Cell Research (IJHOSCR). 2010 Oct; 4(4):25-28.
54. Repp R, Schaekel U, Helm G, Thiede C, Soucek S, Pascheberg U, Wandt H, Aulitzky W, Bodenstein H, Sonnen R, Link H, Ehninger G, Gramatzki M. Immunophenotyping is an independent factor for risk stratification in AML. Cytometry B Clin Cytom. 2003 may; 53(1): 11-9.
55. Walter R, Applbaum F, Estey E, Bernstein I, Acute myeloid leukemia stem cells and CD33-targted immunotherapy. Blood. 2012 Jun; 119(26):6198-6208.
56. Ehninger A, Kramer M, Rolling C, Thiede C, Bornhauser M, Bonin M, Wermke M, Feldmann A, Bachmann M, Ehninger G, Oelschlager U. Distribution and levels of cell surface expression of CD33 and CD123 in acute myeloid leukemia. Blood Cancer J. 2014 June;4,e218.
57. Terwilliger T, Abdul-Hay M. Acute lymphoblastic leukemia: a comprehensive review and 2017 update. Blood Cancer J.2017 Jun; 7(6):e577.
58. Olsen J, Chang C, Herrick L, Zu Y, Ehsan A. Acute leukemia immunohistochemistry: A systematic diagnostic approach. Arch Pathol Lab Med.2008 Mar; 132(3):462-475.
59. AL-Gwaiz L, Bassioni W. Immunophenotyping of acute lymphoblastic leukemia using immunohistochemistry in bone marrow biopsy specimens. Histol Histopathol. 2008 Oct; 23(10): 1223-8
60. Choudhury R, Sreevalli A, Balakrishnan C, Ross C. Clinical and laboratory profile of acute leukemia with special reference to flow cytometry. Medica Innovatica. 2017 Dec; 6(2):10-19.