International Journal of Advanced Biomedicine

http://dx.doi.org/10.18576/ab/020104

Hematological Studies in Egyptian Children with Acute Lymphoid and Myeloid leukemia

Ahmed S. El-Shafey[*,1], *Saida M. Amer*[1], *Nanis G. Allam*[1] *and Mohsen S. El-Alfy*[2]

[1] Microbiology Section, Faculty of Science, Tanta University, Egypt
[2] Hematology & Oncology unit, Pediatrics Department, Faculty of Medicine, Ain shams University, Egypt

Received: 21 Nov. 2016, Revised: 12 Dec. 2016, Accepted: 14 Dec. 2016.
Published online: 1 Jan. 2017.

Introduction: Acute leukemia is the most common form of childhood cancer and is the primary cause of cancer-related mortality in children.
Material and methods: Complete blood count investigations were done to patients with acute leukemia and healthy control by manual method.
Results: A significant decrease in hematological parameters as total leucocytes count, hemoglobin, Red blood cell count, platelets count, hematocrit and neutrophils percent during chemotherapy compared to before chemotherapy as action of chemotherapy.
Conclusion: This study proved that children with acute leukemia suffered from leucopenia, anemia, and thrombocytopenia during chemotherapy that made them susceptible to infections and bleeding complications.
Keywords: A lymphoid, Myeloid leukemia, leucopenia, anemia, and thrombocytopenia

1 Introduction

Appelbaum et al., (2011); Kantarjian and O'Brien, (2011) reported that leukemia is a type of blood cancer that begins in the bone marrow. Bone marrow is the soft tissue in the center of the bones, where blood cells are produced. The term leukemia means white blood. White blood cells (leukocytes) are used by the body to fight infections and other foreign substances. Leukocytes are made in the bone marrow. Leukemia leads to an uncontrolled increase in the number of white blood cells .The cancerous cells prevent healthy red cells, platelets, and mature white cells (leukocytes) from being made. Life-threatening symptoms can then develop as normal blood cells decline. The cancer cells can spread to the bloodstream and lymph nodes. They can also travel to the brain and spinal cord (the central nervous system) and other parts of the body. The main leukemia types are: acute lymphocytic leukemia (ALL), acute myelogenous leukemia (AML), chronic lymphocytic leukemia (CLL) and chronic myelogenous leukemia (CML).

Acute lymphoblastic leukemia (ALL) is a fast-growing cancer of a type of white blood cells called lymphoblasts. These cells are usually found in the bone marrow. ALL occurs when the body produces a large number of immature lymphoblasts. The cancer cells grow quickly and replace normal cells in the bone marrow. Bone marrow is the soft tissue in the center of bones that helps form all blood cells.

ALL prevents healthy blood cells from being made. Life-threatening symptoms can occur as normal blood counts drop. ALL makes the person more likely to bleed and develop infections. Symptoms include: bone and joint pain, easy bruising and bleeding (such as bleeding gums, skin bleeding, nosebleeds, abnormal periods), feeling weak or tired, fever, loss of appetite and weight loss, paleness, pain or feeling of fullness below the ribs, pinpoint red spots on the skin (petechiae), swollen glands (lymphadenopathy) in the neck, under arms, and groin and night sweats. A physical exam may reveal the following: bruising, swollen liver, lymph nodes, and spleen, signs of bleeding (petechiae, purpura). Blood tests may include: complete blood count (CBC), bone marrow aspiration and biopsy, lumbar puncture (spinal tap) to check for leukemia cells in the spinal fluid, tests are also done to look for changes in the DNA inside the abnormal white cells. Certain DNA changes may determine how well a patient does (prognosis), and what kind of treatment is recommended (Jeha and Pui, 2012).

*Corresponding author e-mail: ahmedsamymmd@gmail.com

Acute myeloid leukemia (AML) is cancer that starts inside bone marrow. This is the soft tissue in the center of bones that helps form all blood cells. The cancer grows from cells that would normally turn into white blood cells. Symptoms of AML may include any of the following: bleeding from the nose, bleeding gums, bruising, bone pain or tenderness, fatigue, fever, heavy menstrual periods, pallor, shortness of breath (gets worse with exercise), skin rash or lesion, swollen gums (rare), Weight loss. There may be signs of a swollen spleen, liver, or lymph nodes. A complete blood count (CBC) may show anemia and a low number of platelets. A white blood cell count (WBC) can be high, low, or normal. Bone marrow aspiration and biopsy will show if there are any leukemia cells. Subtypes are based on specific genetic changes or mutations and how the leukemia cells appear under the microscope (Appelbaum et al., 2013).

2 Material and Methods

2.1 Sampling

Blood samples were taken from patients and healthy children at the Hematology & Oncology unit, pediatric hospital, Ain Shams University and Pediatric Department, Tanta Cancer Center. Blood samples were collected in EDTA tubes for complete blood count.

2.2 Patients and controls

- This study was done according to guidelines of Egyptian minister of health and population decree 95/year 2005 for medical research, good clinical practice, Declaration of Helsinki and World Health Organization Guidelines

-This study included

1-Group I: included 30 patients with acute leukemia (5-15 years old) (27 acute lymphoid leukemia + 3 acute myeloid leukemia) and classified into:

-Group Ia: included 9 children with leukemia newly diagnosed before chemotherapy.

-Group Ib: included 21 children with leukemia during chemotherapy.

2-Group II: included 20 healthy control children with the same age range of children with leukemia.

2.3: Measuring of complete blood count parameters

2.3.1: Count of white blood cells (Cheesbrough, 2005)

Reagent:

WBC diluting fluid: This is a weak acid solution to which gentian violet is added which stains the nucleus of white cells

Principle of test

Whole blood is diluted 1 in 20 in an acid reagent which haemolyzes the red cells (not the nucleus of nucleated red cells), leaving the white cells to be counted. White cells are counted microscopically using an Improved Neubauer ruled counting chamber (haemocytometer) and the number of WBCs per litre of blood calculated.

Blood sample:

EDTA anticoagulated blood or capillary blood can be used for counting white cells. Heparin or sodium citrate anticoagulated blood must not be used. The count should be performed within 6 hours (blood should not be refrigerated).

Method:

1. Measure 0.38 ml of diluting fluid and dispense it into a small container or tube.

2. Add 20 µL (0.02 ml, 20 cmm) of well-mixed EDTA anticoagulated venous blood or free flowing capillary blood and mix. Important: The volume of blood used in the test must be correct.

3. Assemble the counting chamber:

– Make sure the central grid areas of the chamber and the special haemocytometer cover glass are completely clean and dry.

– Slide the cover glass into position over the grid areas and press down on each side until rainbow colors (Newton's rings) are seen. Prior moistening of the chamber surface on each side of the grid areas will help the cover glass to adhere to the chamber.

4. Re-mix the diluted blood sample. Using a capillary, Pasteur pipette, or plastic bulb pastette held at an angle of about 45°, fill one of the grids of the chamber with the sample, taking care not to overfill the area.

5. Leave the chamber undisturbed for 2 minutes to allow time for the white cells to settle.

6. Dry the underside of the chamber and place it on the microscope stage. Using the 10 X objective with the condenser iris closed sufficiently to give good contrast, focus the rulings of the chamber and white cells. Focus the cells until they appear as small black dots.

7. Count the cells in the four large corner squares of the chamber marked W1, W2, W3, W4 .Include in the count the cells lying on the lines of two sides of each large square.

8. Report the number of white cells per litre of blood using the following simple calculation:

– Divide the total number of cells counted by 2.

– Divide the number obtained by 10.

The number obtained x 10^{9} is the white cell count

2.3.2 Count of platelets (Cheesbrough, 2005)

Reagent:

Ammonium oxalate 10 g/l (1% w/v) diluting fluid.

Principle of test:

Blood is diluted 1 in 20 in a filtered solution of ammonium oxalate reagent which lyses the red cells. Platelets are counted microscopically using an Improved Neubauer ruled counting chamber and the number of platelets per litre of blood calculated.

Blood sample: Use EDTA anticoagulated venous blood.

Method:

Perform a platelet count within 2 hours of collecting the blood.

1. Measure 0.38 ml of filtered ammonium oxalate diluting fluid and dispense it into a small container or tube.

2. Add 20 µL (0.02 ml, 20 cmm) of well-mixed anticoagulated venous blood and mix.

3. Assemble the counting chamber and fill it with well-mixed sample as described previously in steps 3 and 4 of the method for counting white cells.

4. Leave the chamber undisturbed for 20 minutes. To prevent drying of the fluid, place the chamber in a petri dish or plastic container on dampened tissue or blotting paper and cover with a lid.

5. Dry the underside of the chamber and place it on the microscope stage. Using the 10 X objective, focus the rulings of the grid and bring the central square of the chamber into view. Change to the 40X objective and focus the small platelets. They will be seen as small bright fragments.

6. Count the platelets in the small squares marked.

7. Report the number of platelets in 1 litre of blood. This is the actual number of platelets counted 10^9

2.3.3 Measuring of red blood cell count (Cheesbrough, 2005)

Procedures:

1- Add 4 ml of R.B.Cs counting soluting solution into small stoppered tube

2- Using Automatic pipette draw 0.02 (20 micron) capillary or venous blood

3- Add the blood into 4 ml of R.B.Cs counting soluting solution and mix well

4- Securely attach to counting chamber the special cover glass provided

5- Using a fine bore Pasteur pipette ,fill the chamber with the well mixed diluted blood

6- Allow the cells to settle for 5 minutes

7- Using a 40 X objective .count the number of cells in 1/5 sq.mm; using 5 of the small squares of the large center square.

Number of R. B. C s /mm^3= number of R.B.Cs counted in five small squares x 10.000

2.3.4 Measure of Hemoglobin (Cheesbrough, 2005)

Specimen: Capillary blood or EDTA anticoagulated venous blood can be used.

Principle of test:

Hemoglobin (oxyhemoglobin, methemoglobin, carboxy-hemoglobin) is converted to cyanomethemoglobin according to follwing reactions:

Hemoglobin —(Potassium ferricynide)→ Methemoglobin

Methemoglobin —(Potassium cynide)→ Cyanomethemoglobin

Reagents composition:

Drabkin reagent contain

Potassium ferricyanide 30 mmol/L & Potassium cyanide 38 mmol/L & Monopotassium phosphate 50 mmol/L

Procedure:

This method is done by spectrophotometer

Wavelength: 540 nm & Temperature 37 c

Cuvette : 1 Cm light path

Read against reagent blank

Working reagent	5 µL
Sample	20 µL

Mix and read the optical density within 1 hour

2.3.5 Measuring of packed cell volume (PCV/HCT) and Red cell induces (Cheesbrough, 2005)

2.3.5.a Packed cell volume

Principle of test:

The packed cell volume is that proportion of whole blood occupied by red cells, expressed as a ratio (litre/litre). Anticoagulated blood in a glass capillary of specified length, bore size, and wall-thickness is centrifuged in a microhaematocrit centrifuge at RCF 12 000–15 000 xg for 3–5 minutes to obtain constant packing of the red cells. A

small amount of plasma remains trapped between the packed red cells. The PCV value is read from the scale of a microhaematocrit reader or calculated by dividing the height of the red cell column by the height of the total column of blood.

Specimen:

To measure the PCV, either well mixed well oxygenated EDTA anticoagulated blood can be used or capillary blood collected into a heparinized capillary.

Method:

Whenever possible perform the test in duplicate.

1. About three quarters fill either:

– a plain capillary with well mixed EDTA anticoagulated blood (tested within 6 hours of collection), or

– a heparinized capillary with capillary blood.

2. Seal the unfilled end of the capillary using a sealant material

3. Carefully locate the filled capillary in one of the numbered slots of the microhaematocrit rotor with the sealed end against the rim gasket (to prevent breakage). Write the number of the slot on the patient's form. Position the inner lid carefully to avoid dislodging the tubes.

4. Centrifuge for 5 minutes (RCF 12 000– 15 000 xg).

5. Immediately after centrifuging, read the PCV. First check that there has been no leakage of blood from the capillary or breakage

$$\frac{\text{Length of red cell column (mm)}}{\text{Length of total column (mm)}} = \text{PCV}$$

2.3.5.b Measuring of red cell indices

Red cell indices most frequently used in the investigation of anemia are: Mean cell hemoglobin concentration (MCHC) & Mean cell volume (MCV) & Mean cell hemoglobin (MCH)

MCHC: Providing a laboratory is able to measure a PCV as previously described and perform an accurate hemoglobin test, an MCHC can be calculated (see following text). MCV and MCH: To calculate these indices, an accurate red blood cell (RBC) count is required. To perform an accurate RBC count, an electronic cell analyzer is needed. Most district laboratories will not therefore be able to calculate these indices; however, examining a well-stained blood film can help to detect macrocytosis or microcytosis.

$$\frac{\text{PCV (l/l)}}{\text{RBC} \times 10^{12}/\text{l}} = \text{MCV fl*}$$

$$\frac{\text{Hbg/l}}{\text{PCV (l/l)}} = \text{MCHC g/l}$$

$$\frac{\text{Hb in g/l}}{\text{RBC} \times 10^{12}/\text{l}} = \text{MCH pg*}$$

2.6 Blood films (Cheesbrough, 2005)

Technique of making a thin blood film

1. Make a blood spreader from a slide which has ground glass polished sides as follows:

-Examine each end of the slide and select the end which is completely smooth, i.e. no chips in the glass. If one end of the slide is frosted, use the non-frosted end (ensure it is smooth).

– Using a glass marker, etch across a corner of the slide.

– Holding the slide between a piece of cloth, break off the corner and discard safely the broken off piece of glass.

2 .Place a drop of blood on the end of a clean dry slide .Avoid making the drop too large (if too large, use a drop from the excess blood to make the film).

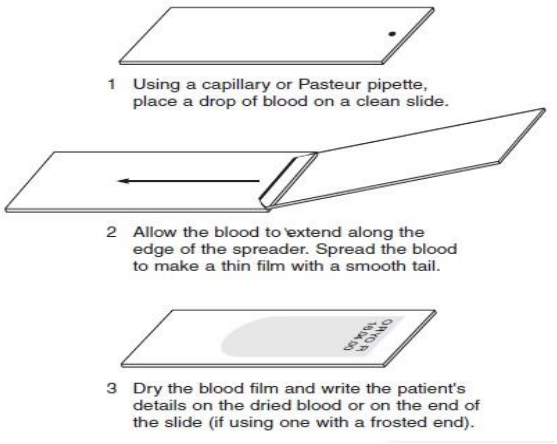

Fig.1: Spreading a thin blood film.

3. using a clean smooth edged spreader, draw the spreader back to touch the drop of blood and allow the blood to extend along the edge of the spreader. Holding the spreader at an angle of about 30°, spread the drop of blood to make a film about 40–50 mm in length (two thirds of the slide).

4. Wipe clean the end of the spreader.

5. Immediately air dry the film by waving the slide back and forth. Protect the dried film from dust and insects.

6. When completely dry and within a few minutes of making the blood film, fix it in absolute methanol.

3 Result

3.1 Determination of complete blood count (CBC) in children with leukemia children compared to healthy control children

3.1.1 Effect of leukemia and chemotherapy on total leukocyte count

The results in **Table (1)** showed that the total leukocyte count before chemotherapy was ranging from 25 x 10^3/cmm to 50 x 10^3/cmm but during chemotherapy was ranging from 0.9 x 10^3/cmm to 2.9 x 10^3/cmm and in healthy control was ranging from 5.2 x 10^3/cmm to 11.9 x 10^3/cmm. The results of total leucocytes count were significant decrease during chemotherapy compared to their count before chemotherapy and in healthy control (P value <0.001), (P value <0.001) respectively and also there were significant increase in total leukocyte counts (TLC) before chemotherapy compared to healthy control (P value <0.001).

Table 1: Effect of leukemia and chemotherapy on total leukocyte count

Groups	Total leukocyte count by (x 10^3/cmm)			ANOVA	
	Range by (x 10^3/cmm)	Mean ± SD		F	P-value
Before chemotherapy	25 - 50	34.622 ± 9.583		203.571	<0.001*
During Chemotherapy	0.9 - 2.9	2.014 ± 0.576			
Healthy Control	5.2 - 11.9	7.730 ± 1.700			
TUKEY'S Test					
Before chemotherapy & During chemotherapy		Before chemotherapy & Healthy control		During chemotherapy & Healthy control	
<0.001*		<0.001*		<0.001*	

Table 2: Effect of leukemia and chemotherapy on hemoglobin concentration

Groups	Hemoglobin (HB) by (gm/dl)			ANOVA	
	Range by gm/dl	Mean ± SD		F	P-value
Before chemotherapy	5.5 - 12.5	9.333 ± 2.436		29.091	<0.001*
During Chemotherapy	2.9 - 11.5	7.419 ± 2.015			
Healthy control	9.8 - 14.8	11.975 ± 1.515			
TUKEY'S Test					
Before chemotherapy & During chemotherapy		Before chemotherapy & Healthy control		During chemotherapy & Healthy control	
0.041*		0.003*		<0.001*	

Table 3: Effect of leukemia and chemotherapy on red blood cells count

Groups	Red blood cells count by (Million / cmm)			ANOVA	
	Range by (Million / cmm)	Mean ± SD		F	P-value
Before chemotherapy	2.1 - 4.11	3.112 ± 0.759		79.064	<0.001*
During chemotherapy	1.9 - 4.19	2.264 ± 0.508			
Healthy control	3.77 - 5.6	4.383 ± 0.457			
TUKEY'S Test					
Before chemotherapy & During chemotherapy		Before chemotherapy & Healthy control		During chemotherapy & Healthy control	
0.001*		<0.001*		<0.001*	

3.1.2 Effect of leukemia and chemotherapy on hemoglobin concentration

The results in **Table (2)** showed that the hemoglobin concentration before chemotherapy was ranging from 5.5 to 12.5 gm/dl but during chemotherapy was ranging from 2.9 to 11.5 gm/dl and in healthy control were ranging from 9.8 to 14.8 gm/dl. The results of hemoglobin concentrations were significant decrease during chemotherapy compared to their concentrations before chemotherapy and in healthy

Table 4: Effect of leukemia and chemotherapy on platelets count

Groups	Platelets count by (x 10^3/cmm)						ANOVA	
	Range by (x 10^3/cmm)			Mean	±	SD	F	P-value
Before chemotherapy	61	-	193	126.333	±	34.128	127.584	<0.001*
During chemotherapy	16	-	42	28.333	±	7.625		
Healthy Control	155	-	360	250.300	±	65.945		

TUKEY'S Test		
Before chemotherapy & During chemotherapy	Before chemotherapy & Healthy control	During chemotherapy & Healthy control
<0.001*	<0.001*	<0.001*

Table 5: Effect of leukemia and chemotherapy on Packed Cell volume

Groups	Packed Cell volume by (%)						ANOVA	
	Range by %			Mean	±	SD	F	P-value
Before chemotherapy	16.5	-	38	26.244	±	7.305	49.901	<0.001*
During Chemotherapy	10.3	-	30.3	21.048	±	3.440		
Healthy control	31.2	-	49.2	36.230	±	4.949		

TUKEY'S Test		
Before chemotherapy & During chemotherapy	Before chemotherapy & Healthy control	During chemotherapy & Healthy control
0.028*	<0.001*	<0.001*

Table 6: Effect of leukemia and chemotherapy mean cell volume (MCV)

Groups	Mean cell volume by (FL)						ANOVA	
	Range by FL			Mean	±	SD	F	P-value
Before chemotherapy	53.2	-	114.6	85.700	±	19.177	3.323	0.045*
During Chemotherapy	52.3	-	144.3	96.033	±	22.803		
Healthy control	70.9	-	90.2	82.565	±	5.331		

TUKEY'S Test		
Before chemotherapy & During chemotherapy	Before chemotherapy & Healthy control	During chemotherapy & Healthy control
0.296	0.893	0.041*

control children (P value =0.041), ((P value < 0.001) respectively, and also there were significant decrease in hemoglobin concentrations before chemotherapy compared to healthy control (P value = 0.003).

3.1.3 Effect of leukemia and chemotherapy on red blood cells count

Table 7: Effect of leukemia and chemotherapy on mean cell hemoglobin

Groups	Mean cell hemoglobin by (PG)						ANOVA	
	Range by PG			Mean	±	SD	F	P-value
Before chemotherapy	17.7	-	41.2	30.511	±	7.063	3.826	0.029*
During Chemotherapy	15	-	54.8	33.452	±	9.715		
Healthy Control	22.8	-	30.4	27.325	±	1.993		

TUKEY'S Test		
Before chemotherapy & During chemotherapy	Before chemotherapy & Healthy control	During chemotherapy & Healthy control
0.555	0.507	0.022*

Table 8: Effect of leukemia and chemotherapy mean cell hemoglobin concentration

Groups	Mean cell hemoglobin concentration by (g/dl)						ANOVA	
	Range by g/dl			Mean	±	SD	F	P-value
Before chemotherapy	24.6	-	51.5	36.456	±	8.936	0.894	0.416
During Chemotherapy	18.2	-	56.7	35.590	±	9.527		
Healthy control	30.1	-	35.7	33.105	±	1.570		

Table 9: Effect of leukemia and chemotherapy on neutrophils percent

Groups	Neutrophils by (%)						ANOVA	
	Range by %			Mean	±	SD	F	P-value
Before chemotherapy	20	-	35	28.000	±	5.025	130.991	<0.001*
During chemotherapy	15	-	40	21.256	±	5.596		
Healthy Control	44	-	71	53.250	±	7.793		

TUKEY'S Test		
Before chemotherapy & During chemotherapy	Before chemotherapy & Healthy control	During chemotherapy & Healthy control
0.033*	<0.001*	<0.001*

The results in **Table (3)** showed that the red blood cells count before chemotherapy was ranging from 2.1 Million / cmm to 4.11 Million / cmm but during chemotherapy was ranging from 1.9 Million / cmm to 4.19 Million / cmm but in healthy control was ranging from 3.77 Million / cmm to 5.6 Million / cmm. The results of red blood cells count were significant decrease during chemotherapy compared to their counts before chemotherapy and in healthy control children (P value =0.001) (P value < 0.001) respectively and also there were significant decrease in red blood cells count before chemotherapy compared to healthy control (P value < 0.001).

3.1.4 Effect of leukemia and chemotherapy on platelets count

The results in **Table (4)** showed that the platelets count before chemotherapy was ranging from 61 x 10^3/cmm to 193 x 10^3/cmm but during chemotherapy was ranging from 16 x 10^3/cmm to 42 x 10^3/cmm and in healthy control was ranging from 155 x 10^3/cmm to 360 x 10^3/cmm. The results of platelets count were significant decrease during chemotherapy compared to their count before chemotherapy and in healthy control children (P value <0.001), (P value <0.001) respectively and also there were significant decrease in platelets count before chemotherapy compared to healthy

Table 10: Effect of leukemia and chemotherapy on lymphocytes percent

Groups	Lymphocytes by (%)		ANOVA	
	Range by %	Mean ± SD	F	P-value
Before chemotherapy	18 - 60	41.000 ± 14.009	78.737	<0.001*
During chemotherapy	50 - 79	68.619 ± 6.734		
Healthy Control	21 - 46	37.250 ± 6.719		
TUKEY'S Test				
Before chemotherapy & During chemotherapy	**Before chemotherapy & Healthy control**	**During chemotherapy & Healthy control**		
<0.001*	0.514	<0.001*		

Table 11: Effect of leukemia and chemotherapy on eosinophils percent

Groups	Eosinophils by (%)		ANOVA	
	Range by %	Mean ± SD	F	P-value
Before chemotherapy	1 - 2	1.556 ± 0.527	22.422	<0.001*
During chemotherapy	2 - 4	2.952 ± 0.921		
Healthy Control	2 - 5	3.950 ± 0.999		
TUKEY'S Test				
Before chemotherapy & During chemotherapy	**Before chemotherapy & Healthy control**	**During chemotherapy & Healthy control**		
0.001*	<0.001*	0.003*		

Table 12: Effect of leukemia and chemotherapy on monocytes percent

Groups	Monocytes by (%)		ANOVA	
	Range by %	Mean ± SD	F	P-value
Before chemotherapy	2 - 3	2.444 ± 0.527	9.522	<0.001*
During chemotherapy	2 - 10	5.714 ± 2.283		
Healthy Control	2 - 10	5.450 ± 1.986		
TUKEY'S Test				
Before chemotherapy & During chemotherapy	**Before chemotherapy & Healthy control**	**During chemotherapy & Healthy control**		
<0.001*	0.001*	0.903		

control (P value < 0.001).

3.1.5 Effect of leukemia and chemotherapy on Packed Cell volume

The results in **Table (5)** showed that the packed cell volume before chemotherapy was ranging from 16% to 38 % but during chemotherapy was ranging from 10.3% to 30.3 % and in healthy control was ranging from 31.2% to 49.2 %. The results of packed cell volume were significant decrease during chemotherapy compared to their percent before chemotherapy and in healthy control children (P value = 0.028), (P value <0.001) respectively and also there were significant decrease in packed cell volume (PCV/ HCT) before chemotherapy compared to healthy control (P value < 0.001).

3.1.6 Effect of leukemia and chemotherapy mean cell volume (MCV)

The results in **Table (6)** showed that the mean cell volume before chemotherapy was ranging from 53.2 FL to 114.6 FL but during chemotherapy was ranging from 52.3 FL to 144.3 FL and in healthy control were ranging from 70.9 FL to 90.2 FL. The results also revealed that, there were significant increase in mean cell volume (MCV) during chemotherapy compared to healthy control children (P value =0.041).

3.1.7 Effect of leukemia and chemotherapy on mean cell hemoglobin

The results in **Table (7)** showed that the mean cell hemoglobin before chemotherapy was ranging from 17.7 PG to 41.2 PG but during chemotherapy was ranging from 15 PG to 54.8 PG and in healthy control was ranging from 22.8 PG to 30.4 PG. The results also revealed that, there were significant increase in mean cell volume (MCV) during chemotherapy compared to healthy control children (P value =0.022).

3.1.8 Effect of leukemia and chemotherapy mean cell hemoglobin concentration

The results in **Table (8)** showed that mean cell hemoglobin concentrations before chemotherapy were ranging from 24.6 g/dl to 51.5 g/dl but during chemotherapy were ranging from 18.2 g/dl to 56.7 g/dl and in healthy control were ranging from 30.1 g/dl to 35.7 g/dl. The results also revealed that, there was no significant difference in mean cell hemoglobin concentration (MCHC) between before chemotherapy and during chemotherapy compared to healthy control (P value = 0.416).

3.1.9 Effect of leukemia and chemotherapy on neutrophils percent

The results in **Table (9)** showed that neutrophils percent before chemotherapy was ranging from 20% to 35% but during chemotherapy was ranging from 15% to 40 % and in healthy control was ranging from 44% to 71 %. The results of neutrophils percent were significant decrease during chemotherapy compared to their percent before chemotherapy and in healthy control children (P value =0.033), (P value <0.001) respectively and also there were significant decrease in neutrophils percent before chemotherapy compared to healthy control (P value < 0.001).

3.1.10 Effect of leukemia and chemotherapy on lymphocytes percent

The results in **Table (10)** showed that lymphocytes percent before chemotherapy was ranging from 18% to 60 % but during chemotherapy was ranging from 50% to 79 % and in healthy control was ranging from 21% to 46 %. The results of lymphocytes percent were significant increase during chemotherapy compared to their percent before chemotherapy and in healthy control children (P value <0.001), (P value <0.001) respectively.

3.1.11 Effect of leukemia and chemotherapy on eosinophils percent

The results in **Table (11)** showed that eosinophils percent before chemotherapy was ranging from 1% to 2 % but during chemotherapy was ranging from 2% to 4 % and in healthy control was ranging from 2% to 5 %. The results of eosinophils percent were significant decrease during chemotherapy compared to healthy control children (P value =0.003), and also there were significant decrease in eosinophils percent before chemotherapy compared to their percent during chemotherapy and in healthy control (P value = 0.001), (P value < 0.001) respectively.

3.1.12 Effect of leukemia and chemotherapy on monocytes percent

The results in **Table (12)** showed that monocytes percent before chemotherapy was ranging from 2% to 3 % but during chemotherapy was ranging from 2% to 10 % and in healthy control was ranging from 2% to 10 %. The results of monocytes percent were significant increase during chemotherapy compared to before chemotherapy (P value <0.001), and also there were significant decrease in monocytes percent before chemotherapy compared to healthy control (P value = 0.001).

3.1.13 Effect of chemotherapy on basophiles percent

The results in **Table (13)** showed that basophiles percent before chemotherapy was 0 % to all cases but during chemotherapy was ranging from 1% to 3 % and in healthy control was 1% to all cases. The results also revealed that, there were no significant difference in basophiles percent between children with leukemia during chemotherapy and

healthy control (P value = 0.188).

3.1.14 Effect of chemotherapy on abnormal blood cells

The results in **Table (14)** showed that, basophiles percent

Table 13: Effect of leukemia and chemotherapy on basophiles percent

Groups	Basophiles by (%)						T-Test	
	Range by %			Mean	±	SD	t	P-value
During chemotherapy	1	-	3	1.833	±	0.753	1.485	0.188
Healthy control	1	-	1	1.000	±	0.000		

Table 14: Effect of chemotherapy on abnormal blood cells

Groups	Abnormal blood cells by (%)						T-Test	
	Range by %			Mean	±	SD	t	P-value
Before chemotherapy	20	-	52	30.375	±	12.270	5.388	<0.001*
During chemotherapy	1	-	5	3.000	±	1.265		

before chemotherapy was ranging from 20% to 52 % but during chemotherapy was ranging from 1% to 5 % and in healthy control was 0% to all cases. The results also revealed that, there was significant decrease in abnormal blood cells during chemotherapy compared to before chemotherapy (P value <0.001).

4 Discussion

Leukemia is the most common type of childhood cancer. It accounts for 30% of all cancers diagnosed in children younger than 15 years. Within this population, acute lymphocytic leukemia (ALL) occurs approximately five times more frequently than acute myelogenous leukemia (AML) and accounts for approximately 78% of all childhood leukemia diagnoses. Epidemiologic studies of acute leukemias in children have examined possible risk factors, including genetic, infectious, and environmental, in an attempt to determine etiology. Only one environmental risk factor (ionizing radiation) has been significantly linked to ALL or AML (Belson et al., 2007).

In the present work, complete blood count (CBC) showed that there were significant decrease in total leukocyte count (TLC) during chemotherapy compared to before chemotherapy, significant decrease in HB, RBCS count, platelets count, HCT, neutrophils percent during chemotherapy compared to before and healthy control, significant decrease in HB, RBCS count, platelets count, HCT, neutrophils percent, eosinophils percent, monocytes percent before chemotherapy compared to healthy control, significant increase in MCV and MCH during chemotherapy compared to healthy control, significant decrease in eosinophils during chemotherapy compared to healthy control, significant increase in lymphocytes percent during chemotherapy compared to before and healthy control and significant increase in monocytes during chemotherapy compared to before chemotherapy.

In the same context, Lex et al. (2001) studied infectious complications in children with acute lymphoblastic leukemia and T-cell lymphoma, they reported that the incidence of febrile episodes during therapy appeared to be correlated with certain chemotherapeutic drug combinations. The highest rate was found after high-dose cytarabine and asparaginase causing a long period of leukopenia. However, after other chemotherapy courses with a similar duration of leukopenia the incidence of febrile episodes was significantly lower, suggesting that specific interactions of different chemotherapeutic agents with the immune response might be an important factor in development of infections, in the same respect, Levinsen et al. (2015), reported that the degree of leukopenia, neutropenia, thrombocytopenia and rise in aminotransferases were all significantly related to 6-mercaptopurine (6MP) dose

The obtained results were in agreement with Yilmaz et al. (2008) who studied the assessment of febrile neutropenia episodes in children with acute leukemia .They reported that febrile neutropenia was observed mostly during consolidation therapy. Mucositis was the most identified focus; gram-negative microorganisms were the most identified pathogens. Five patients developed invasive fungal infections. For the same consideration Wiley et al. (1990) evaluated the courses of 115 consecutive cases of pediatric acute leukemia treated with induction chemotherapy. Seventy-two patients developed fever associated with neutropenia; 15 developed systemic fungal infections.

The obtained results are with the same line with those obtained from Neville et al. (1984) who reported that children with acute leukemia who administrated by

consolidation chemotherapy, with a combination of cytosine arabinoside (Ara-C) and 6-thioguanine (TG), was associated with a fall in the erythrocyte concentration in peripheral blood

In the same context, Bhatia et al. (2007) reported that thrombocytopenia occurs at various grades of severity in patients with leukemia undergoing myelosuppressive chemotherapy

References

[1] Appelbaum, F.R. (2013): Acute leukemias in adults. In: Niederhuber JE, Armitage JO, Doroshow JH, eds. Abeloff's Clinical Oncology. 5th ed. Philadelphia, PA: Elsevier Churchill Livingstone; chap 98.pp:120-132

[2] Appelbaum, F.R. (2011): The acute leukemias. In: Goldman L, Schafer AI, eds. Goldman's Cecil Medicine. 24th ed. Philadelphia, Pa: Elsevier Saunders; chap 189.PP:354-361

[3] Belson, M.; Kingsley, B. and Holmes, A.(2007): Risk factors for acute leukemia in children: a review. Environ Health Perspect. 115(1):138-45

[4] Bhatia, M.; Davenport, V. and Cairo, M.S.(2007): The role of interleukin-11 to prevent chemotherapy-induced thrombocytopenia in patients with solid tumors, lymphoma, acute myeloid leukemia and bone marrow failure syndromes. Leuk Lymphoma. 48(1):9-15.

[5] Cheesbroug, Monica. (2005): Hematological Tests .in: District laboratory practice in tropical countries part2. 2nd ed. Cambridge university press, UK, PP: 38: 326

[6] Jeha, S. and Pui, C.H.(2012): Clinical manifestations and treatment of acute lymphoblastic leukemia in children. In: Hoffman R, Benz EJ Jr, Silberstein LE. Hematology*:* Basic Principles and Practice. 6th ed.Philadelphia, Pa: Elsevier Saunders; chap 64. PP: 224

[7] Kantarjian, H. and O'Brien S.(2011): The chronic leukemias. In: Goldman L, Schafer AI, eds. Goldman's Cecil Medicine. 24th ed. Philadelphia, Pa: Elsevier Saunders; chap 190.PP:381:390

[8] Levinsen, M.; Rosthøj, S.; Nygaard, U.; Heldrup, J.; Harila-Saari, A.; Jonsson, OG.; Bechensteen, A.G.; Abrahamsson, J; Lausen, B.; Frandsen, T.L.; Weinshilboum, R.M. and Schmiegelow, K.(2015): Myelotoxicity after high-dose methotrexate in childhood acute leukemia is influenced by 6-mercaptopurine dosing but not by intermediate thiopurine methyltransferase activity. Cancer Chemother Pharmacol. 75(1):59-66.

[9] Lex, C.; Körholz, D.; Kohlmüller, B.; Bönig, H.; Willers, R.; Kramm, C.M. and Göbel ,U: (2001): Infectious complications in children with acute lymphoblastic leukemia and T-cell lymphoma--a rationale for tailored supportive care. Support Care Cancer. 9(7):514-21.

[10] Neville, A.J.; Rand, C.A.; Barr R.D.and Mohan, Pa.i K.R.(1984): Drug-induced stomatocytosis and anemia during consolidation chemotherapy of childhood acute leukemia. Am J Med Sci. 287(1):3-7.

[11] Wiley, J.M.; Smith, N.; Leventhal, B.G.; Graham, M.L.; Strauss, L.C.; Hurwitz, C.A.; Modlin, J.; Mellits, D.; Baumgardner, R. and Corden, B.J.(1990): Invasive fungal disease in pediatric acute leukemia patients with fever and neutropenia during induction chemotherapy: a multivariate analysis of risk factors. J Clin Oncol. 8(2):280-6.

[12] Yilmaz, S.; Oren H.; Demircioğlu, F. and Irken, G.(2008): Assessment of febrile neutropenia episodes in children with acute leukemia treated with BFM protocols. Pediatr Hematol Oncol. 25(3):195-204.