

## **AIM OF THE STUDY**

The present study aimed to study serum level of Angiotensin II receptor type 1 and the soluble Angiotensin Converting Enzyme (CD143) in patients with Acute Leukemia in order to extrapolate their possible prognostic value.

## **SUBJECTS AND METHODS**

### **Subjects**

Individuals submitted to this study were divided into three groups:

**Group I:** Involved 20 healthy volunteers clinically free from any disease (control group), their mean age was 33.25 years and were chosen from the staff members of MRI, Alexandria University, and clinical research center, Faculty of Medicine, Alexandria University and their relatives.

**Group II:** Involved 27 patients with newly diagnosed AML.

**Group III:** Involved 10 patients with newly diagnosed ALL.

Patients in group II and III were of matched age as the control group and were recruited from Hematology department, MRI, Alexandria University and clinical research center, Faculty of Medicine, Alexandria University. An informed consent was taken from all contributors in this study.

This work was conducted according to the guidelines of the local ethical committee of MRI.

### **Methods**

To all patients the following investigations were done:

- 1- Full history recording
- 2- Thorough clinical examination
- 3- Routine laboratory investigations including: complete blood picture, bone marrow examination<sup>(179)</sup>, liver and kidney functions.
- 4- Radiological investigations including: chest x ray, abdominal ultrasound and ECG study.

### **Exclusion criteria:**

Patients with preceding clonal hematological diseases such as myeloproliferative disorders, myelodysplastic syndromes, multiple myeloma, lymphoproliferative disorders, aplastic anemia, patients with hypertension or taking ACE inhibitors or patients who previously received chemo or radio-therapy for solid tumors were excluded from this study.

### **Regimens of treatment**

Patients with AML received 3 + 7 protocol of induction including: Daunomycin 45 mg/m<sup>2</sup> for 3 days, Cytosine arabinoside 100 mg/ m<sup>2</sup> x2 / day for 7 days.

Patients with ALL received induction protocol as follows: Vincristine 1.4 mg/ m<sup>2</sup> days 1, 8, 15, 22, Prednisolone 1 mg / kg / day x 28 days, Doxorubicin 25 mg/ m<sup>2</sup> days 1, 2, 3.

After completion of the cycles and restoration of bone marrow cellularity, bone marrow aspiration was done. Patients who achieved complete remission had a BM blasts less than 5%. Those who did not achieve complete remission received a 2<sup>nd</sup> induction cycle.

**Blood sampling:**

Five milliliters venous blood samples were collected in the morning from normal control subjects and patients in groups II and III (at presentation and after the first induction cycle of chemotherapy). Blood samples were allowed to clot for 20 minutes before centrifugation, centrifuged at 3000 rpm for 20 minutes to isolate serum. The serum was stored at -80c until assayed. In each blood sample, ACE and Ang IIT1R were measured by ELISA kit.

## Determination of serum Human ACE

### Principle of the assay

The kit assay human ACE1 level in the sample, use purified human ACE1 antibody to coat microtiter plate wells, make solid – phase antibody, then add ACE1 to wells, combined ACE1 antibody which with HRP labeled, become antibody-antigen-enzyme-antibody complex, add TMB substrate solution, TMB become blue color at HRP enzyme-catalyzed, reaction is terminated by the addition of a sulphuric acid solution and the color change is measured spectrophotometrically at a wavelength of 450 nm. The active of ACE1 in the sample is then determined by comparing the O.D. of the sample to the standard curve.<sup>(180)</sup>

### Reagents

One bottle standard	90u/l
One bottle standard diluents	1.5ml
One bottle HRP-conjugate reagent	6ml
One bottle sample diluents	6ml
One bottle chromagen solution A	6ml
One bottle chromagen solution B	6ml
One bottle stop solution	6ml
One bottle wash solution	20mlx3fold

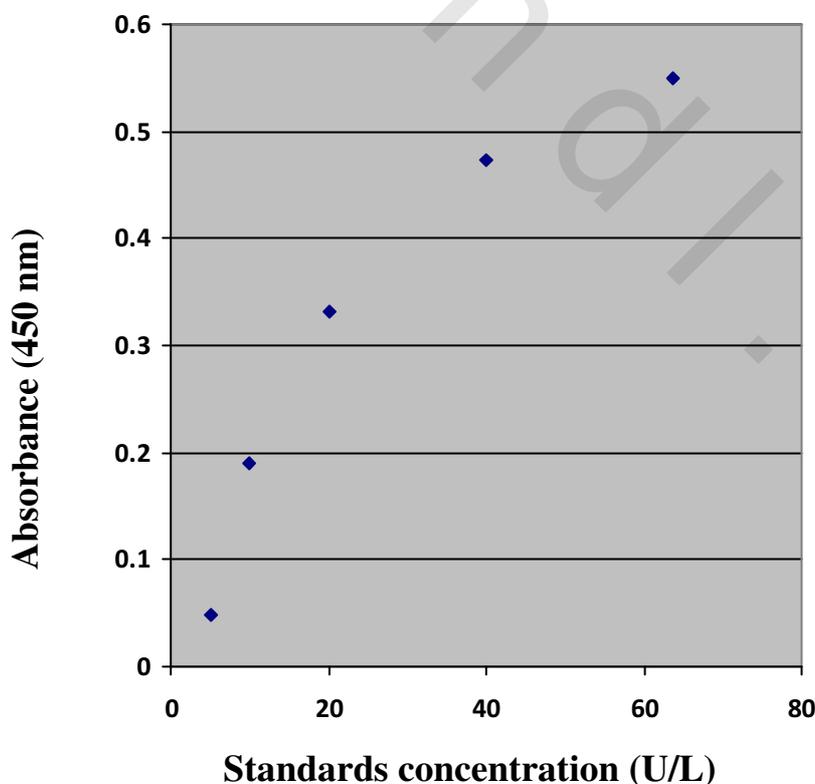
### Assay Procedure

- 1- Each sample, standard, blank should be assayed in a duplicate
- 2- ACE1 standard dilution was made as follows:
  - 50  $\mu$ l of standard diluents were added in duplicate to all standard wells
  - 100  $\mu$ l of prepared standard were pipetted (concentration 720mg/l) in duplicate in the first and second well, the contents of the two wells were gently mixed (concentration of standard S1= 480mg/l). 100  $\mu$ l were taken out and transferred to the third and fourth well separately. After mixing, 50  $\mu$ l of the contents from well 3 and 4 were decanted. 50  $\mu$ l were the added to the fifth and sixth well. This process was repeated two times. Creating two rows of human ACE1 standard dilution ranging from 480 to 40 mg/l. 50  $\mu$ l of the contents from the last microwell used were discarded.
- 3- 50  $\mu$ l of sample diluents were pipetted into the blank wells.
- 4- 40  $\mu$ l of sample diluents and 10  $\mu$ l of testing sample were added to testing sample well and gently mixed (sample final dilution was 5 fold).
- 5- The plate was closed with closure plate membrane and incubated for 30 minutes at 37c.
- 6- Wash solution was diluted 30 fold with distilled water and reserved.
- 7- Closure plate membrane was removed and wells were emptied. Microwell strips were washed 5 times with approximately 400  $\mu$ l wash buffer per well with through aspiration of microwell contents between washes. Wash buffer was allowed to sit in wells for about 10-15 seconds before aspiration.
- 8- After the last wash step, wells were emptied and microwell strips were tapped on absorber paper to remove excess wash buffer.

- 9- 50  $\mu$ l of HRP-conjugate reagent were added to each well except blank well.
- 10- Plate was closed with closure plate membrane and incubated for 30 minutes at room temperature.
- 11- Closure plate membrane was removed and wells were emptied. Microwell strips were washed 5 times.
- 12- Chromagen solution A and chromagen solution B were added to each well, the light preservation was evaded for 15 minutes at 37c.
- 13- 50  $\mu$ l of stop solution was added to each well to stop the reaction (the blue color change to yellow color).
- 14- The absorbance was read at 450nm after adding stop solution and within 15 minute.

### Calculation of Results

- 1- Average absorbance values were calculated for each set of duplicate standards and samples.
- 2- A standard curve was created by plotting the mean absorbance for each standard concentration on the Y axis against ACE1 concentrations on the X axis as log. log paper. A best fit curve was drawn through the points of the graph (Fig ) represents the standard curve of ACE1.
- 3- The concentration of ACE1 for each sample was determined from the standard curve.
- 4- As the sample were diluted 1:5 (10  $\mu$ l sample+40  $\mu$ l sample diluents), the concentration read from the standard curve was multiplied by the dilution factor (x5).



**Figure (8):** Standard curve of ACE

## Determination of serum Human Ang IIT1R

### Principle of the assay

The kit assay human Ang IIT1R level in the sample, use purified human Ang IIT1R antibody to coat microtiter plate wells, make solid-phase antibody, then add Ang IIT1R to wells, combined Ang IIT1R antibody which with HRP labeled, become antibody-antigen-enzyme-antibody complex, after washing completely, add TMB substrate solution, TMB substrate become blue color at HRP enzyme-catalyzed, reaction is terminated by the addition of a sulphuric acid solution and the color change is measured spectrophotometrically at a wavelength of 450 nm. The concentration of Ang IIT1R in the samples is then determined by comparing the O.D. of the samples to the standard curve.<sup>(180)</sup>

### Reagents

Micro ELISA strip plate	12wellx8strips
One bottle standard	0.5ml
One bottle standard diluents	1.5ml
One bottle HRP-conjugate reagent	6ml
One bottle sample diluents	6ml
One bottle chromagen solution A	6ml
One bottle chromagen solution B	6ml
One bottle stop solution	6ml
One bottle wash solution	20mlx30fold

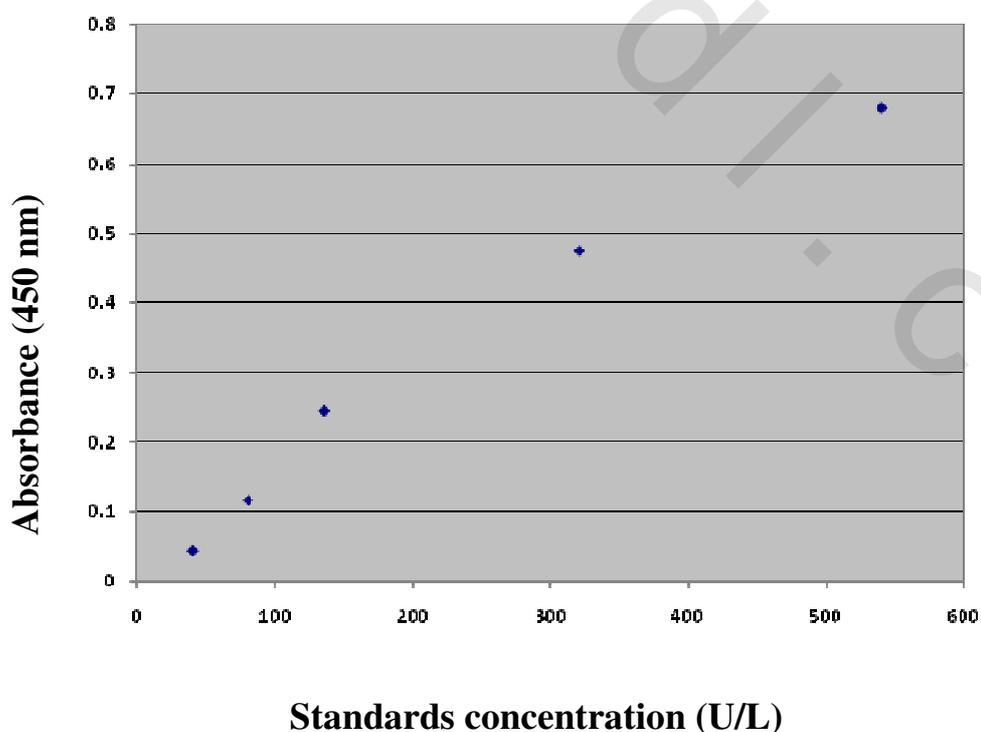
### Assay Procedure

- 1- Each sample, standard, blank should be assayed in a duplicate
- 2- AngIIR-1 standard dilution was made as follows:
  - 50  $\mu$ l of standard diluents were added in duplicate to all standard wells.
  - 100  $\mu$ l of prepared standard were pipetted (concentration 720mg/l) in duplicate in the first and second well, the contents of the two wells were gently mixed (concentration of standard S1= 480mg/l). 100  $\mu$ l were taken out and transferred to the third and fourth well separately. After mixing, 50  $\mu$ l of the contents from well 3 and 4 were decanted. 50  $\mu$ l were the added to the fifth and sixth well. This process was repeated two times. Creating two rows of human AngIIR-1 standard dilution ranging from 480 to 40 mg/l. 50  $\mu$ l of the contents from the last microwell used were discarded.
- 3- 50  $\mu$ l of sample diluents were pipetted into the blank wells.
- 4- 40  $\mu$ l of sample diluents and 10  $\mu$ l of testing sample were added to testing sample well and gently mixed (sample final dilution was 5 fold).
- 5- The plate was closed with closure plate membrane and incubated for 30 minutes at 37c.
- 6- Wash solution was diluted 30 fold with distilled water and reserved.

- 7- Closure plate membrane was removed and wells were emptied. Microwell strips were washed 5 times with approximately 400  $\mu$ l wash buffer per well with through aspiration of microwell contents between washes. Wash buffer was allowed to sit in wells for about 10-15 seconds before aspiration.
- 8- After the last wash step, wells were emptied and microwell strips were tapped on absorber paper to remove excess wash buffer.
- 9- 50  $\mu$ l of HRP-conjugate reagent were added to each well except blank well.
- 10- Plate was closed with closure plate membrane and incubated for 30 minutes at room temperature.
- 11- Closure plate membrane was removed and wells were emptied. Microwell strips were washed 5 times.
- 12- Chromagen solution A and chromagen solution B were added to each well, the light preservation was evaded for 15 minutes at 37c.
- 13- 50  $\mu$ l of stop solution was added to each well to stop the reaction (the blue color change to yellow color).
- 14- The absorbance was read at 450nm after adding stop solution and within 15 minutes.

### Calculation of Results

- 1- The standard density was taken as the horizontal and the optical density value for the vertical.
- 2- The standard curve was drawn on graph paper.
- 3- The corresponding density was found according the same optical density value by the sample curve, multiplied by the dilution multiple.



**Figure (9):** Standard curve of Ang IIT1R

## **Statistical analysis**

Statistical analysis was carried out using SPSS statistics software version 20. Quantitative data were tested for normality using Kolmogorov-Smirnov test. Abnormally distributed data was given as range (minimum-maximum). Non-parametric statistical tests of significance were applied; Mann-Whitney test was used to compare two independent groups. All applied statistical tests of significance were two-tailed.

Receiver operating characteristic (ROC), carried out using MedCalc statistical software, was used to evaluate the diagnostic and prognostic accuracy of a test to correctly pick cured and non- cured subjects. The larger the area under the curve (AUC) i.e. closer to 1, the better the performance of a diagnostic test. Youden index was used to find the cut-off point i.e. the point that gives maximum correct classification. At this cut- off point, sensitivity; defined as the probability that the test is positive in patients with the disease and specificity; defined as the probability that the test is negative in patients without the disease were determined. Also, positive predictive value; defined as probability that the patient has the disease when the test is positive and negative predictive value; defined as probability that the patient will not have the disease when the test is negative were identified.

## RESULTS

### 1 – BIOCHEMICAL RESULTS

#### 1.1- ACE activity (U/L) in normal control subjects and patients groups with either AML or ALL before and after therapy

Range and mean values  $\pm$  SE of ACE activity (U/L) in normal control subjects and patients groups with either AML or ALL before and after therapy were shown in table (1) and illustrated in figure (10). Statistical analyses of these results were represented in table (1).

As presented in table (1), serum ACE activity (U/L) was ranged from 43.0 - 47.25 with a mean value  $45.51 \pm 0.29$  in normal control group, from 48.25 - 71.0 with a mean value  $53.56 \pm 1.20$  in AML group before therapy, from 43.0 - 65.0 with a mean value  $48.12 \pm 1.02$  in AML group after therapy, from 49.25 - 69.0 with a mean value  $53.10 \pm 1.81$  in ALL group before therapy, and from 43.75 - 63.0 with a mean value  $49.20 \pm 1.61$  in ALL group after therapy.

The statistical analyses of these results revealed that the activity of ACE (U/L) in patients groups with either AML or ALL before therapy was significantly higher than in control group. After therapy, the activity of this enzyme in both groups of patients was significantly decreased but still significantly higher than in normal control subjects.

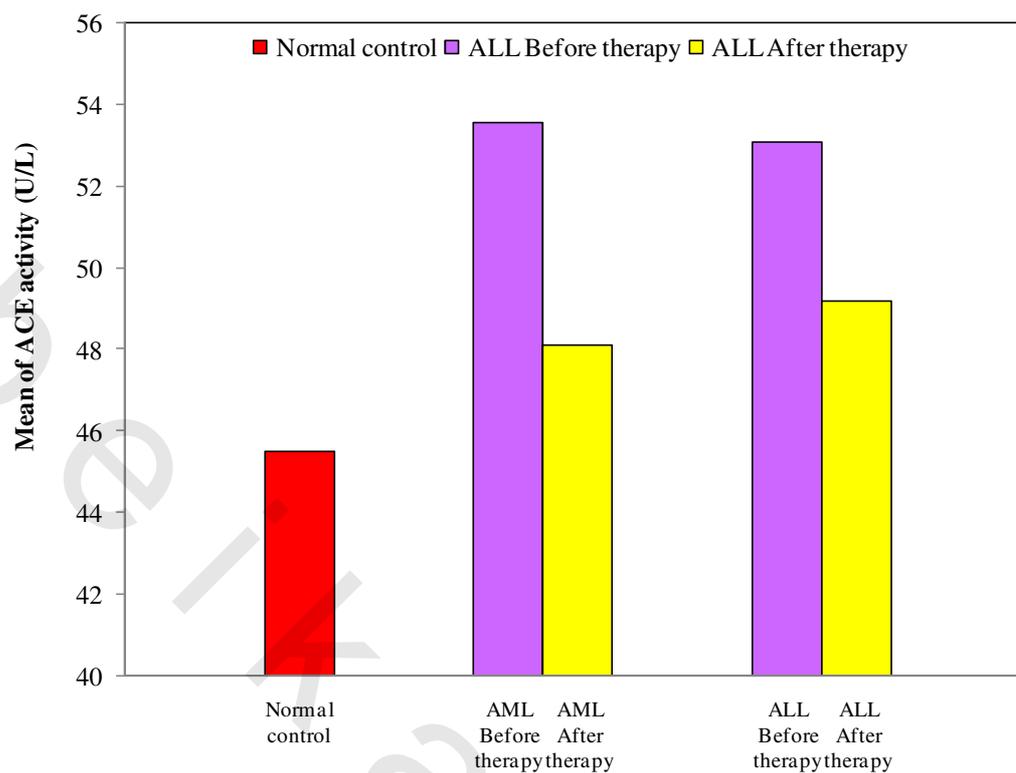
**Table (1): Statistical analyses of serum ACE activity (U/L) in normal control subjects and patients groups either with AML or ALL before and after therapy**

	Normal control (n= 20)	Patients with AML (n= 27)		Patients with ALL (n= 10)	
		Before therapy	After therapy	Before therapy	After therapy
<b>ACE (U/L)</b>					
Range	43.0 - 47.25	48.25 - 71.0	43.0 - 65.0	49.25 - 69.0	43.75 - 63.0
Mean $\pm$ SE	$45.51 \pm 0.29$	$53.56 \pm 1.20$	$48.12 \pm 1.02$	$53.10 \pm 1.81$	$49.20 \pm 1.61$
<b>p<sub>1</sub></b>		<0.001*	0.031*	<0.001*	<0.001*
<b>p<sub>2</sub></b>		<0.001*		0.005*	

p<sub>1</sub>: p comparing mean values with control group

p<sub>2</sub>: p comparing mean values before and after therapy

\*: Statistically significant at  $p \leq 0.05$



**Figure (10):** ACE activity (U/L) in all studied groups

## 1.2- Ang IIT1R level (U/L) in normal control subjects and patients groups with either AML or ALL before and after therapy

Range and mean values  $\pm$  SE of Ang IIT1R level (U/L) in control and patients groups with either AML or ALL before and after therapy were shown in table (2) and illustrated in figure (11). Statistical analyses of these results is represented in table (2).

As presented in table (2), serum Ang IIT1R level (U/L) was ranged from 324.0 - 360.0 with a mean value  $344.60 \pm 1.61$  in normal control group, from 370.0 - 520.0 with a mean value  $418.33 \pm 7.39$  in AML group before therapy, from 336.0 - 400.0 with a mean value  $358.81 \pm 3.50$  in AML group after therapy, from 378.0 - 550.0 with a mean value  $432.0 \pm 18.14$  in ALL group before therapy, and from 338.0 - 392.0 with a mean value  $361.40 \pm 5.66$  in ALL group after therapy.

The statistical analyses of these results revealed that the level of Ang IIT1R (U/L) in patients groups with either AML or ALL before therapy was significantly higher than in control group. After therapy, it was noticed that the level of this receptor in both groups of patients was significantly decreased than in their corresponding values before therapy and still significantly higher than in normal control group.

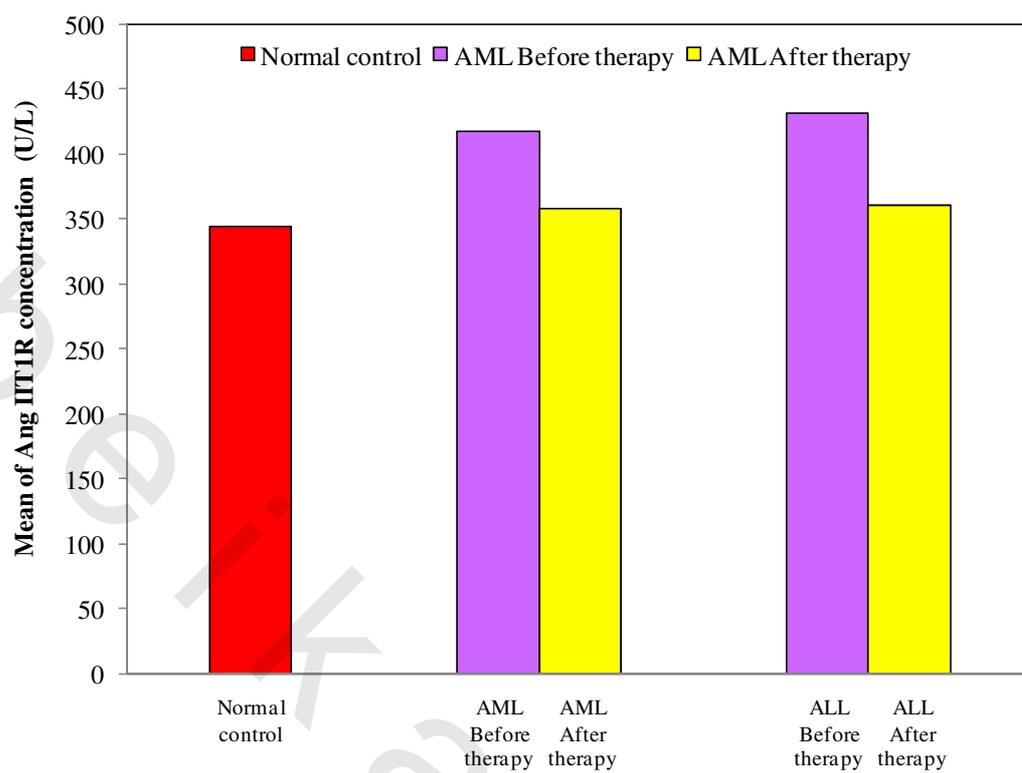
**Table (2): Statistical analyses of serum Ang IIT1R concentration (U/L) in normal control subjects and patients groups either with AML or ALL before and after therapy**

	Normal control (n= 20)	Patients with AML (n= 27)		Patients with ALL (n= 10)	
		Before therapy	After therapy	Before therapy	After therapy
<b>AngIIT1R (U/L)</b>					
Range	324.0 - 360.0	370.0 - 520.0	336.0 - 400.0	378.0 - 550.0	338.0 - 392.0
Mean $\pm$ SE	$344.60 \pm 1.61$	$418.33 \pm 7.39$	$358.81 \pm 3.50$	$432.0 \pm 18.14$	$361.40 \pm 5.66$
<b>p<sub>1</sub></b>		<0.001*	0.001*	<0.001*	0.016*
<b>p<sub>2</sub></b>		<0.001*		0.004*	

p<sub>1</sub>: p comparing mean values with control group

p<sub>2</sub>: p comparing mean values before and after therapy

\*: Statistically significant at  $p \leq 0.05$



**Figure (11):** Ang IIT1R concentration (U/L) in all studied groups

### **1.3- Liver function parameters in normal control subjects and patients groups with either AML or ALL**

Range and mean values  $\pm$  SE of liver function parameters in control and patients groups with either AML or ALL were shown in table (3) and illustrated in figures (12, 13, 14, 15, 16, 17 and 18). Statistical analyses of these results were represented in table (3).

As presented in table (3), the mean value of direct bilirubin concentration (mg/dl) was ranged from 0.10 - 0.20 with a mean value  $0.12 \pm 0.01$  in normal control group, from 0.10 - 0.40 with a mean value  $0.16 \pm 0.02$  in AML group, and from 0.10 - 1.00 with a mean value  $0.33 \pm 0.09$  in ALL group.

Also table (3) showed that, the mean value of indirect bilirubin concentration (mg/dl) was ranged from 0.15 - 0.30 with a mean value  $0.23 \pm 0.01$  in normal control group, from 0.10 - 0.50 with a mean value  $0.36 \pm 0.02$  in AML group, and from 0.10 - 0.60 with a mean value  $0.34 \pm 0.05$  in ALL group.

Also table (3) showed that, the mean value of total bilirubin concentration (mg/dl) was ranged from 0.30 - 0.40 with a mean value  $0.35 \pm 0.01$  in normal control group, from 0.30 - 0.80 with a mean value  $0.52 \pm 0.03$  in AML group, and from 0.30 - 0.90 with a mean value  $0.58 \pm 0.07$  in ALL group.

Also table (3) showed that, the mean value of total serum protein concentration (mg/dl) was ranged from 6.80 - 7.50 with a mean value  $7.21 \pm 0.04$  in normal control group, from 1.90 - 9.50 with a mean value  $6.87 \pm 0.32$  in AML group, and from 2.0 - 8.30 with a mean value  $6.52 \pm 0.55$  in ALL group.

Also table (3) showed that, the mean value of serum albumin concentration (mg/dl) was ranged from 3.90 - 4.60 with a mean value  $4.29 \pm 0.04$  in normal control group, from 1.80 - 4.30 with a mean value  $3.09 \pm 0.12$  in AML group, and from 2.40 - 3.50 with a mean value  $3.07 \pm 0.14$  in ALL group.

Also table (3) showed that, the mean value of SGOT concentration (u/l) was ranged from 12.0 - 25.0 with a mean value  $17.45 \pm 0.82$  in normal control group, from 11.0 - 55.0 with a mean value  $21.67 \pm 1.96$  in AML group, and from 12.0 - 141.0 with a mean value  $46.0 \pm 12.83$  in ALL group.

Also table (3) showed that, the mean value of SGPT concentration (u/l) was ranged from 14.0 - 25.0 with a mean value  $18.35 \pm 0.74$  in normal control group, from 10.0 - 82.0 with a mean value  $31.11 \pm 3.60$  in AML group, and from 7.0 - 89.0 with a mean value  $46.90 \pm 8.36$  in ALL group.

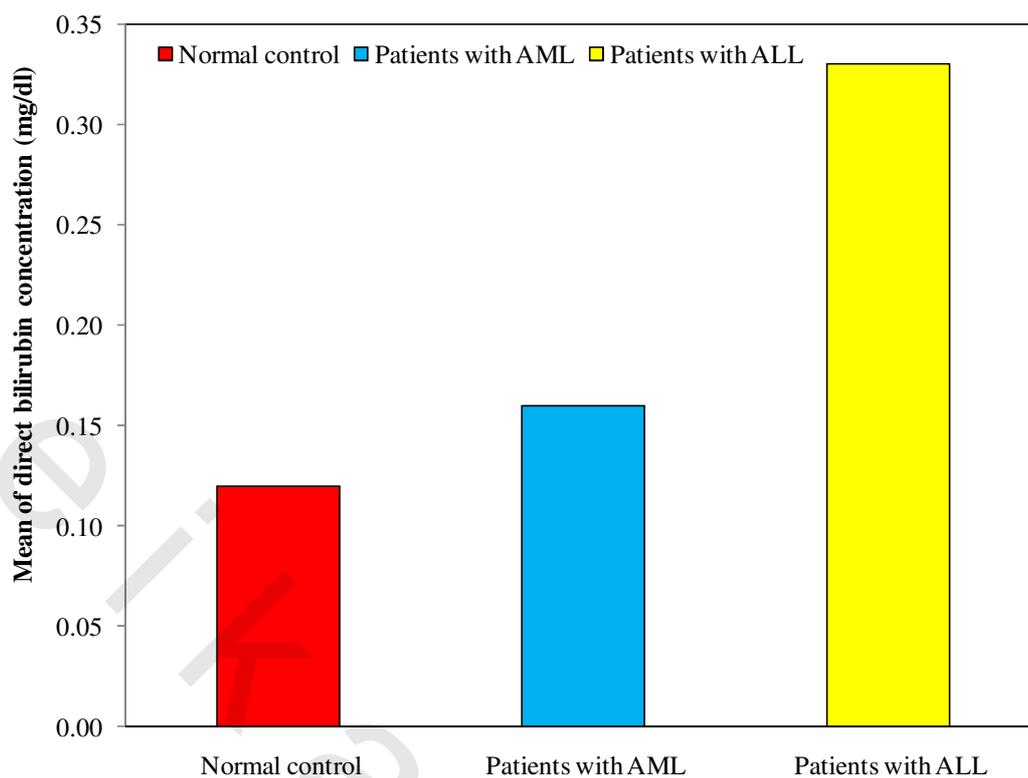
The statistical analyses of these results revealed that the mean values of indirect bilirubin (mg/dl), total bilirubin (mg/dl) and SGPT (u/l) concentrations in both groups of patients were significantly higher than normal control group. In addition, the concentrations of direct bilirubin (mg/dl) as well as SGOT (u/l) in patients with ALL were significantly higher than normal control group. On the other hand, serum albumin (mg/dl) concentrations in both groups of patients was significantly less than in normal subjects.

**Table (3): Statistical analyses of liver function parameters in normal control subjects and patients groups either with AML or ALL**

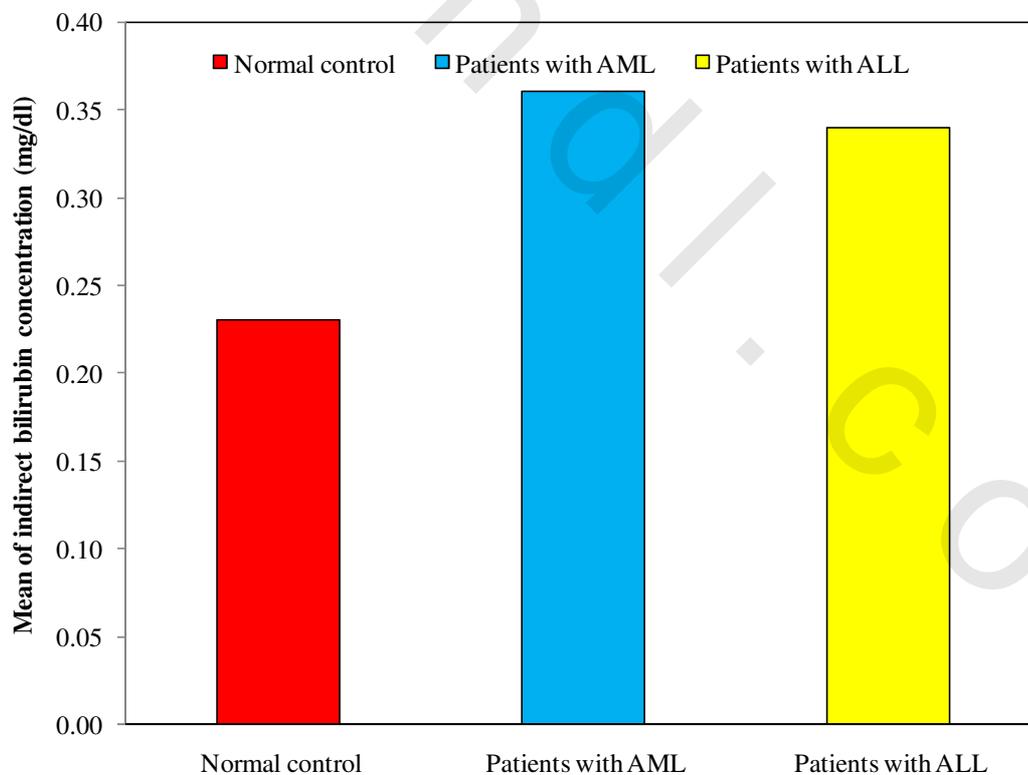
	Normal control (n= 20)	Patients with AML (n= 27)	Patients with ALL (n= 10)
<b>Direct bilirubin (mg/dl)</b>			
Range	0.10 – 0.20	0.10 – 0.40	0.10 – 1.0
Mean ± SE	0.12 ± 0.01	0.16 ± 0.02	0.33 ± 0.09
<b>P1</b>		p <sub>1</sub> = 0.347	p <sub>2</sub> = 0.008*
<b>Indirect bilirubin (mg/dl)</b>			
Range	0.15 – 0.30	0.10 – 0.50	0.10 – 0.60
Mean ± SE	0.23 ± 0.01	0.36 ± 0.02	0.34 ± 0.05
<b>P1</b>		p <sub>1</sub> <0.001*	p <sub>2</sub> = 0.011*
<b>Total bilirubin (mg/dl)</b>			
Range	0.30 - 0.40	0.30 – 0.80	0.30 – 0.90
Mean ± SE	0.35 ± 0.01	0.52 ± 0.03	0.58 ± 0.07
<b>P1</b>		p <sub>1</sub> <0.001*	p <sub>2</sub> <0.001*
<b>Total serum protein (mg/dl)</b>			
Range	6.80 - 7.50	1.90 – 9.50	2.0 - 8.30
Mean ± SE	7.21 ± 0.04	6.87 ± 0.32	6.52 ± 0.55
<b>P1</b>		p <sub>1</sub> = 0.640	p <sub>2</sub> = 0.411
<b>Albumin (mg/dl)</b>			
Range	3.90 – 4.60	1.80 – 4.30	2.40 – 3.50
Mean ± SE	4.29 ± 0.04	3.09 ± 0.12	3.07 ± 0.14
<b>P1</b>		p <sub>1</sub> <0.001*	p <sub>2</sub> <0.001*
<b>SGOT (u/l)</b>			
Range	12.0 - 25.0	11.0 – 55.0	12.0 – 141.0
Mean ± SE	17.45 ± 0.82	21.67 ± 1.96	46.0 ± 12.83
<b>P1</b>		p <sub>1</sub> = 0.133	p <sub>2</sub> = 0.010*
<b>SGPT (u/l)</b>			
Range	14.0 – 25.0	10.0 – 82.0	7.0 – 89.0
Mean ± SE	18.35 ± 0.74	31.11 ± 3.60	46.90 ± 8.36
<b>P1</b>		p <sub>1</sub> = 0.002*	p <sub>2</sub> = 0.005*

p<sub>1</sub>: p comparing mean values with control group

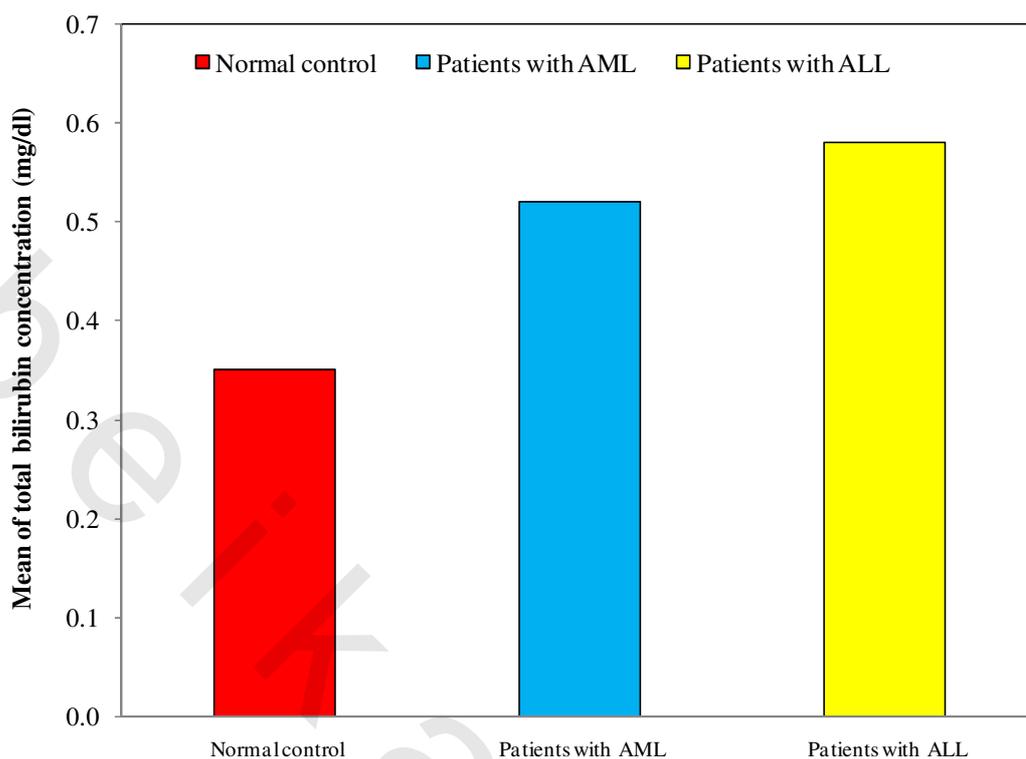
\*: Statistically significant at p ≤ 0.05



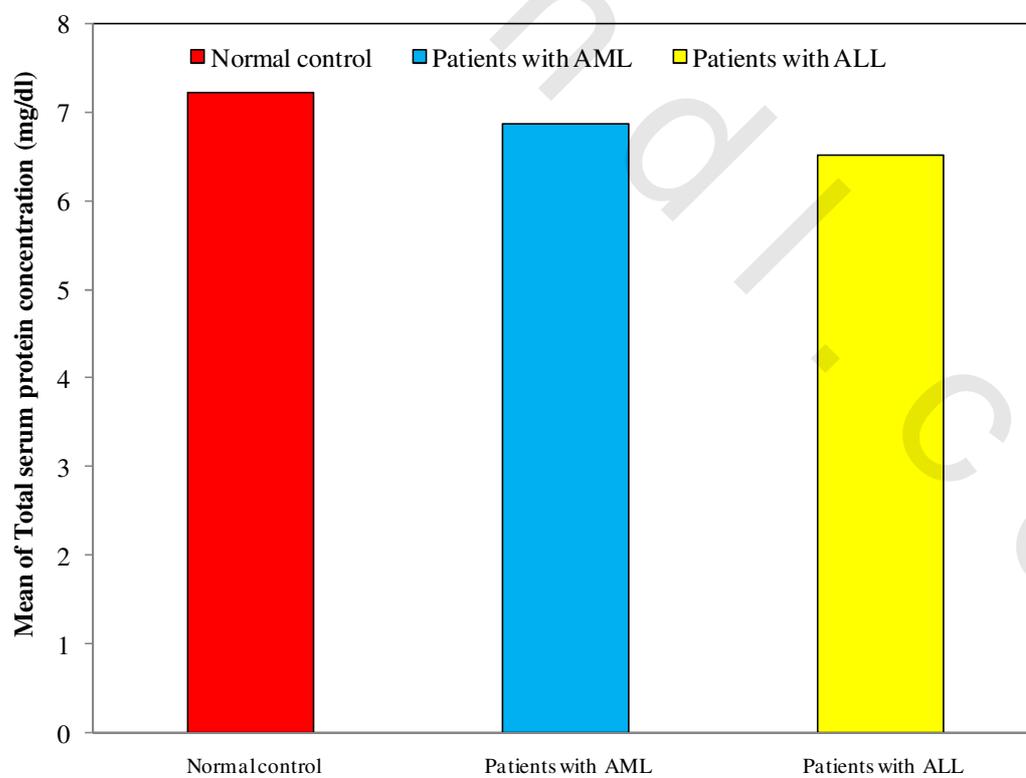
**Figure (12):** Direct bilirubin concentration (mg/dl) in all studied groups



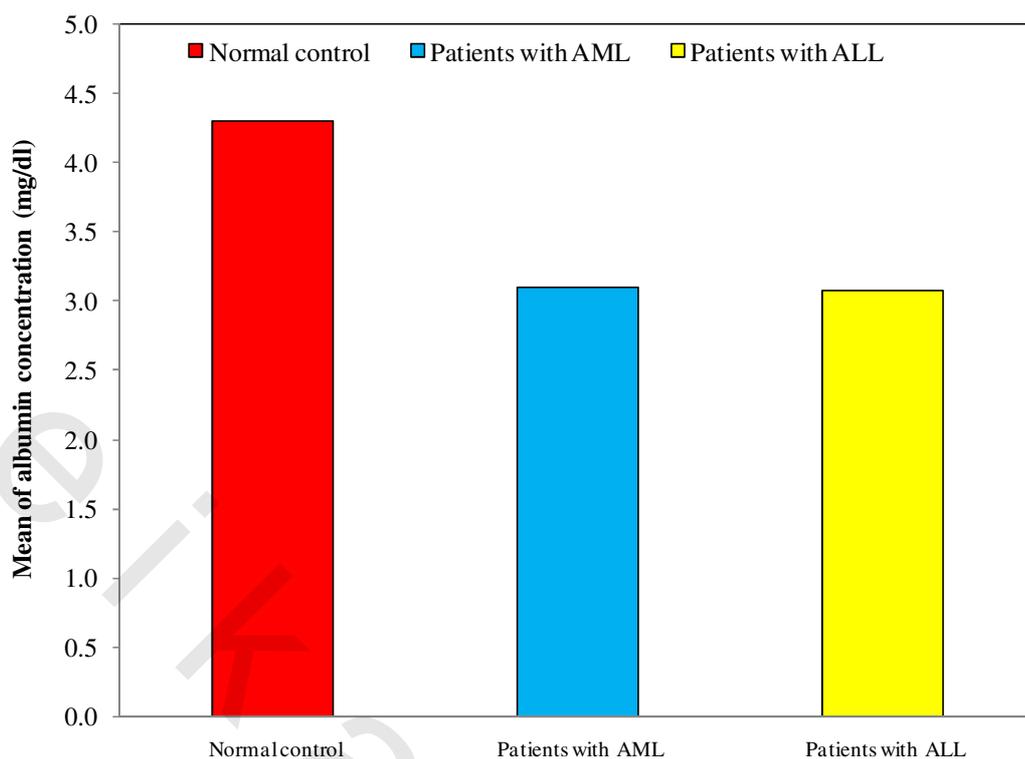
**Figure (13):** Indirect bilirubin concentration (mg/dl) in all studied groups



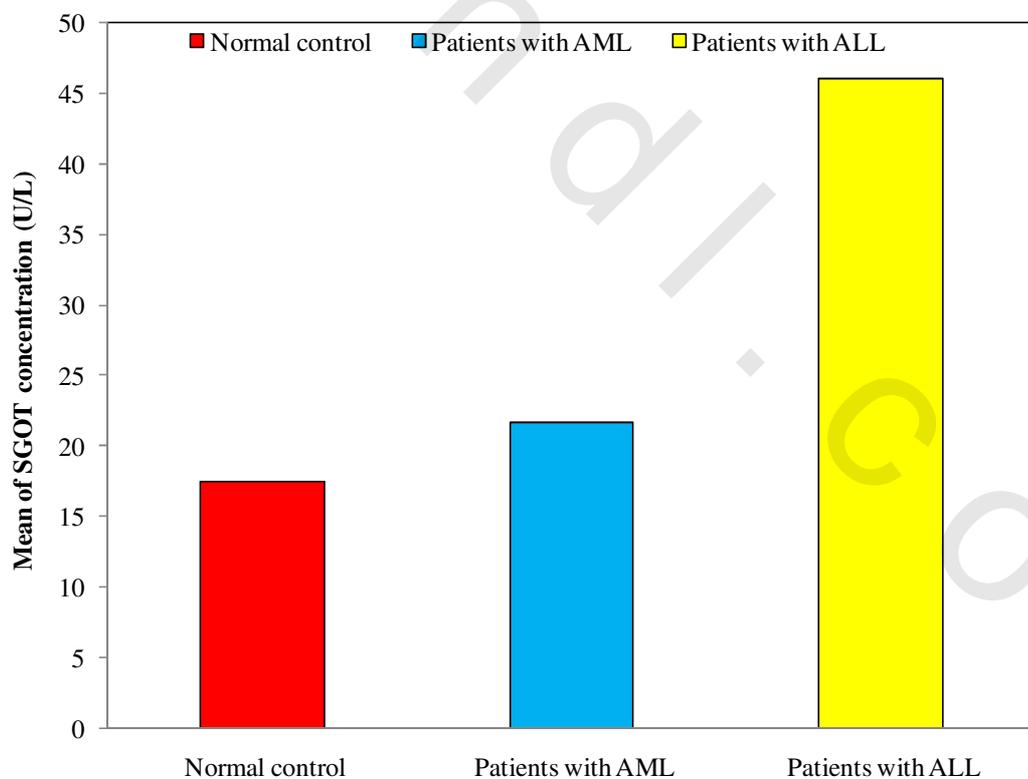
**Figure (14):** Total bilirubin concentration (mg/dl) in all studied groups



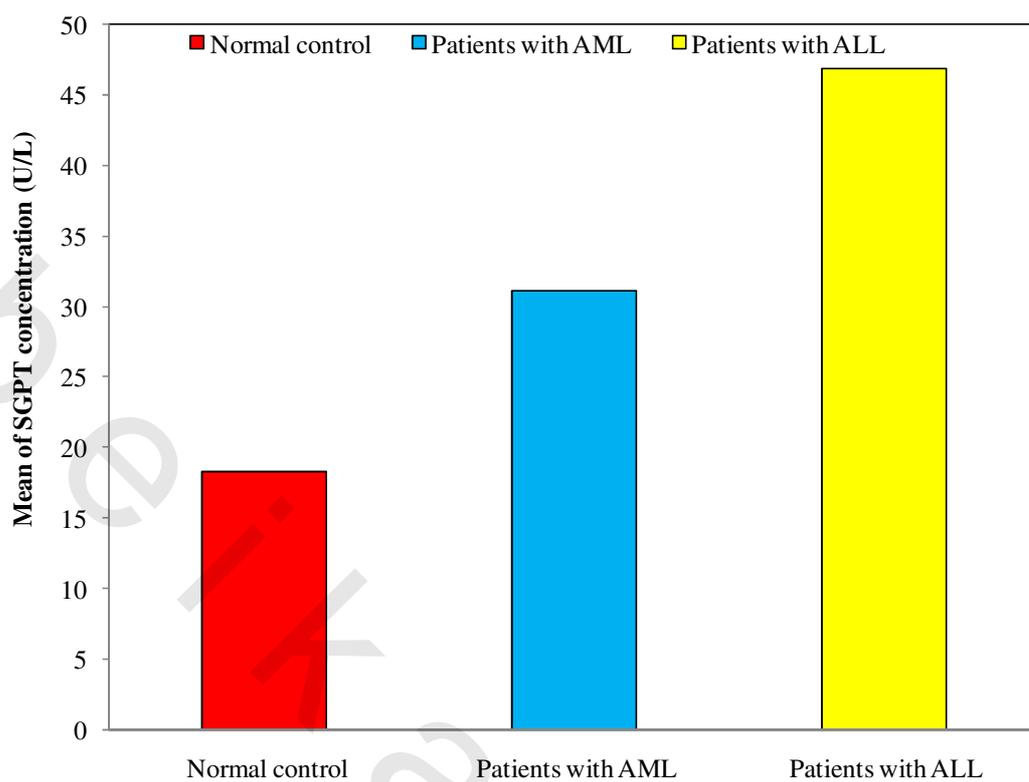
**Figure (15):** Total serum protein concentration (mg/dl) in all studied groups



**Figure (16):** Albumin concentration (mg/dl) in all studied groups



**Figure (17):** SGOT concentration (u/l) in all studied groups



**Figure (18):** SGPT concentration (u/l) in all studied groups

#### **1.4- Kidney function parameters in normal control subjects and patients groups with either AML or ALL**

Range and mean values  $\pm$  SE of kidney function levels in control and patients groups with either AML or ALL were shown in table (4) and illustrated in figures (19, 20, 21 and 22). Statistical analyses of these results were represented in table (4).

As presented in table (4), the mean value of Alkaline phosphatase concentration (u/l) was ranged from 65.0 - 85.0 with a mean value  $76.35 \pm 1.15$  in normal control group, from 40.0 - 113.0 with a mean value  $79.52 \pm 3.18$  in AML group, and from 40.0 - 92.0 with a mean value  $69.30 \pm 5.49$  in ALL group.

Also table (4) showed that, the mean value of uric acid concentration (mg/dl) was ranged from 2.30 - 3.50 with a mean value  $2.87 \pm 0.09$  in normal control group, from 1.90 - 5.10 with a mean value  $2.78 \pm 0.18$  in AML group, and from 1.30 - 6.0 with a mean value  $2.89 \pm 0.43$  in ALL group.

Also table (4) showed that, the mean value of urea concentration (mg/dl) was ranged from 22.0 - 35.0 with a mean value  $27.90 \pm 0.88$  in normal control group, from 13.0 - 42.0 with a mean value  $26.74 \pm 1.33$  in AML group, from 24.0 - 35.0 with a mean value  $28.56 \pm 1.38$  in ALL group.

Also table (3) showed that, the mean value of creatinine concentration (mg/dl) was ranged from 1.20 - 1.60 with a mean value  $1.38 \pm 0.03$  in normal control group, from 0.40 - 1.90 with a mean value  $1.03 \pm 0.09$  in AML group, and from 0.50 - 1.40 with a mean value  $0.97 \pm 0.10$  in ALL group.

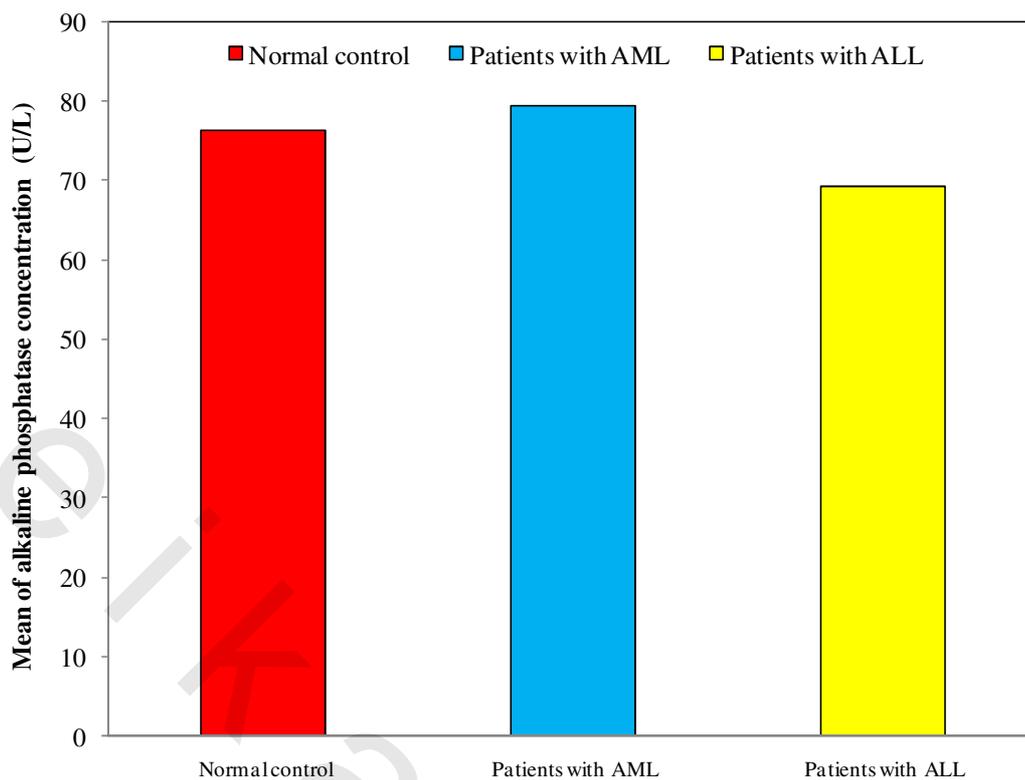
The statistical analyses of these results revealed that in both groups of patients, the mean values of creatinine (mg/dl) concentrations was significantly lower than in normal control group.

**Table (4): Statistical analyses of kidney function parameters in normal control subjects and patients groups either with AML or ALL**

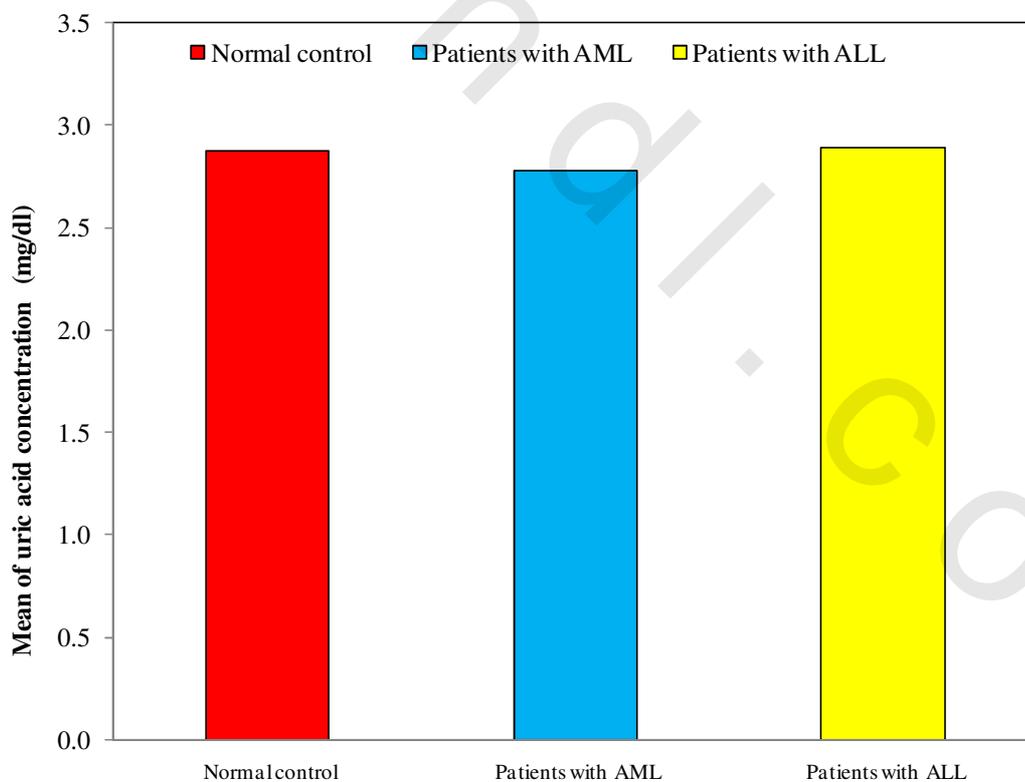
	<b>Normal control (n= 20)</b>	<b>Patients with AML (n= 27)</b>	<b>Patients with ALL (n= 10)</b>
<b>Alkaline phosphatase (U/L)</b>			
Range	65.0 – 85.0	40.0 – 113.0	40.0 – 92.0
Mean ± SE	76.35 ± 1.15	79.52 ± 3.18	69.30 ± 5.49
<b>P1</b>		p <sub>1</sub> = 0.440	p <sub>2</sub> = 0.193
<b>Uric acid (mg/dl)</b>			
Range	2.30 – 3.50	1.90 – 5.10	1.30 – 6.0
Mean ± SE	2.87 ± 0.09	2.78 ± 0.18	2.89 ± 0.43
<b>P1</b>		p <sub>1</sub> = 0.076	p <sub>2</sub> = 0.414
<b>Urea (mg/dl)</b>			
Range	22.0 - 35.0	13.0 – 42.0	24.0 – 35.0
Mean ± SE	27.90 ± 0.88	26.74 ± 1.33	28.56 ± 1.38
<b>P1</b>		p <sub>1</sub> = 0.531	p <sub>2</sub> = 0.673
<b>Creatinine (mg/dl)</b>			
Range	1.20 – 1.60	0.40 – 1.90	0.50 – 1.40
Mean ± SE	1.38 ± 0.03	1.03 ± 0.09	0.97 ± 0.10
<b>P1</b>		p <sub>1</sub> = 0.003*	p <sub>2</sub> <0.001*

p<sub>1</sub>: p value for comparing between control and patients

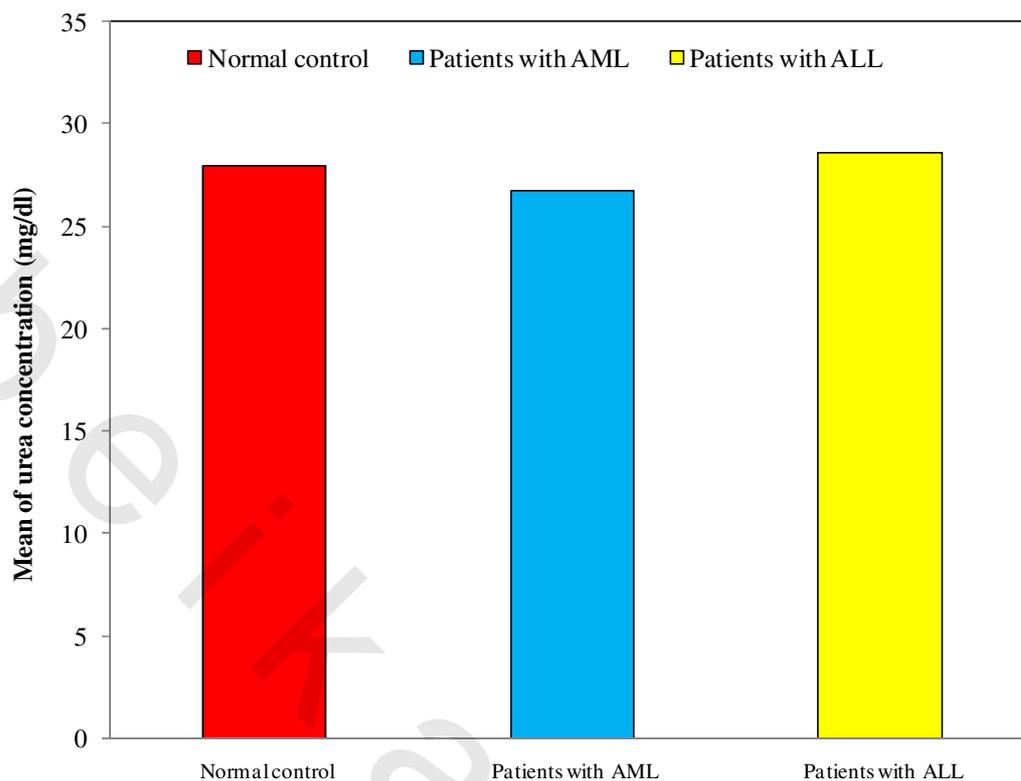
\*: Statistically significant at  $p \leq 0.05$



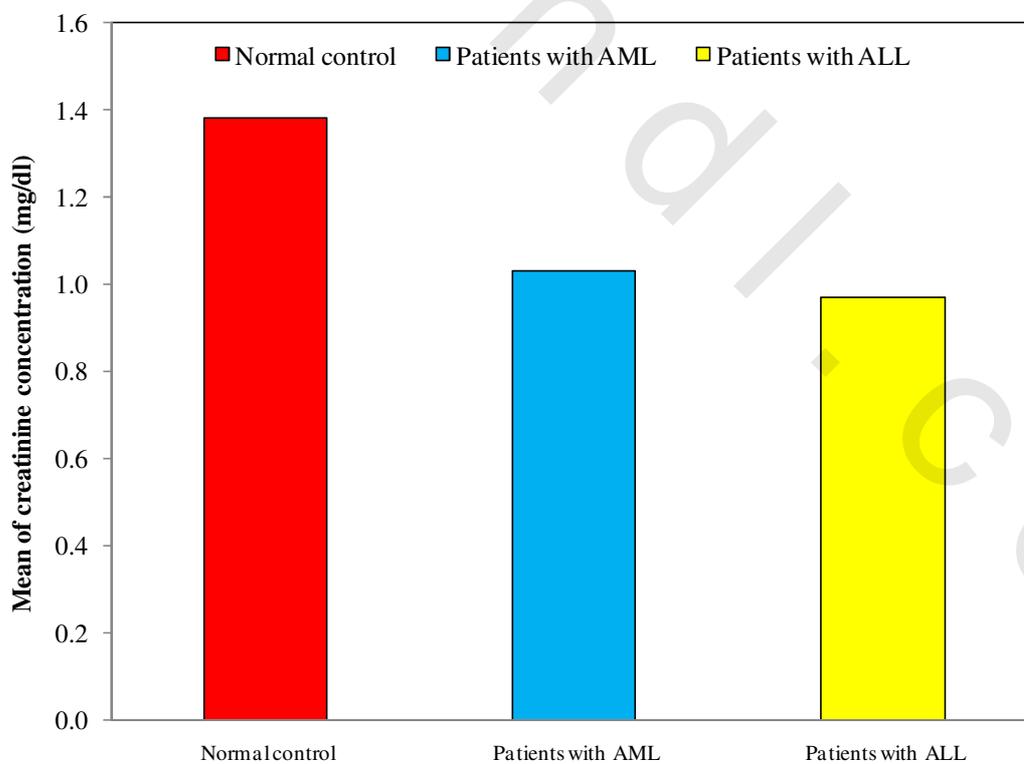
**Figure (19):** Alkaline phosphatase concentration (u/l) in all studied groups



**Figure (20):** Uric acid concentration (mg/dl) in all studied groups



**Figure (21):** Urea concentration (mg/dl) in all studied groups



**Figure (22):** Creatinine concentration (mg/dl) in all studied groups

### **1.5- Sodium, Potassium, Calcium and Phosphorus levels in normal control subjects and patients groups with either AML or ALL**

Range and mean values  $\pm$  SE of electrolytes levels in control and patients groups with either AML or ALL were shown in table (5) and illustrated in figures (23, 24, 25 and 26). Statistical analyses of these results were represented in table (5).

As presented in table (5), the mean value of sodium concentration (meq/l) was ranged from 130.0 - 145.0 with a mean value  $138.95 \pm 0.75$  in normal control group, from 114.0 - 139.0 with a mean value  $132.48 \pm 1.15$  in AML group, and from 120.0 - 135.0 with a mean value  $128.60 \pm 1.90$  in ALL group.

Also table (5) showed that, the mean value of potassium concentration (meq/l) was ranged from 3.50 - 4.90 with a mean value  $4.33 \pm 0.08$  in normal control group, from 2.80 - 5.10 with a mean value  $3.69 \pm 0.12$  in AML group, and from 3.30 - 5.10 with a mean value  $3.72 \pm 0.19$  in ALL group.

Also table (5) showed that, the mean value of calcium concentration (meq/l) was ranged from 9.50 - 10.60 with a mean value  $10.20 \pm 0.07$  in normal control group, from 7.10 - 9.60 with a mean value  $8.53 \pm 0.14$  in AML group, and from 4.30 - 9.70 with a mean value  $8.28 \pm 0.49$  in ALL group.

Also table (5) showed that, the mean value of phosphorus concentration (meq/l) was ranged from 4.80 - 5.30 with a mean value  $5.04 \pm 0.03$  in normal control group, from 2.70 - 5.10 with a mean value  $3.95 \pm 0.10$  in AML group, and from 2.50 - 4.60 with a mean value  $3.90 \pm 0.22$  in ALL group.

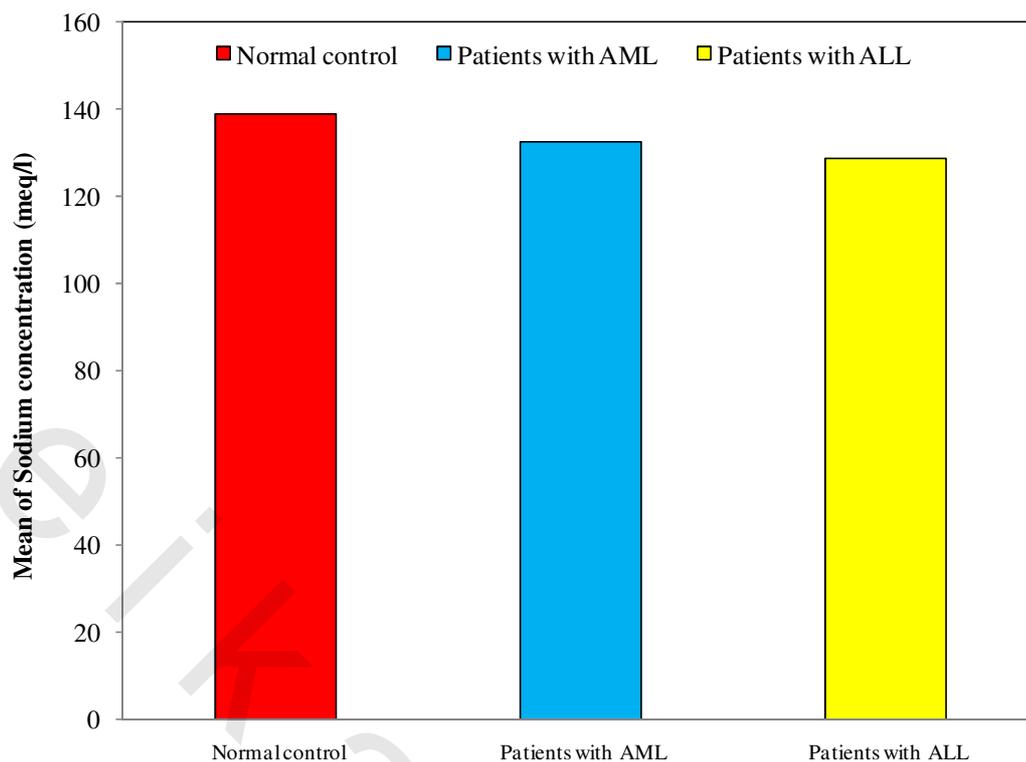
The statistical analyses of these results revealed that the mean values of sodium, potassium, calcium and phosphorus concentrations in both groups of patients were significantly lower than in normal control group.

**Table (5): Statistical analyses of Sodium, Potassium, Calcium and Phosphorus levels in normal control subjects and patients groups either with AML or ALL**

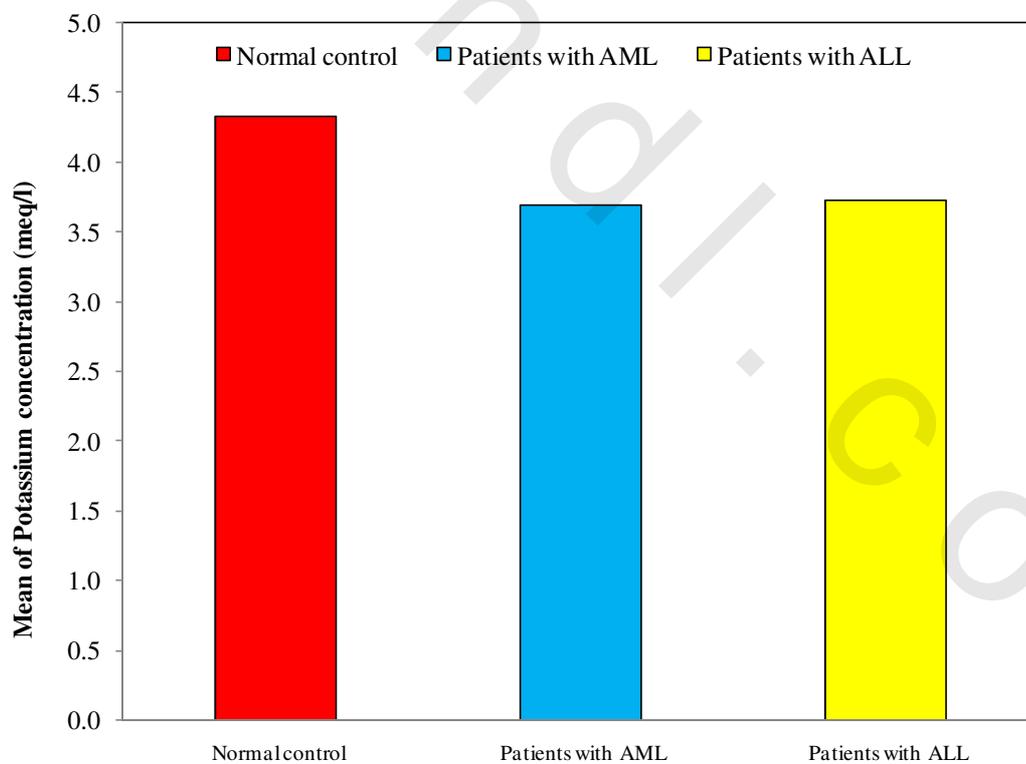
	Normal control (n= 20)	Patients with AML (n= 27)	Patients with ALL (n= 10)
<b>Sodium (meq/l)</b>			
Range	130.0 - 145.0	114.0 - 139.0	120.0 - 135.0
Mean $\pm$ SE	138.95 $\pm$ 0.75	132.48 $\pm$ 1.15	128.60 $\pm$ 1.90
<b>P1</b>		p <sub>1</sub> <0.001*	p <sub>2</sub> <0.001*
<b>Potassium (meq/l)</b>			
Range	3.50 - 4.90	2.80 - 5.10	3.30 - 5.10
Mean $\pm$ SE	4.33 $\pm$ 0.08	3.69 $\pm$ 0.12	3.72 $\pm$ 0.19
<b>P1</b>		p <sub>1</sub> <0.001*	p <sub>2</sub> =0.003*
<b>Calcium (meq/l)</b>			
Range	9.50 - 10.60	7.10 - 9.60	4.30 - 9.70
Mean $\pm$ SE	10.20 $\pm$ 0.07	8.53 $\pm$ 0.14	8.28 $\pm$ 0.49
<b>P1</b>		p <sub>1</sub> <0.001*	p <sub>2</sub> <0.001*
<b>Phosphorus (meq/l)</b>			
Range	4.80 - 5.30	2.70 - 5.10	2.50 - 4.60
Mean $\pm$ SE	5.04 $\pm$ 0.03	3.95 $\pm$ 0.10	3.90 $\pm$ 0.22
<b>P1</b>		p <sub>1</sub> <0.001*	p <sub>2</sub> <0.001*

p<sub>1</sub>: p value for comparing between control and patients

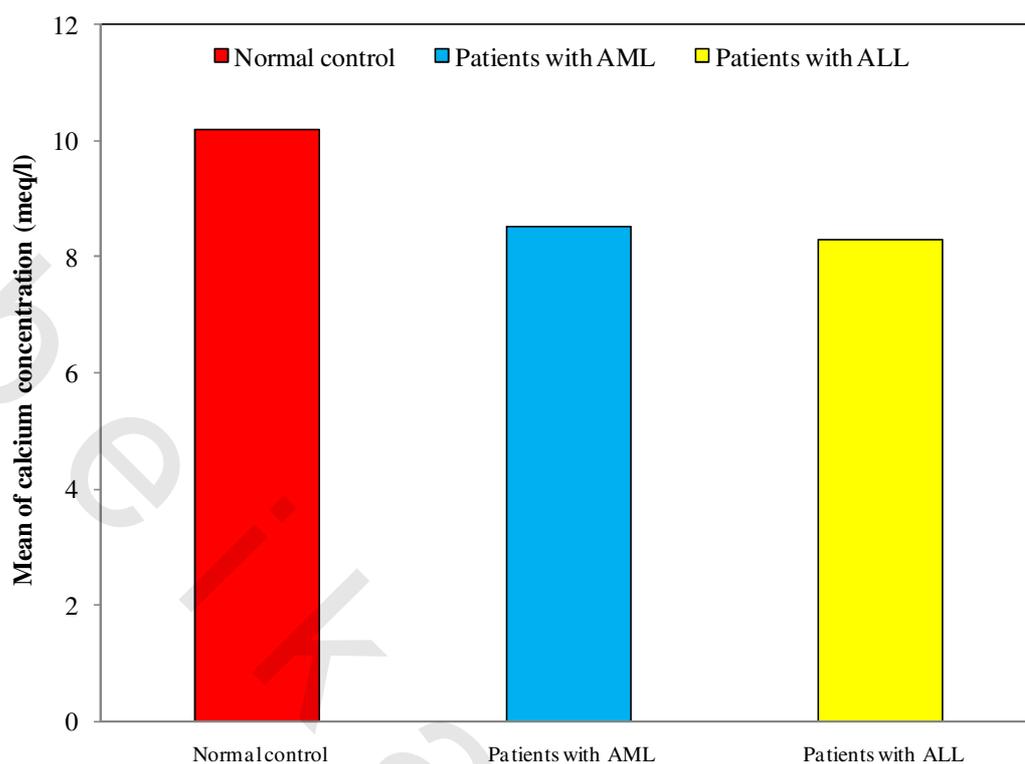
\*: Statistically significant at p  $\leq$  0.05



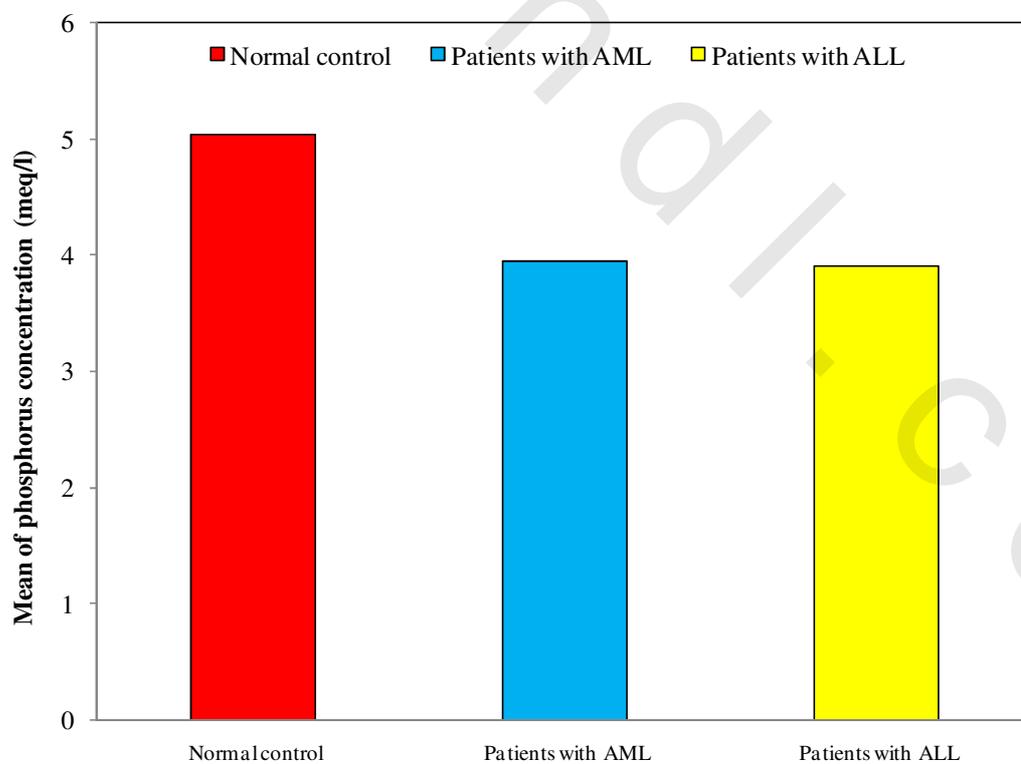
**Figure (23):** Sodium concentration (meq/l) in all studied groups



**Figure (24):** Potassium concentration (meq/l) in all studied groups



**Figure (25):** Calcium concentration (meq/l) in all studied groups



**Figure (26):** Phosphorus concentration (meq/l) in all studied groups

## 2 – HEMATOLOGICAL RESULTS

### 2.1- Mean values of WBC count (x10e9), Platelets count (x10e9) and Hemoglobin concentration (g/dl) in normal control subjects and patients groups with either AML or ALL

Range and mean values  $\pm$  SE of hematological parameters in control and patients groups with either AML or ALL before therapy were shown in table (6) and illustrated in figures (27, 28 and 29). Statistical analyses of these results were represented in table (6).

As presented in table (6), the mean value of WBC count (x10e9) was ranged from 3.50 - 10.0 with a mean value  $7.21 \pm 0.40$  in normal control group, from 0.41 - 86.20 with a mean value  $15.40 \pm 4.19$  in AML group, and from 1.03 - 86.16 with a mean value  $22.58 \pm 9.95$  in ALL group.

Also table (6) showed that, the mean value of platelets count (x10e9) was ranged from 127.0 - 328.0 with a mean value  $233.0 \pm 11.96$  in normal control group, from 4.0 - 66.0 with a mean value  $22.42 \pm 2.76$  in AML group, and from 8.0 - 44.0 with a mean value  $28.34 \pm 3.75$  in ALL group.

Moreover table (6) showed that the mean value of Hb concentration (g/dl) was ranged from 12.40 - 15.50 with a mean value  $13.23 \pm 0.18$  in normal control group, from 4.40 - 11.20 with a mean value  $8.11 \pm 0.26$  in AML group, and from 5.40 - 10.0 with a mean value  $7.71 \pm 0.49$  in ALL group.

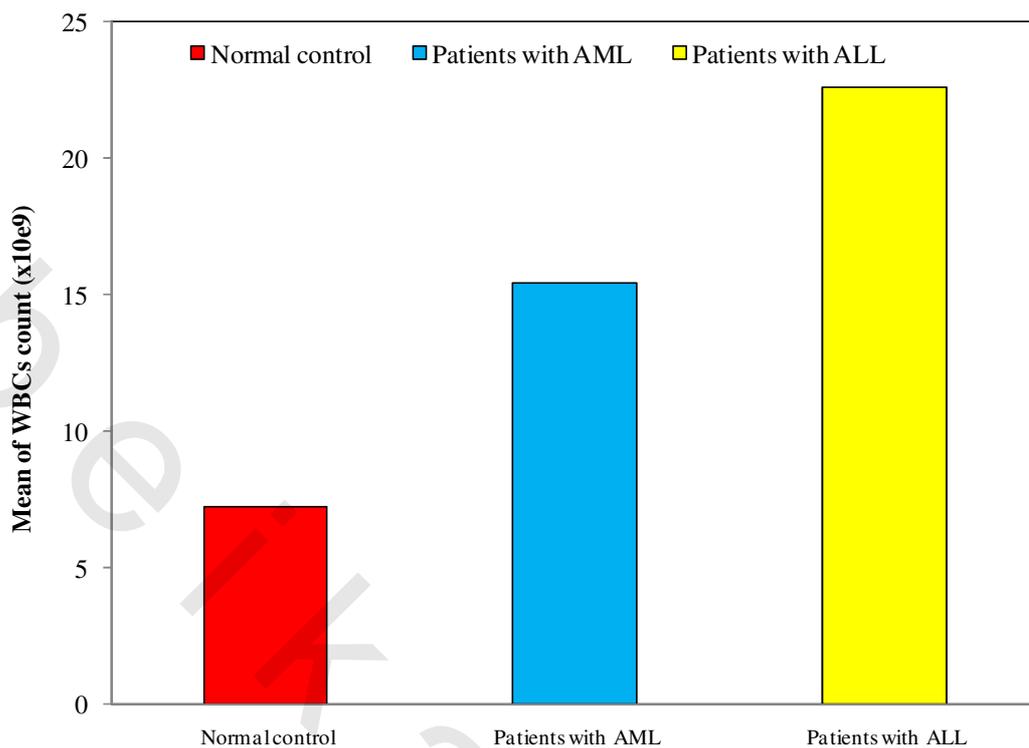
The statistical analyses of these results revealed that the mean value of WBCs count in AML and ALL patients were higher than in control group. On the other hand, Platelets count and Hb concentration in both groups of patients were significantly less than in normal subjects.

**Table (6): Statistical analyses of hematological parameters in normal control subjects and patients groups either with AML or ALL**

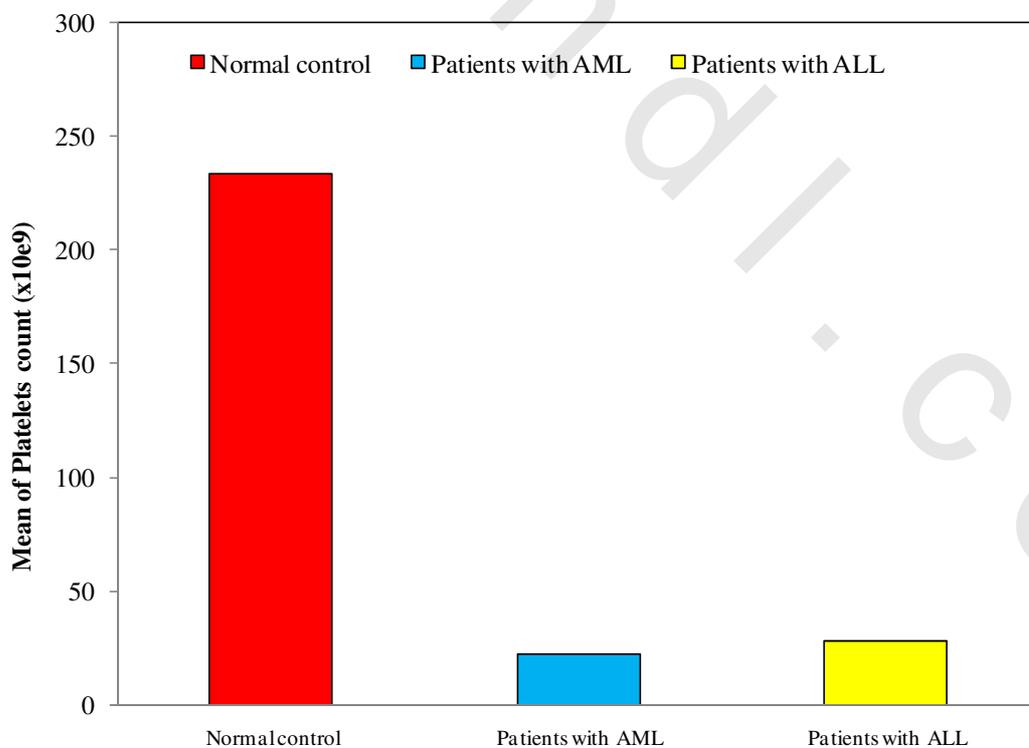
	Normal control (n= 20)	Patients with AML (n= 27)	Patients with ALL (n= 10)
<b>WBCs count (x10e9)</b>			
Range.	3.50 - 10.0	0.41 - 86.20	1.03 - 86.16
Mean $\pm$ SE	$7.21 \pm 0.40$	$15.40 \pm 4.19$	$22.58 \pm 9.95$
P1		0.208	0.397
<b>Platelets count (x10e9)</b>			
Range	127.0 - 328.0	4.0 - 66.0	8.0 - 44.0
Mean $\pm$ SE	$233.0 \pm 11.96$	$22.42 \pm 2.76$	$28.34 \pm 3.75$
P1		<0.001*	<0.001*
<b>HB concentration (g/dl)</b>			
Range	12.40 - 15.50	4.40 - 11.20	5.40 - 10.0
Mean $\pm$ SE	$13.23 \pm 0.18$	$8.11 \pm 0.26$	$7.71 \pm 0.49$
P1		<0.001*	<0.001*

p<sub>1</sub>: p comparing mean values with control group

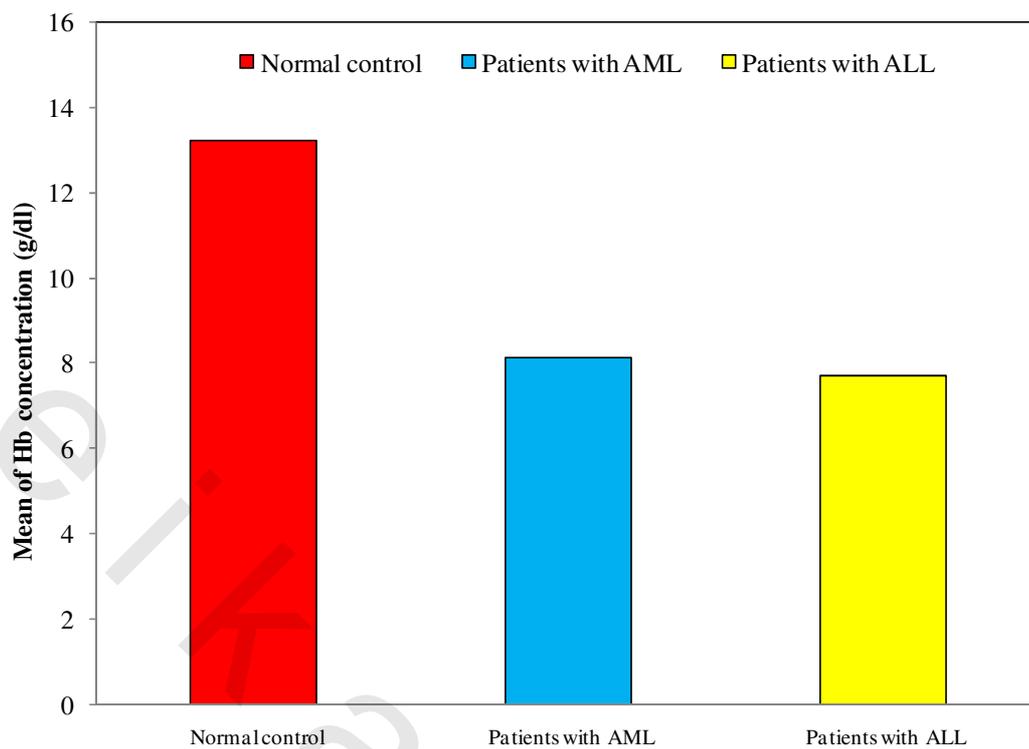
\*: Statistically significant at  $p \leq 0.05$



**Figure (27):** WBCs count (x10e9) in all studied groups



**Figure (28):** Platelets count (x10e9) in all studied groups



**Figure (29):** Hb concentration (g/dl) in all studied groups

## 2.2 - Mean values of blasts cells % in normal control group and patients group with either AML or ALL before and after therapy

Range and mean values  $\pm$  SE of blast cells % in control and patients groups with either AML or ALL before and after therapy were shown in table (7) and illustrated in figure (30). Statistical analyses of these results was represented in table (7).

As presented in table (7), blast cells % was ranged from 1.0 - 3.0 with a mean value  $1.45 \pm 0.14$  in normal control group, from 10.0 - 96.0 with a mean value  $73.93 \pm 3.68$  in AML group before therapy, from 1.0 - 60.0 with a mean value  $8.81 \pm 2.34$  in AML group after therapy, from 15.0 - 90.0 with a mean value  $70.90 \pm 6.73$  in ALL group before therapy, and from 2.0 - 20.0 with a mean value  $10.50 \pm 2.27$  in ALL group after therapy.

The statistical analyses of these results revealed that the blast cells % in patients with either AML or ALL before therapy was significantly higher than in control group. After therapy, the % of blast cells in both groups of patients were significantly decreased than their corresponding values before therapy and still significantly higher than in control group.

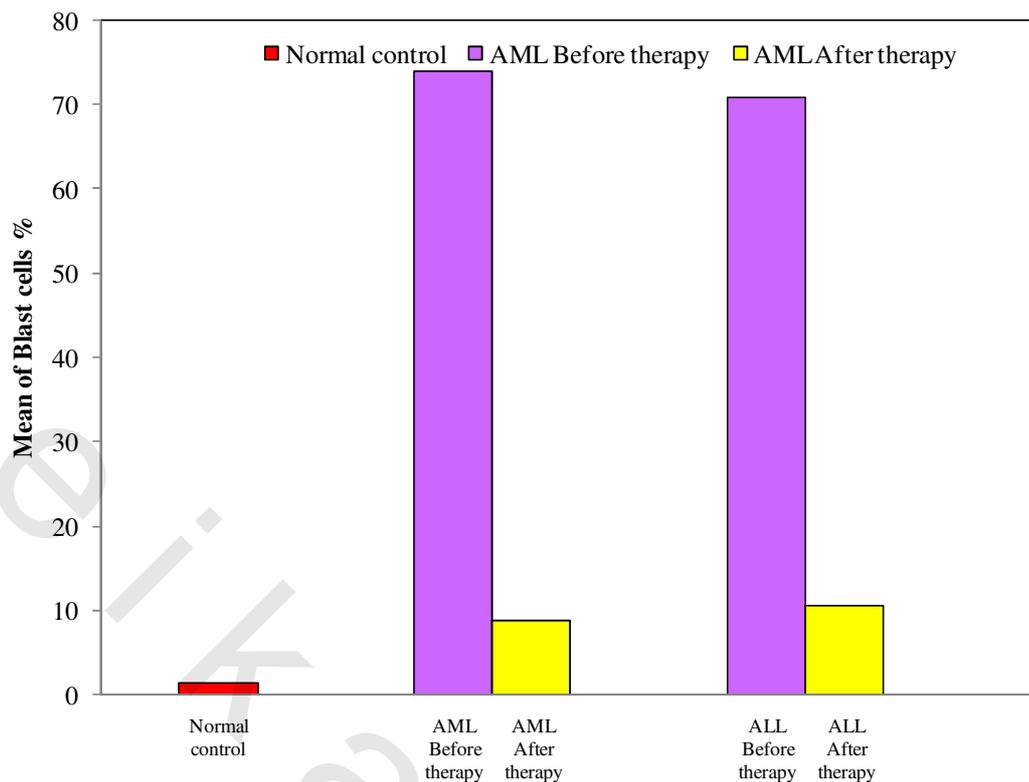
**Table (7): Statistical analyses of blasts cells % in normal control subjects and patients groups either with AML or ALL before and after therapy**

	Normal control (n= 20)	Patients with AML (n= 27)		Patients with ALL (n= 10)	
		Before therapy	After therapy	Before therapy	After therapy
<b>Blast cells %</b>					
Range	1.0 - 3.0	10.0 - 96.0	1.0 - 60.0	15.0 - 90.0	2.0 - 20.0
Mean $\pm$ SE	$1.45 \pm 0.14$	$73.93 \pm 3.68$	$8.81 \pm 2.34$	$70.90 \pm 6.73$	$10.50 \pm 2.27$
<b>p<sub>1</sub></b>		<0.001*	<0.001*	<0.001*	<0.001*
<b>p<sub>2</sub></b>		<0.001*		0.005*	

p<sub>1</sub>: p comparing mean values with control group

p<sub>2</sub>: p comparing mean values before and after therapy

\*: Statistically significant at  $p \leq 0.05$



**Figure (30):** Blast cells% in all studied groups

### 3- CORRELATION of ACE and Ang IIT1R WITH ALL STUDIED PARAMETERS

#### 3.1 - Correlation between ACE activity (U/L) and all studied parameters:

As presented in table (8), ACE activity (U/L) in serum of AML patients group showed a significant positive correlation with blast cells % at presentation.

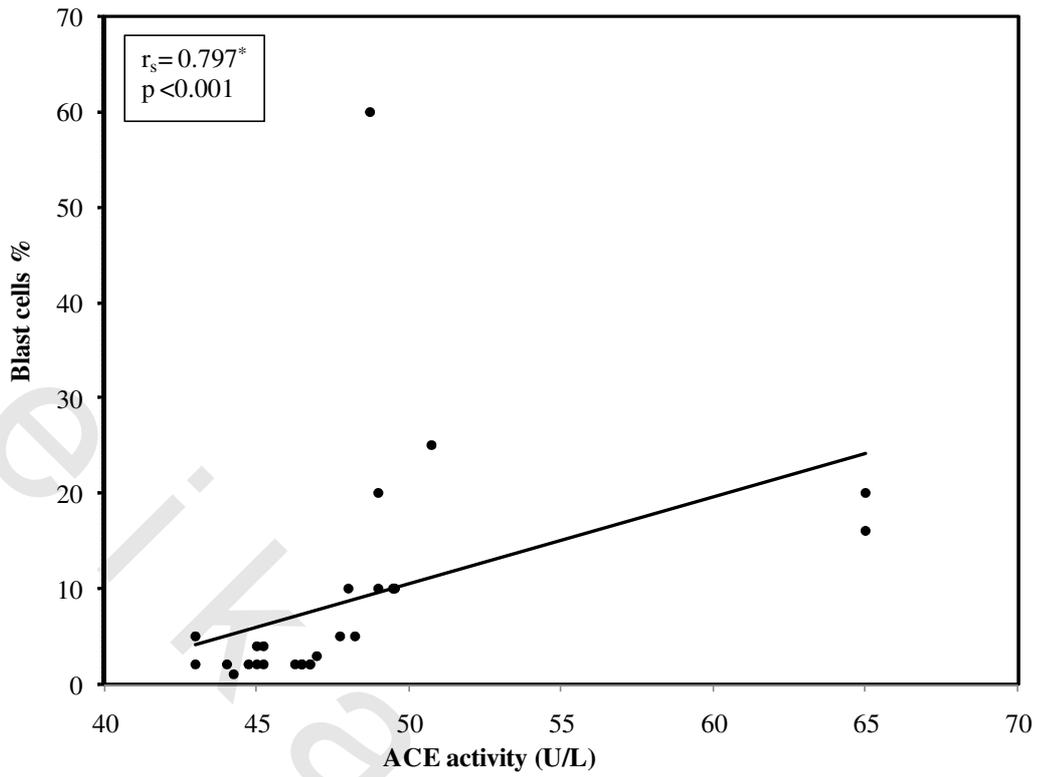
In ALL patients groups, it was noticed that there was a significant positive correlation with blast cells % and Hb concentration (g/dl), and was inversely correlated with Phosphorus concentration (meq/l).

**Table (8): Correlation between ACE activity (U/L) and all studied parameters**

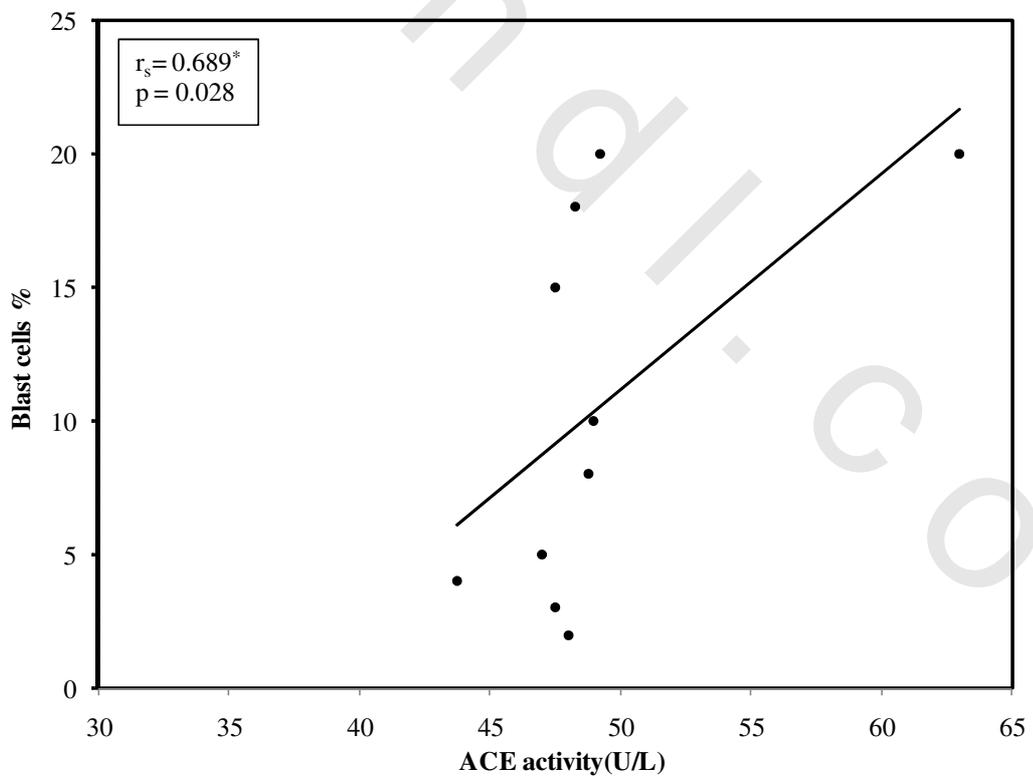
ACE activity (U/L)	AML patients		ALL patients	
	$r_s$	p	$r_s$	p
Blast cells %	0.797*	<0.001	0.689*	0.028
WBCs count (x10e9)	0.073	0.718	-0.237	0.510
HB concentration (g/dl)	0.006	0.978	0.670*	0.034
Platelets count (x10e9)	0.044	0.829	-0.535	0.111
Direct bilirubin concentration (mg/dl)	0.247	0.215	-0.430	0.251
Indirect bilirubin concentration (mg/dl)	-0.376	0.053	-0.405	0.246
Total bilirubin concentration (mg/dl)	-0.147	0.464	-0.165	0.648
Total serum protein concentration (mg/dl)	-0.036	0.857	-0.210	0.561
Albumin concentration (mg/dl)	-0.159	0.428	0.198	0.583
SGOT concentration (u/l)	0.117	0.562	0.598	0.068
SGPT concentration (u/l)	-0.083	0.680	0.615	0.059
Alkaline phosphatase concentration (u/l)	-0.324	0.099	-0.379	0.280
Uric acid concentration (mg/dl)	0.203	0.311	0.232	0.519
Urea concentration (mg/dl)	-0.162	0.421	0.518	0.125
Creatinine concentration (mg/dl)	-0.058	0.773	-0.117	0.748
Sodium concentration (meq/l)	0.032	0.874	-0.437	0.207
Potassium concentration (meq/l)	-0.220	0.271	0.022	0.952
Calcium concentration (meq/l)	0.196	0.328	0.257	0.474
Phosphorus concentration (meq/l)	0.069	0.733	-0.659*	0.038*

$r_s$ : Spearman coefficient

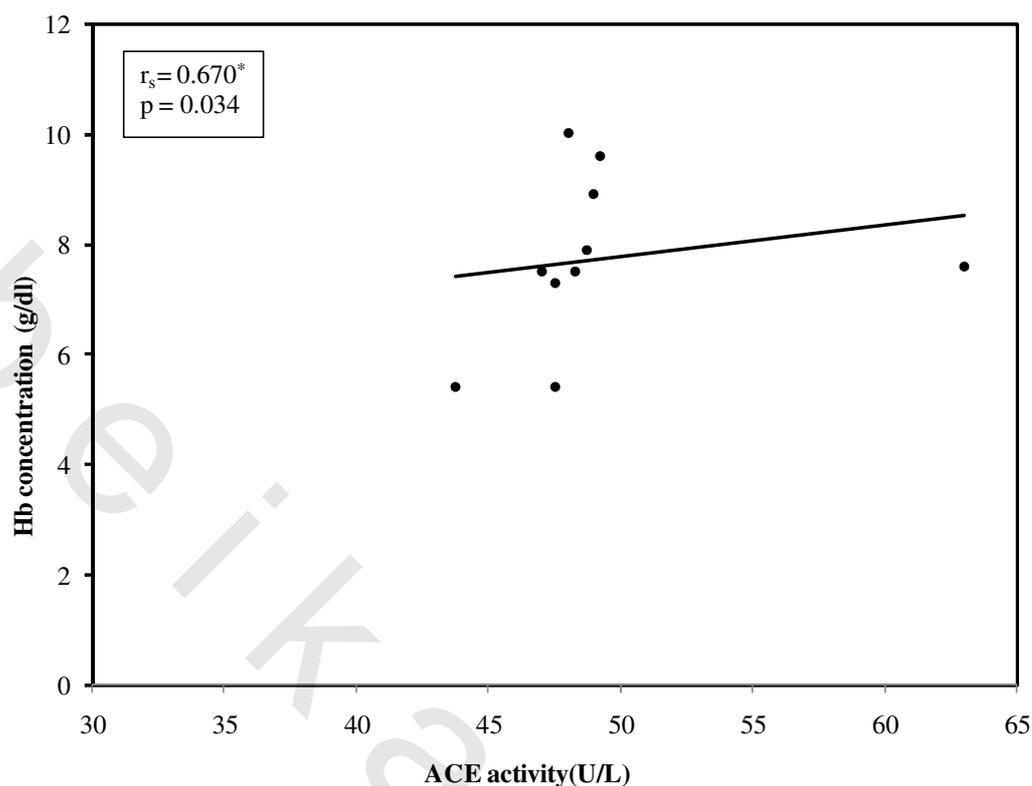
\*: Statistically significant at  $p \leq 0.05$



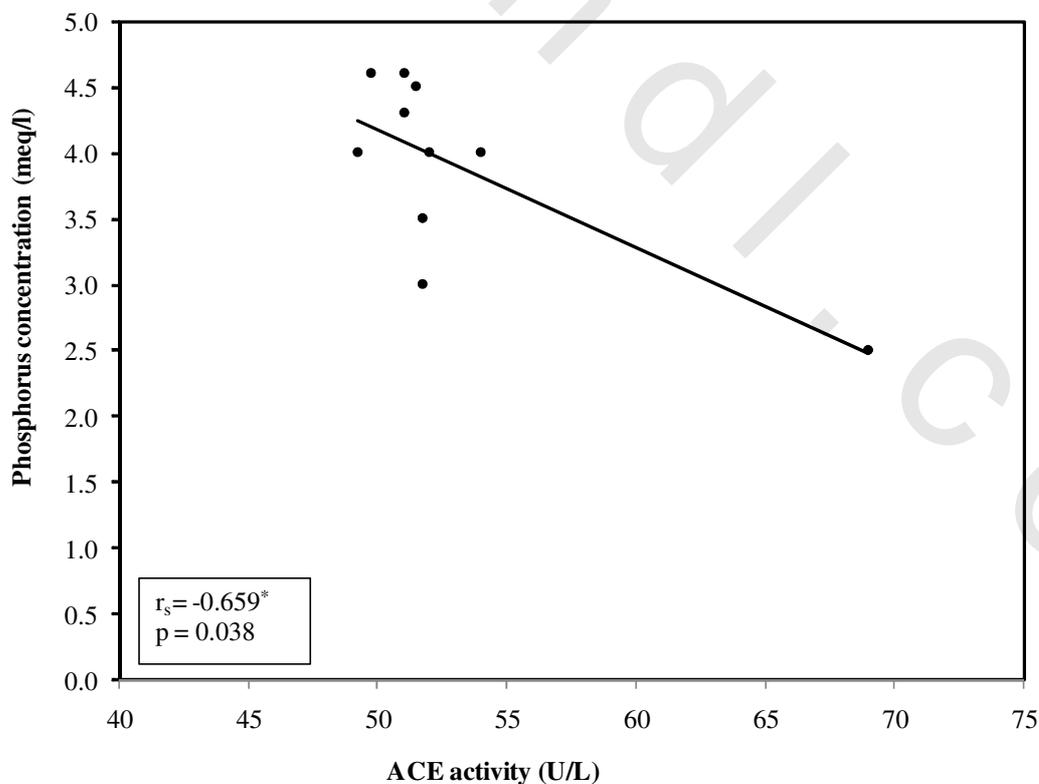
**Figure (31):** Correlation between ACE activity (U/L) and Blast cells % in AML patients



**Figure (32):** Correlation between ACE activity (U/L) and Blast cells % in ALL patients



**Figure (33):** Correlation between ACE activity (U/L) and Hb concentration (g/dl) in ALL patients



**Figure (34):** Correlation between ACE activity (U/L) and phosphorus concentration (meq/l) in ALL patients

### 3.2 - Correlation between Ang IIT1R concentration (U/L) and all studied parameters

As presented in table (9), Ang IIT1R concentration (U/L) in serum of AML patients group showed a significant positive correlation with blast cells % and urea concentration (mg/dl) at presentation.

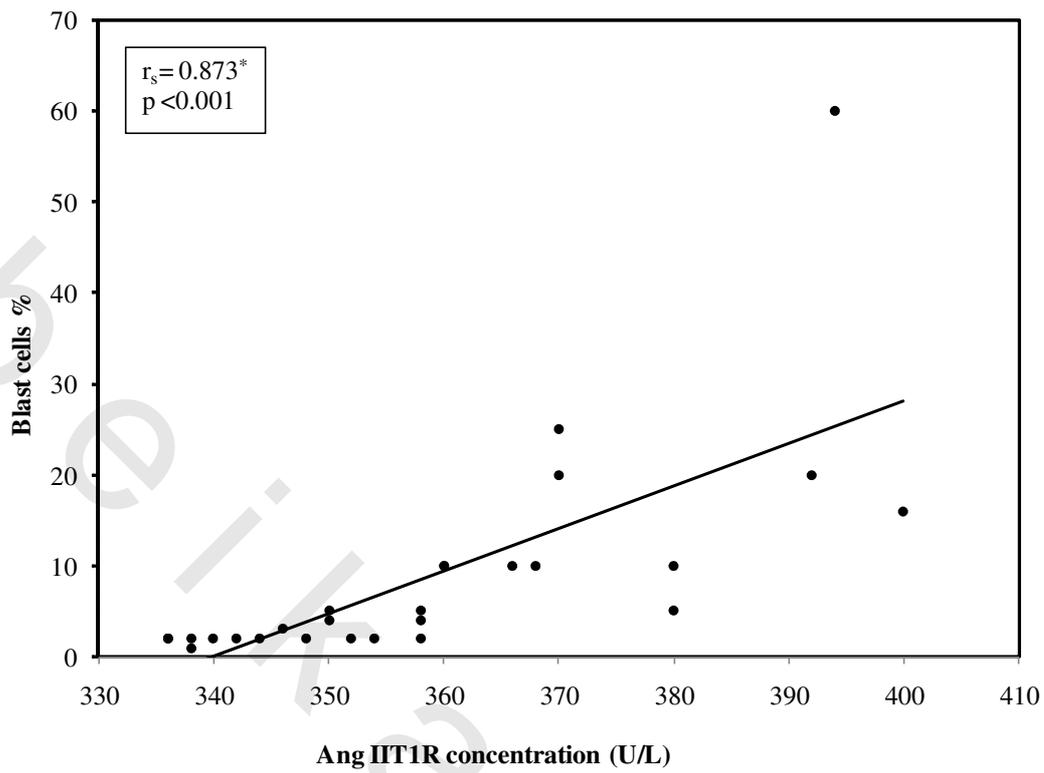
In ALL patients groups, it was noticed that there was a significant positive correlation with blast cells %, and was inversely correlated with WBCs count ( $\times 10^9$ ) and Sodium concentration (meq/l).

**Table (9): Correlation between Ang IIRT1 concentration (U/L) and all studied parameters**

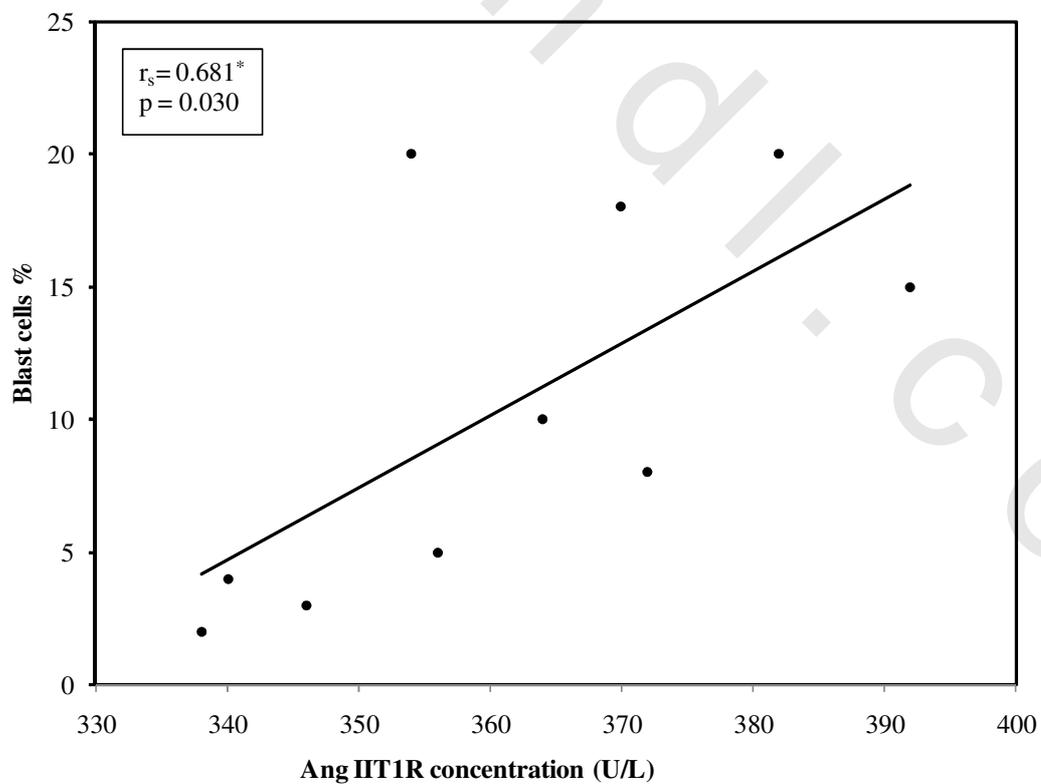
AngIIT1R concentration (U/L)	AML patients		ALL patients	
	Coff.	p	Coff.	p
Blast cells %	$r_s = 0.873^*$	<0.001	$r_s = 0.681^*$	0.030
WBCs count ( $\times 10^9$ )	$r_s = 0.094$	0.641	$r_s = -0.806^*$	0.005
HB concentration (g/dl)	$r_s = -0.216$	0.279	$r_s = -0.029$	0.938
Platelets count ( $\times 10^9$ )	$r_s = -0.052$	0.798	$r_s = -0.512$	0.130
Direct bilirubin concentration (mg/dl)	$r_s = 0.056$	0.781	$r_s = -0.365$	0.299
Indirect bilirubin concentration (mg/dl)	$r_s = -0.027$	0.895	$r_s = -0.279$	0.435
Total bilirubin concentration (mg/dl)	$r_s = 0.019$	0.924	$r_s = -0.081$	0.825
Total serum protein concentration (mg/dl)	$r_s = -0.180$	0.370	$r_s = -0.080$	0.827
Albumin concentration (mg/dl)	$r_s = 0.063$	0.755	$r_s = 0.129$	0.722
SGOT concentration (u/l)	$r_s = 0.253$	0.204	$r_s = -0.115$	0.751
SGPT concentration (u/l)	$r_s = -0.226$	0.257	$r_s = -0.401$	0.250
Alkaline phosphatase concentration (u/l)	$r_s = -0.328$	0.095	$r_s = 0.201$	0.578
Uric acid concentration (mg/dl)	$r_s = -0.116$	0.563	$r_s = -0.018$	0.960
Urea concentration (mg/dl)	$r_s = 0.428^*$	0.026*	$r_s = 0.073$	0.841
Creatinine concentration (mg/dl)	$r_s = 0.135$	0.502	$r_s = -0.140$	0.699
Sodium concentration (meq/l)	$r_s = -0.248$	0.212	$r_s = -0.838^*$	0.002*
Potassium concentration (meq/l)	$r_s = -0.244$	0.220	$r_s = 0.332$	0.348
Calcium concentration (meq/l)	$r_s = 0.073$	0.716	$r_s = -0.079$	0.828
Phosphorus concentration (meq/l)	$r_s = 0.099$	0.624	$r_s = 0.215$	0.550

$r_s$ : Spearman coefficient

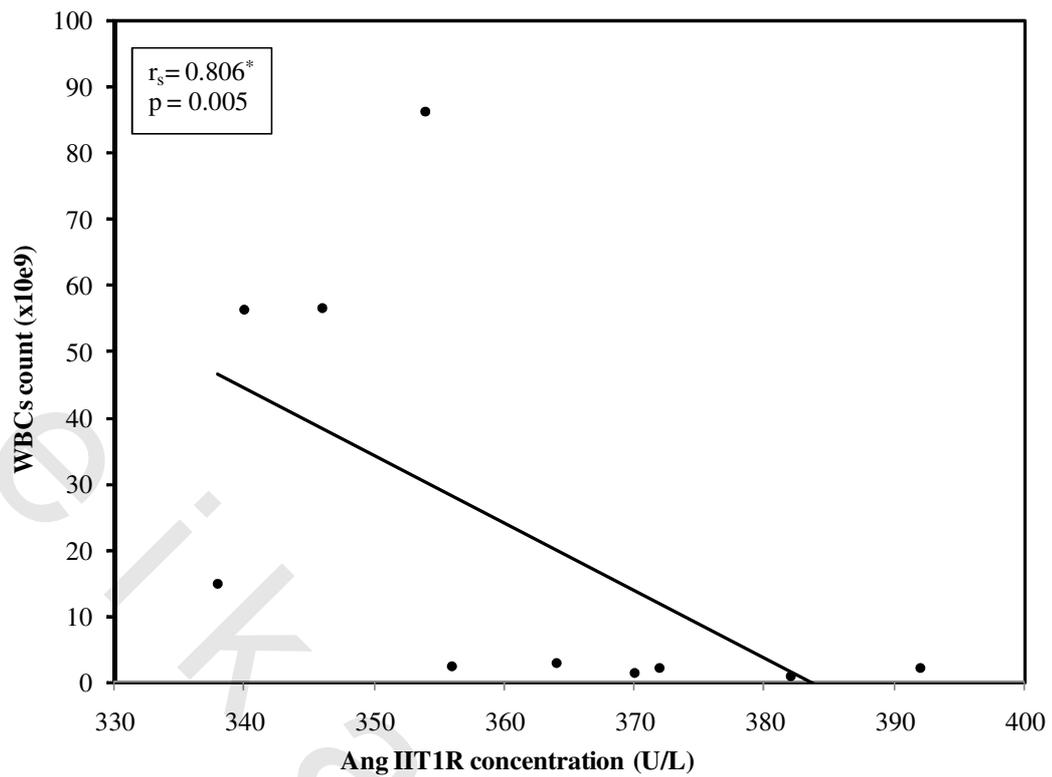
\*: Statistically significant at  $p \leq 0.05$



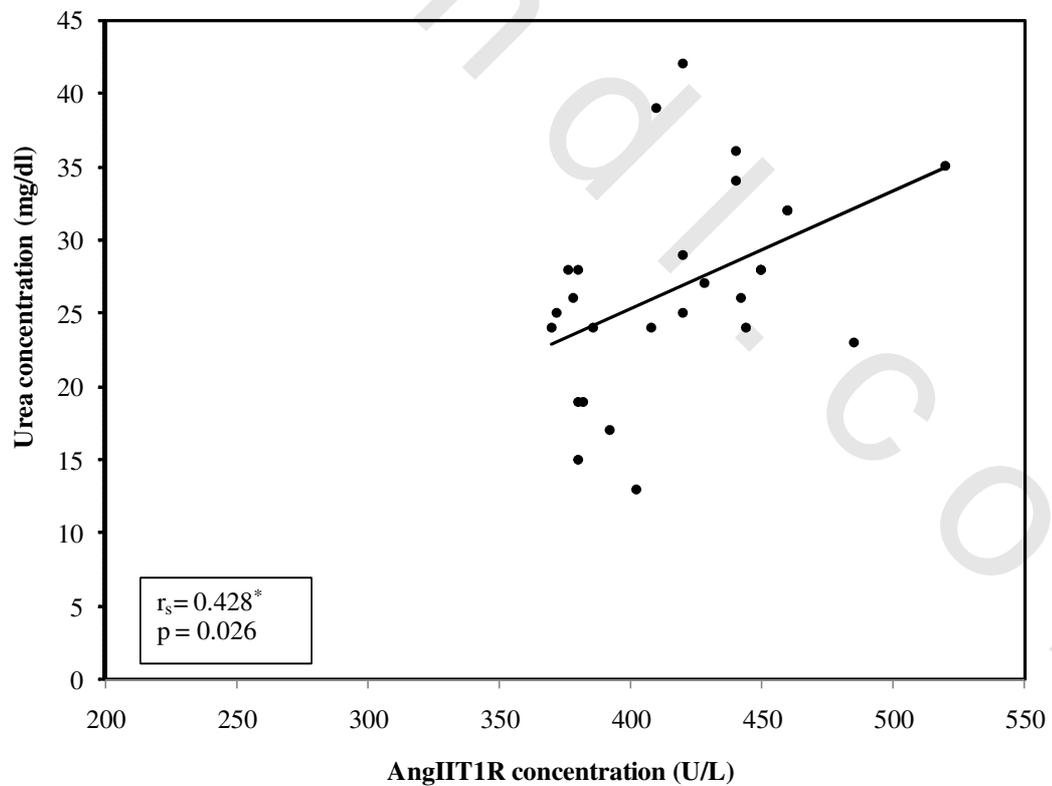
**Figure (35):** Correlation between Ang IIT1R concentration (U/L) and Blast cells % in AML patients



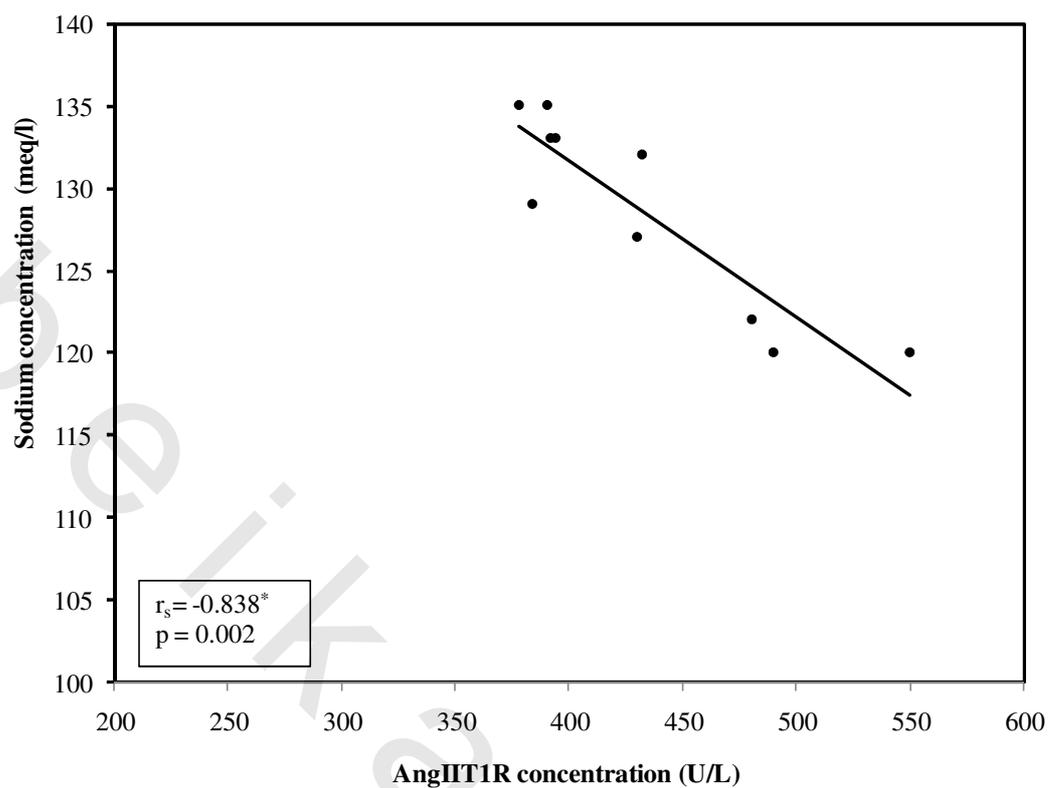
**Figure (36):** Correlation between Ang IIT1R concentration (U/L) and Blast cells % in ALL patients



**Figure (37):** Correlation between Ang IIT1R concentration (U/L) and WBCs count (x10e9) in ALL patients



**Figure (38):** Correlation between Ang IIT1R concentration (U/L) and urea concentration (mg/dl) in AML patients



**Figure (39):** Correlation between Ang II T1R concentration (U/L) and Sodium concentration (meq/l) in ALL patients

#### 4 – COMPARISON BETWEEN THE VALUES OF SERUM ACE ACTIVITY (U/L) AND ANG IIT1R CONCENTRATION (U/L) AS DIAGNOSTIC MARKERS FOR ACUTE LEUKEMIA

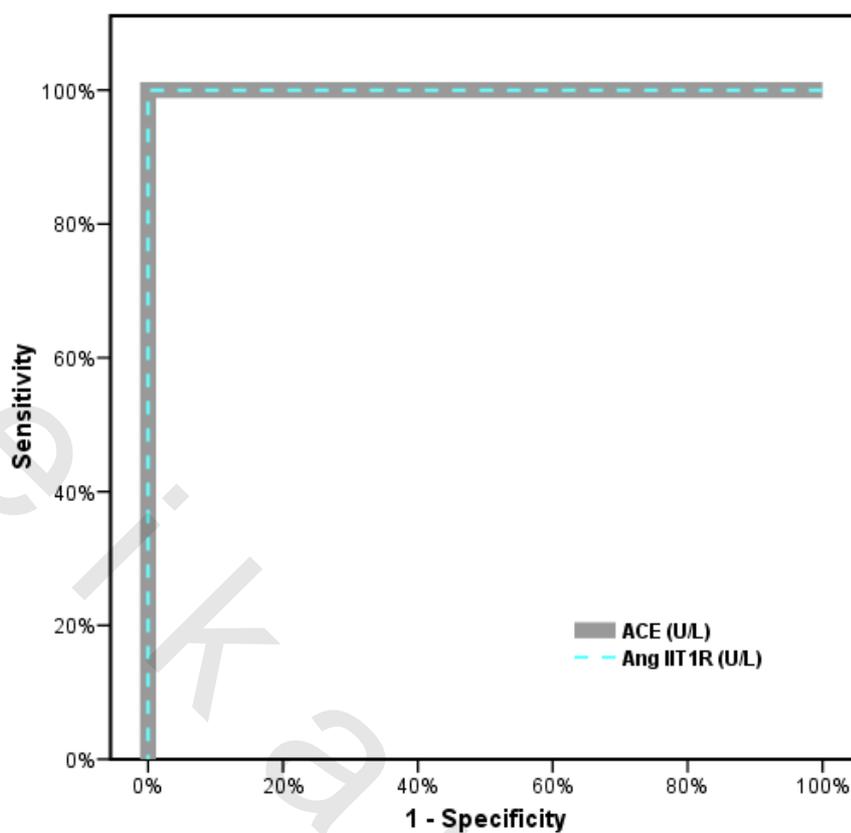
The ROC curves analysis was used to compare the diagnostic values of serum ACE activity (U/L) and Ang IIT1R concentration (U/L) depending on the area under the curves (AUC). The higher AUC corresponds to a better diagnostic test.

Serum ACE activity (U/L) showed significant AUC (100%,  $p < 0.001$ ) with sensitivity 100% and specificity 100% at a cut off value (47.25 U/L).

Serum Ang IIT1R concentration (U/L) showed significant AUC (100%,  $p < 0.001$ ) with sensitivity 100% and specificity 100% at a cut off value (360 U/L).

**Table (10):** The area under the ROC curves, sensitivity and specificity for serum ACE activity (U/L) and Ang IIT1R concentration (U/L) in acute leukemic patients before therapy

Before therapy	Area under the curve	Asymptomatic significance	Cut off values	Sensitivity %	Specificity %
ACE activity (U/L)	100%	<0.001	47.25	100.0	100.0
Ang IIT1R concentration (U/L)	100%	<0.001	360	100.0	100.0



**Figure (40):** Graphical representation of the ROC curves for serum ACE activity (U/L) and Ang IIT1R concentration (U/L) in acute leukemic patients before therapy

## 5 – PROGNOSTIC VALUES OF SERUM ACE ACTIVITY (U/L) AND ANG IIT1R CONCENTRATION (U/L) IN ACUTE LEUKEMIC PATIENTS

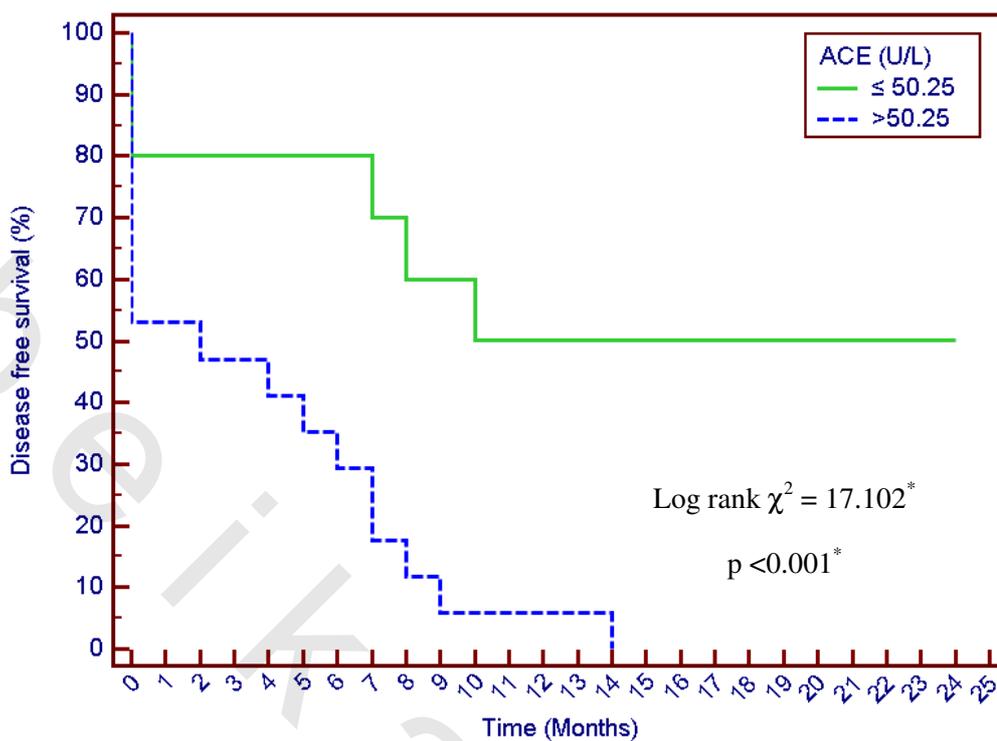
To study the prognostic values of these two parameters the Kaplan-Meier disease free survival (DFS) curves were constructed. As shown in table (11 and 12) and figures (41 and 42) Kaplan-Meier DFS curves for acute leukemic patients groups revealed that, patients with elevated levels of serum ACE activity (U/L) and Ang IIT1R concentration (U/L) than their corresponding cut off points were significantly different from those with low levels according to log rank test ( $p < 0.001^*$  and  $p < 0.001^*$  respectively)

**Table (11): Test of significance of disease free survival of serum ACE activity (U/L) in acute leukemic patients**

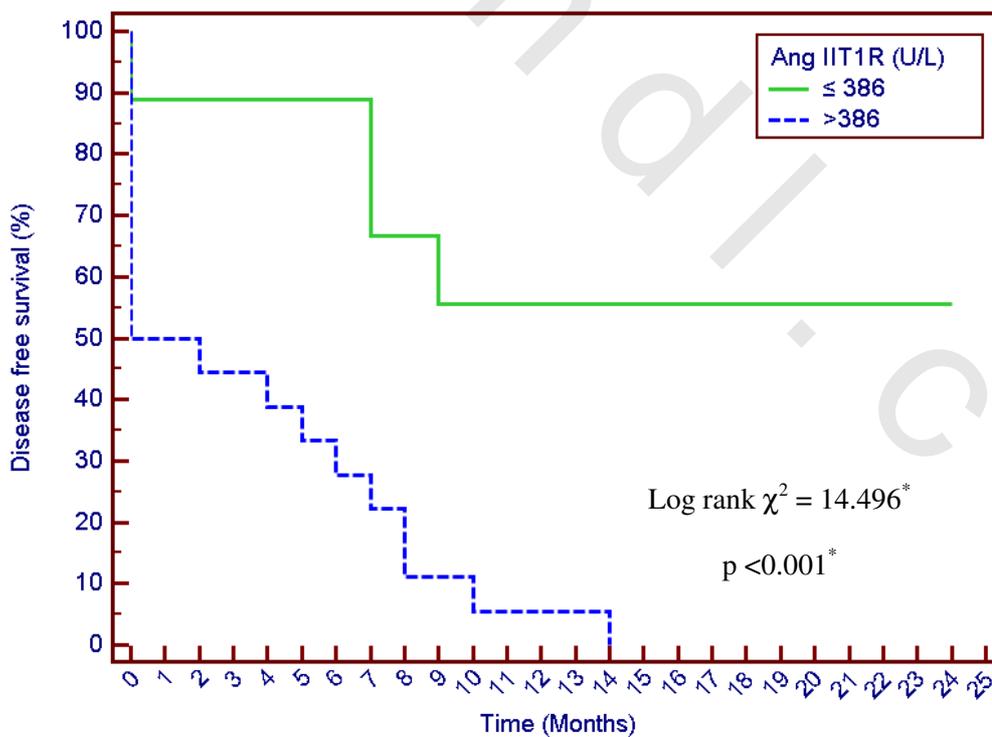
ACE (U/L)	Mean	%	Log rank	
			$\chi^2$	p
$\leq 50.25$	14.75	50.0	17.102*	$< 0.001^*$
$> 50.25$	2.84	0.0		

**Table (12): Test of significance of disease free survival of serum Ang IIT1R concentration (U/L) in acute leukemic patients**

Ang IIT1R (U/L)	Mean	%	Log rank	
			$\chi^2$	p
$\leq 386$	15.18	54.5	14.496*	$< 0.001^*$
$> 386$	3.12	0.0		



**Figure (41):** Kaplan-Meier disease free survival of serum ACE activity (U/L) for acute leukemic patients



**Figure (42):** Kaplan-Meier disease free survival of serum Ang IIT1R concentration (U/L) for acute leukemic patients