AIM OF THE WORK

The aim of the present work was to study telomere length and human telomerase reverse transcriptase (hTERT) level in acute myeloid leukemia and to detect if these parameters might be useful in providing insight into the clinical outcome of AML patients.

SUBJECTS

After the consent of the Ethical Committee of the Medical Research Institute, seventy Individuals were included in the study and divided as follows:

Twenty healthy individuals with comparable age and sex as a control group.

Fifty patients with acute myeloid leukemia.

Inclusion criteria:

Newly diagnosed acute myeloid leukemia cases either Donovo or secondary to myelodysplastic syndrome and myeloproliferative neoplasms in patients older than 16 years were included in the study. Patients were subdivided according to French-American-British (FAB) classification.

Exclusion criteria:

Acute myeloid leukemia cases younger than 16 years.

The patients were treated according to the standard chemotherapy protocol for induction. Patients received Cytarabine 100 mg/m^2 continuous IV infusion for 7days **plus** daunorubicin 60 mg/m^2/day for 3days. Patients were followed up by bone marrow examination at day 28 to determine patient's response. The term complete remission (CR) generally refered to morphologic complete remission, defined by red blood cell transfusion independence, an absolute neutrophil count of >1,000/µl and a platelet count of 100,000/µl or greater, and a morphologic leukemia-free state, defined by <5% blasts in a bone marrow aspirate sample with marrow spicules and with a count of at least 200 nucleated cells, absence of blasts with Auer rods, and absence of extramedullary leukemia. Partial remission is defined by attainment of the blood count criteria for complete remission, and a decrease of at least 50% in the percentage of blasts in the bone marrow aspirate, to 5 to 25%, or ≤5% blasts in the marrow with presence of Auer rods. Patients who have persistent AML in marrow and/or blood were said to have a refractory disease. Relapsed disease defined as the reappearance of leukemia cells in the bone marrow or peripheral blood or elsewhere in the body (other tissues/organs) after the attainment of a CR. [156]

METHODS

All subjects participating in this study were subjected to the following:

I- Thorough history taking.

A full medical history was taken from all subjects with special stress on:

- Age and sex.
- Complaint regarding fatigue, fever, weight loss, abdominal pain or discomfort, bone pain and bleeding attacks.

II- Thorough clinical examination.

With special emphasis on:

a- General examination for pallor, purpura, lymphadenopathy and skin nodules.

b- Spleen size in midclavicular line below the left costal margin, consistency and tenderness.

c- Liver size and consistency.

III-Routine work up:

- Renal function tests [157]
- Liver function tests [157]
- Radiological work up (chest X-ray, U/S abdomen &pelvis and ECHO)

IV- Diagnostic laboratory investigations

a- Complete blood picture (CBP) [158]

CBP from 2.5 ml EDTA-anticoagulated blood was done using automated cell counter. The blood films will be prepared, stained by Leishman stain and then examined for differential WBC and RBC morphology.

b- Bone marrow examination [159]

Bone marrow aspiration was done for all patients either from sternum or posterior iliac crest. Marrow aspirates were then smeared on glass slides and stained by Leishman stain.

c- Immunophenotyping [160]

Bone marrow or blood samples (2-3 mL) with heparin anticoagulation from patients were obtained, and immunophenotypes were identified by flow cytometry using monoclonal antibodies directed to antigens for T cells, B cells, myeloid cells [CD13, CD33, CD117 and myeloperoxidase (MPO)], monocytes (CD14 and CD64), erythroid cells (alpha-glycophorin), platelet cells (CD61 and CD41a), non-specific lineage pan-

leukocytes (CD45) and precursor cells [CD34, human leukocyte antigen-DR (HLA-DR) and terminal deoxynucleotidyl transferase (TdT)].

V- Advanced investigations

A - Quantitative assessment of hTERT by ELISA. [161]

Kit was purchased from GenWay

Principle of the test

The hTERT Elisa kit is a polyclonal - polyclonal sandwich ELISA. An antibody to hTERT is pre-coated to plastic micro well strips. During incubation hTERT from recombinant calibrators and samples bind to the precoated antibody. The wells are washed to remove unbound components and a biotin conjugated antibody to hTERT is added to complete the antibody sandwich. The wells are washed to remove any unbound detection antibody and incubated with streptavidin - HRP. The wells are washed again to remove excess streptavidin -HRP and incubated with tetramethylbenzidine (TMB) substrate resulting in a blue colored solution. A sulfuric acid solution is then added to the wells to stop reaction and turning the solution yellow. The intensity of the yellow is directly proportional to the concentration of hTERT in sample. hTERT levels are quantified by measuring the absorbances at 450 nm and comparing the absorbance vs. concentration generated from the standard curve. The kit range can detect hTERT concentration between 07.81 - 500 ng/ml

Samples

2ml of blood from each AML patient and the control was collected in a tube-allowed to clot for 30 minutes at room temperature then centrifuged for 10 minutes at 5000 rpm.

Serum was removed and collected in an Ependorf, labelled and stored at –20°C till assay

Assay procedure

- All samples and kit reagents were brought to room temperature (20-25°C) two hours before the assay.
- Kit reagents were reconstituted and properly mixed using vortex.
- Decreasing concentrations of the standard were made.
- 100 µl of standards and samples were placed in the designated wells.
- The plate was incubated at room temperature for 1 hour.
- After incubation, each well was filled with 200 µl of 1X Wash Buffer and aspirated. Repeated 5 times.
- 100 µl of Detection antibody was dispensed in each well. The plate was incubated at room temperature for 1 hour.

- Then the plate was washed 5 times.
- 100 µl of streptavidin-HRP was transferred in each well, plate was incubated at room temperature for 1 hour.
- The plate was washed again as described before.
- 100 µl of the mixed substrate solution was transferred in each well.
- The plate was incubated for 15-20 minutes.
- 100 µl stop solution was placed in each well.
- The plate was read by microplate reader at 450 nm.

Calculation of results

- Average from the duplicate reading of standard, control, and sample was calculated.
- Zero reading was subtracted from each averaged value.
- Standard curve was created by reducing the data using computer software capable of generating a four parameter logistic (4-PL) curve –fit.
- The concentration of unknown was determined from the standard curve.

B - A quantitative PCR method for measuring telomere length. [162]

- o Genomic DNA from patients was extracted using QIAamp DNA Blood Mini Kit (50) from Qiagen.
- o Relative telomere length (RTL) were determined using real-time PCR

i - The QIAamp DNA Blood Spin Procedure

All reagents were prepared as followed :

a- QIAGEN Protease stock solution was prepared: 1.2 ml protease solvent was pipetted into the vial containing lyophilized QIAGEN Protease.

b- Buffer Al was mixed thoroughly by shaking before use.

c- Buffer AW1was prepared by adding 25 ml of ethanol (96–100%)

d- Buffer AW2 was prepared by adding 30 ml of ethanol (96–100%) .

- All samples and reagents were equilibrated to room temperature (15–25°C).
- Water bath was heated to 56°C.
- All centrifugation steps were carried out at room temperature.

1. 20 µl QIAGEN Protease was piptted into the bottom of a 1.5 ml microcentrifuge tube.

2. 200 µl whole blood was added to the microcentrifuge tube.

3. 200 µl Buffer AL was added to the sample. Mixed by pulse-vortexing for 15 s.

4. The microcentrifuge tube was incubated at 56°C for 10 min.

5. The 1.5 ml microcentrifuge tube was centrifuged to remove drops from the inside of the lid.

6. 200 µl ethanol (96–100%) was added to the sample, and mixed again by pulse-vortexing for 15 s. After mixing, the 1.5 ml microcentrifuge tube was centrifuged to remove drops from the inside of the lid.

7. The mixture from step 6 carefully applied to the QIAamp Spin Column (in a 2 ml collection tube) without wetting the rim, the cap was closed, and centrifuged at 6000 x *g* (8000 rpm) for 1 min. the QIAamp Spin Column was placed in a clean 2 ml collection tube, and the tube containing the filtrate was discarded.

8. The QIAamp Spin Column carefully opened and add 500 µl Buffer AW1 without wetting the rim then the cap was closed and centrifuged at 6000 x *g* (8000 rpm) for 1 min. The QIAamp Spin Column was placed in a clean 2 ml collection tube, and the collection tube containing the filtrate was discarded.

9. The QIAamp Spin Column was carefully opened and 500 µl Buffer AW2 was added without wetting the rim. Then the cap was closed and centrifuged at full speed (20,000 x *g*; 14,000 rpm) for 3 min.

10. The QIAamp Spin Column was placed in a clean 1.5 ml microcentrifuge tube, and the collection tube containing the filtrate was discarded. The QIAamp Spin Column was carefully opened and 200 µl Buffer AE was added. The tube was incubated at room temperature (15–25°C) for 1 min, and then centrifuge at 6000 x *g* (8000 rpm) for 1min.

11. The DNA eluted in Buffer AE was stored at –20°C.

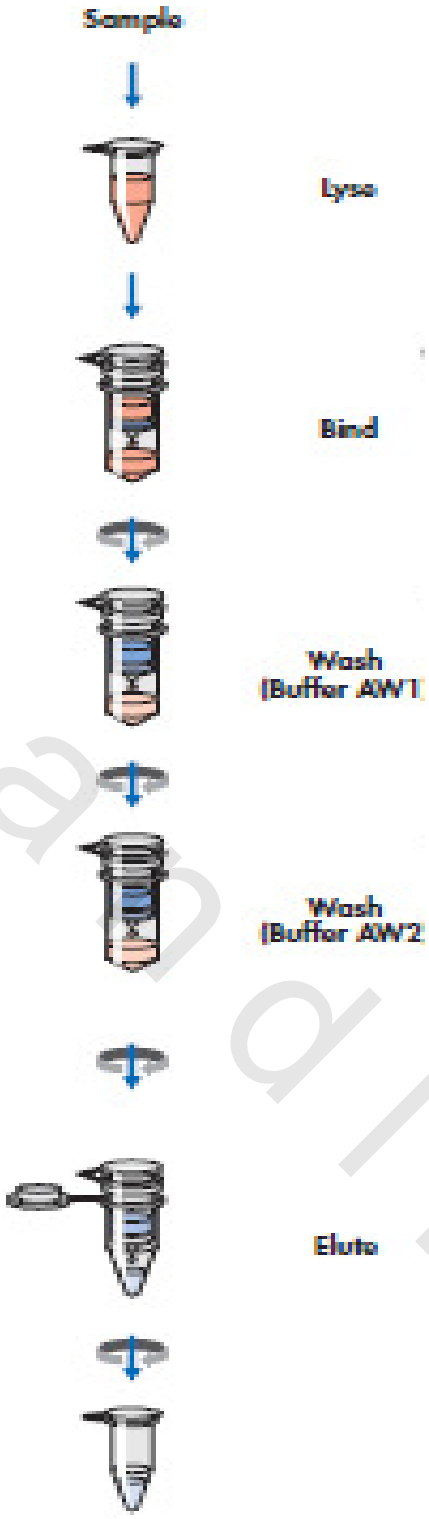


Fig. (3): The QIAamp DNA Spin procedure.

ii- Determination of concentration, yield, and purity

The concentration of DNA in the eluate was determined by measuring the absorbance at 260 nm with a spectrophotometer (Nanodrop ND –2000 spectrophotometer). The Purity was determined by calculating the ratio of absorbance at 260 nm to absorbance at 280 nm. Pure DNA has an *A*260/*A*280 ratio of 1.7–1.9.

Fig. (4): Nanodrop ND –2000 spectrophotometer.

iii- Primer design and optimization

Primers for real-time PCR for the telomere gene (T), and the single copy gene (S), the housekeeping gene 36 B4 which encodes acidic ribosomal phosphoprotein were designed using Primer Premier 5.0 software.

- Human 36 B4 primers were:

  5'-CAGCAAGTGGGAAGGTGTAATCC-3' (forward) and

  5'-CCCATTCTATCAACGGGTACAA-3' (reverse).

- Human telomere primers (T) were :

  5'-GGTTTTTGAGGGTGAGGGTGAGGGTGAGGGTGAGGGT-3' (forward) and

  5'-TCCCGACTATCCCTATCCCTATCCCTATCCCTATCCCTA-3' (reverse).

iv- Real-time PCR

Real-time PCR was performed with a iNTRON Biotechnology Real/MOD^{TM} SYBR Green RT-PCR kit using the Rotor-Gene 1.7. 94.

1. The 2x Real/MOD^{TM} SYBR Green RT-PCR Master Mix was thawed, template and primers . The individual solutions were mixed and placed on ice.

2. A reaction mix was prepared according to Table VII. Samples were kept on ice while preparing the reaction mix.

3. The reaction mix is mixed thoroughly, and appropriate volumes were aliquots into Real-time PCR tubes.

4. Template DNA was added to the individual PCR tubes containing the reaction mix.

5. The real-time cycler was programmed according to the program outlined in Table VIII.

Table VII: Reaction mix setup.

Component	Volume/reaction	Final concentration
2x RealMODTM Green Real-time PCR Master mix Solution	10 µl	1x
PCR Forward Primer	Variable	0.2 µM
PCR Reverse Primer	Variable	0.2 µM
Template DNA	1 µl	≤100 ng/reaction
Total volume	**20 ml**	

Table VIII: Real-time cycler conditions

Hold @ 95°c, 15 min	
Cycling (45 repeats)	Step 1 @ 94°c, hold 20 secs
	Step 2 @ 60°c, hold 30 secs
	Step 3 @ 72°c, hold 30 secs,)

1. The PCR tubes were placed in the real-time cycler, and the cycling program was started.

2. A melting curve analysis of the RT-PCR products was performed to verify the specificity and identity of RT-PCR products and exclude primer dimers.

TL was measured using the real-time quantitative polymerase chain reaction (PCR) method. This method determines, for each DNA sample, the factor by which it differs from a reference DNA sample in its ratio of telomere repeat copy number to a single copy gene copy number.

Using two primer pairs that target telomeric hexamer repeats and a single copy gene (*36B4*, which encodes acidic ribosomal phosphoprotein), the telomere repeat copy number (T) to single copy gene copy number (S) ratio was calculated for each DNA sample. The T/S ratio of each experimental DNA sample was related to a reference DNA sample.

Since the amount of the PCR products doubled in each cycle, the T/S ratio was $[2^{c}_{t\ (telomeres)} / 2^{c}_{t\ (36\ B4)}]^{-1} = 2^{-\Delta C}_{t.}$

The relative T/S ratio (T/S of one sample relative to the T/S of another sample) was $2^{-(\Delta C_{t\ sample} - \Delta C_{t\ control})} = 2^{-\Delta\Delta C}_{t}.$

A -

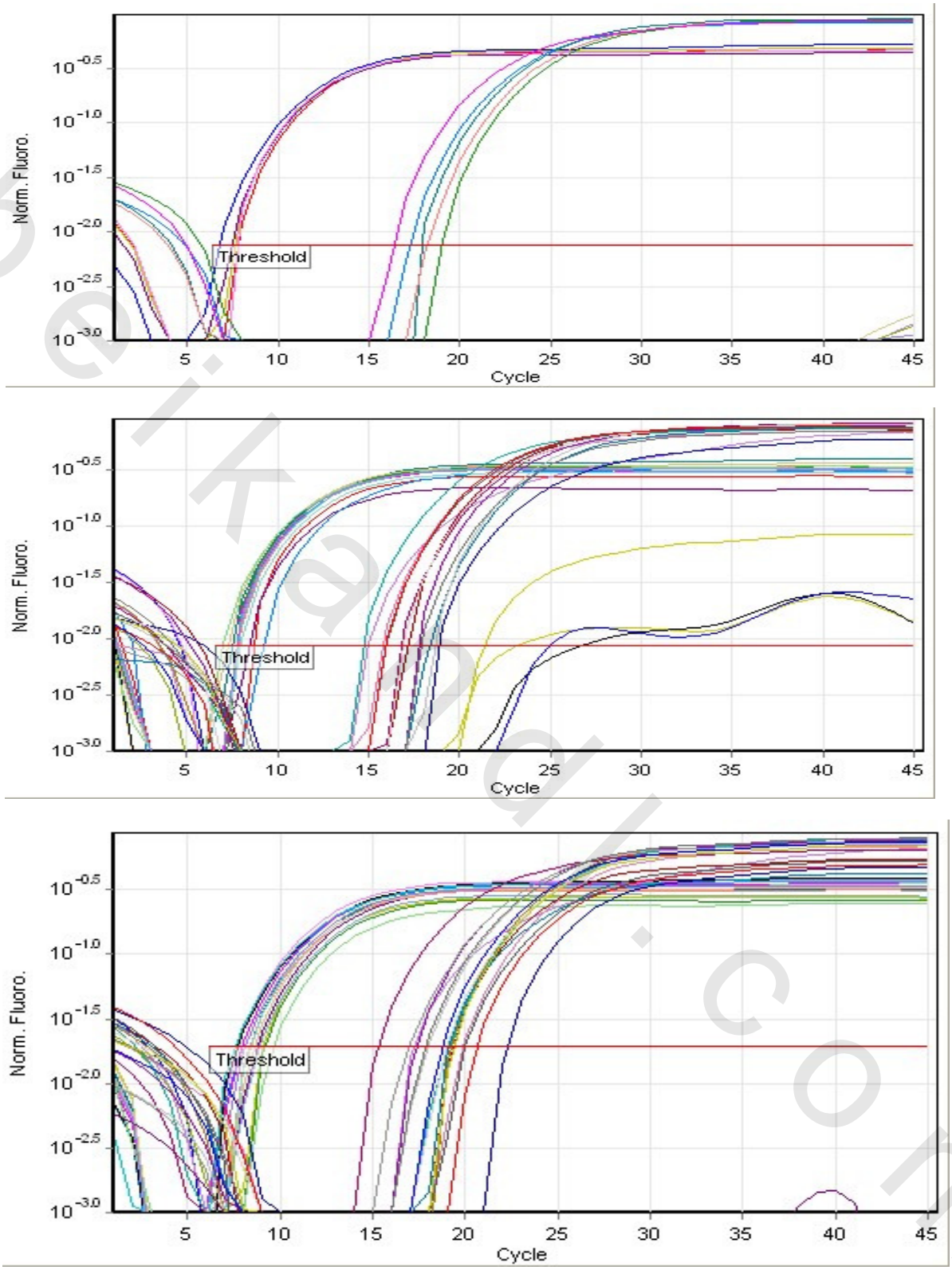


Fig.(5): Cycle threshold for both T and S genes in different samples (from A to C).

VI- Statistical analysis

The statistical analysis was done using an IBM compatible computer and SPSS statistical package for social sciences, version 20.

Continuous variables were expressed as mean ± standard deviation (Mean ± SD), while categorical variables were expressed as numbers and percentages.

Analytical tests used included

- Unpaired student t test (two sided), Mann-Whitney test for comparing means of two groups.
- Statistical correlations between two categorical variables were tested using **Chi- square (χ^2)** or **Fisher Exact tests.** Statistical correlations between two continuous variables were tested using (2-tailed) **Spearman's correlation coefficient (r) and Pearson correlation.**
- **Receiver operating characteristics (ROC)** curves were used to evaluate the specificity, sensitivity and overall accuracy of the predictive variables.
- **Overall survival (OS)** was calculated by **Kaplan-Meier survival** curves and survival differences between subgroups were compared using **log rank test**.

Significance level of **0.05** was used throughout all statistical tests within this study.

RESULTS

The study included Seventy Individuals; twenty of them are healthy subjects with comparable age and sex as a control group, the remaining fifty are acute myeloid leukemia (AML) patients. Thirty six patients were younger than sixty years (72 %). Fourteen patients were sixty years or older (28 %).

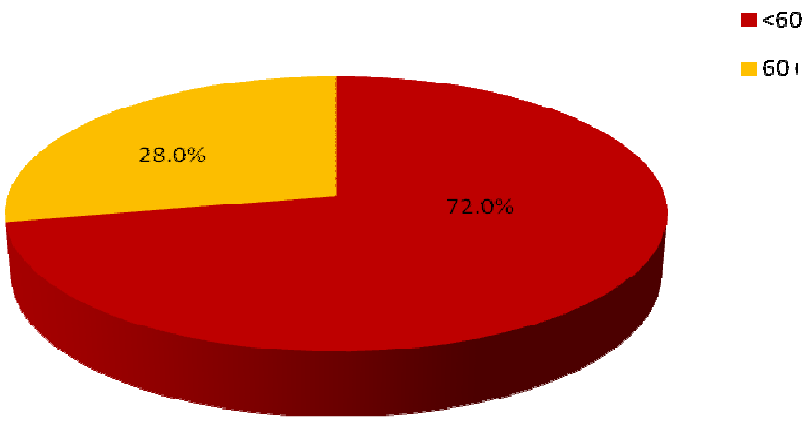
Demographic data

Age and sex distribution illustrated in Tables (IX) and Figures (6 and 7).

The age of AML patients ranged from twenty three to seventy one years with a mean age of 50.8±12.97. Twenty four (48%) patients were males and 26 (52%) were females with male to female ratio of 1: 1.1.

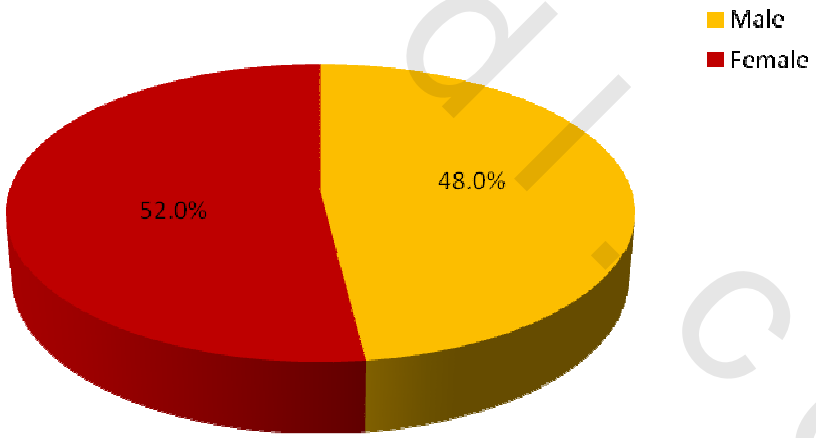
Table (IX): Age and sex distribution of AML patients.

Characteristics	No	%
Age in years		
▪ <60	36	72.0
▪ 60+	14	28.0
Range	23-71	
Mean ± SD	50.8 ± 12.7	
Sex		
▪ Male	24	48.0
▪ Female	26	52.0



Age distribution

Fig. (6): Age distribution of AML patients.



Sex distribution

Fig. (7): Sex distribution of AML patients

Clinical data of AML patients:

The clinical findings in all our patients are enlisted in Table X. The main presenting symptom was fatigue (96% in all AML patients, 97% in adult AML patients), followed by bone pain (84% in all AML patients, 80% in adult AML patients), fever (52% in all AML patients, 55.6% in adult AML patients), and bleeding (50% in all AML patients, 58.3% in adult AML patients).

On the other hand, the main symptoms of elderly AML were bone pains (92.9%), fatigue (92.9%), hepatomegaly (50%), fever (42.9%), urate nephropathy (14.3%). There was no statistically significant difference between both groups of AML patients regarding the clinical presentation of the disease.

Table (X): Clinical data of AML patients included in the study

Clinical presentation	Age				Total		*FEP*
	<60		60+				
	No	%	No	%	No	%	
Fatigue	35	97.2	13	92.9	48	96.0	0.479
Fever	20	55.6	6	42.9	26	52.0	0.420
Bleeding	21	58.3	4	28.6	25	50.0	0.059
Lymphadenopathy	4	11.1	1	7.1	5	10.0	0.675
Hepatomegaly	11	30.6	7	50.0	18	36.0	0.198
Splenomegaly	8	22.2	4	28.6	12	24.0	0.637
Bone pain	29	80.6	13	92.9	42	84.0	0.287
Uric acid nephropathy	2	5.6	2	14.3	4	8.0	0.307

FEP: P value based on Fisher exact probability

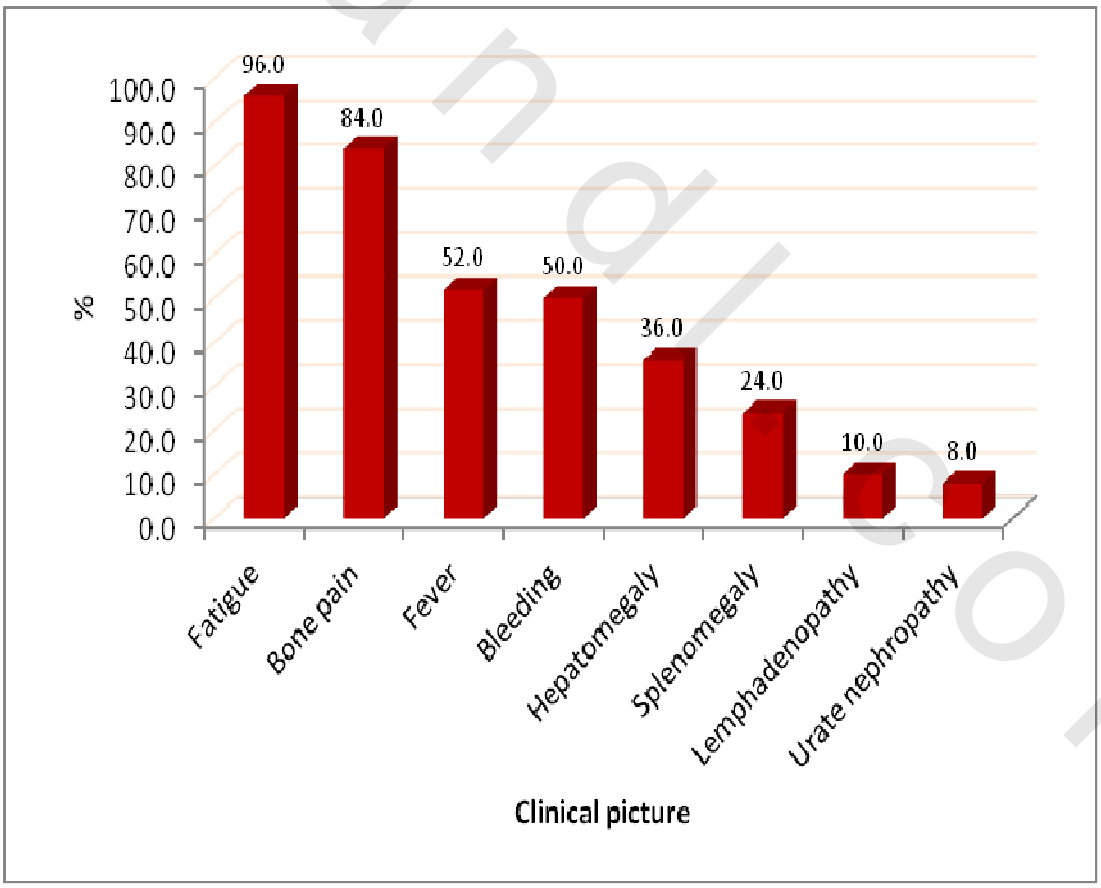


Fig.(8): Clinical data of AML patients included in the study

Haematological parameters

Comparison of haematological parameters in peripheral blood in AML patients in both age groups presented in table XI.

Haemoglobin concentration (Hb) in all AML cases was ranged from 4 g/dl to 11.5 g/dl; mean value was 7.5 g/dL ± 2. Mean Hb concentration in Elderly AML was higher than that in adult AML with mean values of 8±1.9 g/dL ranging from 5.2 to 11.5 g/dL. In adult AML patients Hb concentration ranging from 4 to 11.3 g/dL with a mean value 7.3± 2 g/dL. There was no significant statistical difference between the two groups.

Red blood cell count (RBC) in all AML patients was ranged from 1.7 to 4. ($\times 10^{12}$/L), mean value was 2.5±0.6($\times 10^{12}$/L). While in adult AML patients RBC ranging from 1.7 to 4. ($\times 10^{12}$/L), mean value was 2.4±0.6($\times 10^{12}$/L). In elderly cases RBC was ranging from 1.7 to 4($\times 10^{12}$/L), mean value was 2.6±0.6($\times 10^{12}$/L). There was no statistically significant difference between the two groups.

Platelet count (Plt) was ranged from 5 to 186 ($\times 10^{9}$/L) in all AML patients, mean value was 34.1±33.4 ($\times 10^{9}$/L). Platelet count in elderly group was ranging from 5 to 186 ($\times 10^{9}$/L), mean value was 52.4±46.9($\times 10^{9}$/L). While in younger group Plt count was ranging from 5 to 89($\times 10^{9}$/L), mean value was 27±23.7($\times 10^{9}$/L). Mean value in elderly was statistically significant higher than in young adult group (Z =2.1, p = 0.040).

Total leucocytic count (TLC). TLC in all patients was ranged from 0.3 to 340 ($\times 10^{9}$/L), mean value was 24.1±50.9 ($\times 10^{9}$/L). In elderly group, the mean TLC was 40.0±88 ($\times 10^{9}$/L) with a minimum of 1.1 and maximum of 340 ($\times 10^{9}$/L). In young age group AML cases mean TLC was 17.9±24.8($\times 10^{9}$/L), with minimum of 0.3 ($\times 10^{9}$/L) and maximum of 122($\times 10^{9}$/L). There was no statistical difference between the two groups.

Blast %: blast % in all patients was ranged from 0 % to 95 %, mean value was 50.5±24. In elderly group, Blast % ranged from 15 to 86 %, mean value was 46.5±21. In young age group Blast % ranged from o % to 95%, mean value was 52.1±25.1. There was no statistical difference between the two groups.

Table (XI): Comparison of haematological parameters in peripheral blood in AML patients in both age groups (young adult and elderly).

Peripheral blood counts		Age_c		Total	t	P
		<60	60+			
HB (g/dL)	▪ Minimum	4.0	5.2	4.0	1.1	0.266
	▪ Maximum	11.3	11.5	11.5		
	▪ Mean	7.3	8.0	7.5		
	▪ SD	2.0	1.9	2.0		
	▪ Median	7.0	8.1	7.4		
RBC count ($x10^{12}$)	▪ Minimum	1.7	1.7	1.7	0.96	0.340
	▪ Maximum	4.0	4.0	4.0		
	▪ Mean	2.4	2.6	2.5		
	▪ SD	0.6	0.6	0.6		
	▪ Median	2.4	2.6	2.4		
Plt count ($x10^{9}$)	▪ Minimum	5.0	5.0	5.0	Z=2.1	0.040*
	▪ Maximum	89.0	186.0	186.0		
	▪ Mean	27.0	52.4	34.1		
	▪ SD	23.7	46.9	33.4		
	▪ Median	20.0	47.5	23.0		
TLC($x10^{9}$)	▪ Minimum	0.3	1.1	0.3	Z=0.88	0.381
	▪ Maximum	122.0	340.0	340.0		
	▪ Mean	17.9	40.0	24.1		
	▪ SD	24.8	88.0	50.9		
	▪ Median	7.1	9.3	8.0		
Blast %	▪ Minimum	0.0	15.0	0.0	Z = 0.68	0.498
	▪ Maximum	95.0	86.0	95.0		
	▪ Mean	52.1	46.5	50.5		
	▪ SD	25.1	21.0	24.0		
	▪ Median	58.0	50.0	50.0		

t: independent samples t-test
Z: Mann-Whitney test
* $P < 0.05$ (significant)
Hb: haemoglobin, RBC: red blood cell, plt::platelets,TLC: total leucocytic count

Bone Marrow Examination:

Bone marrow aspiration was performed for all patients included in the study as shown in table (XII).

Table (XII): Myelograms of individual AML patients.

No	Blast %	pro %	Myelo %	Meta %	staff %	seg %	E %	B %	L %	M %	pro-eryt%	Normo %	plasma %	M/E	FAB
1	84	0	0	1	0	0	0	0	7	0	0	8	0	↑	M 1
2	90	0	0	0	0	0	0	0	5	0	0	5	0	↑	M 1
3	42	0	6	13	10	12	0	0	7	4	0	6	0	↑	M 2
4	93	0	0	1	0	0	0	0	4	0	0	2	0	↑	M 1
5	72	1	1	0	2	3	0	0	0	5	1	12	1	↑	M 2
6	29	0	16	4	6	0	1	2	15	1	0	24	2	↑	M 2
7	75	1	4	3	2	5	0	1	3	0	0	6	0	↑	M 2
8	42	2	13	15	3	3	1	0	0	0	0	15	0	↑	M 2
9	76	0	0	2	2	0	0	0	2	0	1	17	0	↑	M 1
10	53	1	5	2	2	4	0	1	6	20	0	6	0	↑	M 4
11	80	1	2	1	0	1	0	0	0	4	1	10	0	↑	M 2
12	12	70	0	0	0	0	0	0	2	0	0	15	1	↑	M 3
13	24	2	4	2	2	0	0	0	3	44	1	15	3	↑	M 5
14	60	0	11	6	6	8	0	1	0	0	0	8	0	↑	M 2
15	74	1	5	3	4	0	0	0	2	0	0	10	1	↑	M 2
16	75	1	6	2	3	1	0	0	4	0	0	10	0	↑	M 2
17	55	2	13	5	7	3	1	0	4	0	0	10	0	↑	M 2
18	80	2	3	2	3	0	0	0	2	1	0	7	0	↑	M 2
19	60	2	13	5	8	2	0	0	0	0	0	10	0	↑	M 2
20	84	0	0	0	0	0	0	0	12	0	0	3	0	↑	M 1
21	65	1	5	3	4	0	0	0	2	0	0	20	0	↑	M 2
22	20	1	5	7	5	10	0	1	5	20	0	25	1	↑	M 4
23	30	2	8	8	7	5	0	1	2	22	0	15	0	↑	M 4
24	66	1	7	5	8	0	0	0	2	0	0	11	0	↑	M 2

Table (XII): Myelograms of individual AML patients (cont.)

No	Blast %	Pro %	Myelo %	Meta %	staff %	seg %	E %	B %	L %	M %	pro-eryt%	Normo %	plasma %	M/E	FAB
25	80	0	2	4	4	0	0	1	1	0	0	8	0	↑	M 7
26	74	1	5	3	4	0	0	0	2	0	0	11	0	↑	M 2
27	45	0	10	10	9	4	1	0	4	2	1	12	2	↑	M 2
28	62	0	2	0	5	2	0	0	12	14	0	3	0	↑	M 4
29	60	2	5	3	6	4	2	1	4	0	0	15	0	↑	M 2
30	75	1	6	2	3	1	0	0	4	0	0	10	0	↑	M 2
31	70	0	5	5	6	4	0	0	0	0	0	10	0	↑	M 2
32	80	1	2	1	0	1	0	0	0	4	1	10	0	↑	M 2
33	75	1	6	2	3	1	0	0	4	0	0	10	0	↑	M 2
34	42	2	13	15	3	3	1	0	0	0	0	15	0	↑	M 2
35	54	1	2	2	8	13	0	0	10	0	0	10	0	↑	M 2
36	74	1	5	3	4	0	0	0	2	0	0	11	0	↑	M 2
37	72	1	1	0	2	3	0	0	0	5	1	12	1	↑	M 2
38	74	1	5	3	4	0	0	0	2	0	0	11	0	↑	M 2
39	50	0	7	4	4	9	1	0	10	1	0	12	0	↑	M 2
40	58	0	7	0	1	2	0	0	8	18	0	6	0	↑	M 4
41	22	0	5	6	6	9	0	1	4	23	0	23	1	↑	M 4
42	14	1	5	4	6	0	0	0	4	3	2	60	1	↓	M 6
43	70	0	6	4	3	2	1	1	0	0	1	10	2	↑	M 2
44	98	0	0	0	0	0	0	0	0	0	0	2	0	↑	M 1
45	80	1	2	1	0	1	0	0	0	4	1	10	0	↑	M 2
46	84	0	0	0	0	0	0	0	12	0	0	3	0	↑	M 1
47	92	0	0	0	0	0	0	0	7	0	0	1	0	↑	M 1
48	95	0	2	0	0	0	0	0	0	0	0	3	0	↑	M 1
49	42	0	6	13	10	12	0	0	7	4	0	6	0	↑	M 2
50	84	0	0	0	0	0	0	0	12	0	0	3	0	↑	M 1

Immunophenotyping of AML patients illustrated in table (XIII):

Bone marrow or blood samples (2-3 mL) with heparin anticoagulation from patients were obtained, and immunophenotypes were identified by flow cytometry using monoclonal antibodies directed to antigens for T cells, B cells, myeloid cells [CD13, CD33, CD117 and myeloperoxidase (MPO)], monocytes (CD14 and CD64), erythroid cells (alpha-glycophorin), platelet cells (CD61 and CD41a), non-specific lineage pan-leukocytes (CD45) and precursor cells [CD34, human leukocyte antigen-DR (HLA-DR) and terminal deoxynucleotidyl transferase (TdT)].

Table XIII: Immunophenotyping of AML patients.

	CD 13	CD 33	CD 117	MPO	CD 14	CD 64	glycophorin	CD 61	CD 41 a	CD 45	CD 34	HLA-DR	TdT	OTHERS
1	+	+	-	+	-	-	-	-	-	+	+	+	-	
2	+	+	+	+	-	-	-	-	-	-	+	+	-	
3	+	+	+	+	-	-	-	-	-	-	+	+	-	
4	+	+	+	+	-	-	-	-	-	-	+	+	+	
5	+	+	-	+	-	-	-	-	-	-	+	+	-	
6	+	+	-	+	-	-	-	-	-	-	+	+	-	
7	+	+	-	+	-	-	-	-	-	+	+	+	-	
8	+	+	-	+	-	-	-	-	-	+	+	+	+	
9	+	+	-	+	-	-	-	-	-	+	+	+	-	
10	+	+	-	+	-	+	-	-	-	+	+	+	-	
11	+	+	-	+	-	-	-	-	-	+	+	+	-	
12	+	+		+						+	+	-		
13	+	+								+		+		7
14	+	+		+	-					+	+	-		
15	+	+		+						+				
16	+	+		+							+			
17	+	+		+								+		
18	+	+		+						+	+	+		
19	+	+								+	+	+		
20	+	+	+	+	-	-	-	-	-	-	+	+	-	
21	+	+		+						+				
22	+	+									+	+		

Table XIII: Immunophenotyping of AML patients (cont.)

	CD 13	CD 33	CD 117	MPO	CD 14	CD 64	glycophorin	CD 61	CD 41 a	CD 45	CD 34	HLA-DR	TdT	OTHERS
23	+	+								+	+	+		
24	+	+		+						+				
25		+							+		+			
26	+	+		+						+				
27	+	+		+						+	+	+		
28	+	+			+	+				+	+	+		11b
29	+	+								+	+	+		
30	+	+		+						+	+	+		
31	+	+								+	+	+		
32	+	+	-	+	-	-	-	-	-	+	+	+	-	
33	+	+		+							+			
34	+	+	-	+	-	-	-	-	-	+	+	+	+	
35	+	+			+	+				+	+	+		
36	+	+		+						+				
37	+	+	-	+	-	-	-	-	-	-	+	+	-	
38	+	+		+						+				
39	+	+			+	+				+	+	+		
40	+	+			+	+				+	+	+		
41	+	+									+	+		
42														
43	+	+		+						+	+			
44	+	+		+						+		+		7
45	+	+	-	+	-	-	-	-	-	+	+	+	-	19
46	+	+	+	+	-	-	-	-	-	-	+	+	-	
47	+	+		+							+	+		
48	+	+								+	+	+		
49	+	+	+	+	-	-	-	-	-	-	+	+	-	
50	+	+	+	+	-	-	-	-	-	-	+	+	-	

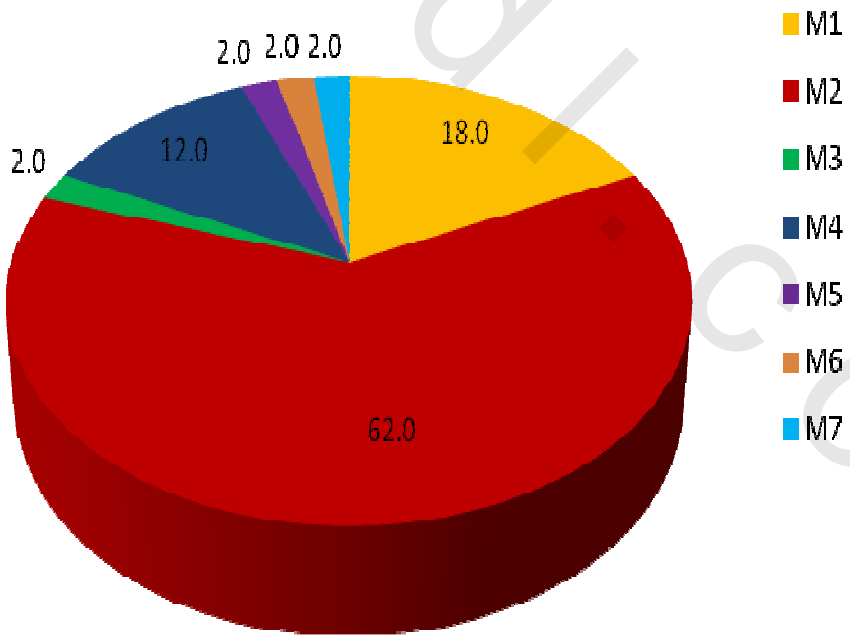
FAB classification illustrated in table XIV:

AML patients were subclassified according to the French-American-British (FAB) classification system using morphologic and cytochemical criteria. The most common type in all patients was M2 (62 %, 31 cases), followed by M1 (18 %, 9 cases), then M4 (12 %, 6 cases).other types (M3,M5,M6,M7) representd by only one case for each.

Table XIV: FAB classification of AML patients

FAB classification	Age				Total		*MCP*
	<60		60+				
	No	%	No	%	No	%	
M1	5	13.9	4	28.6	9	18.0	
M2	24	66.7	7	50.0	31	62.0	
M3	1	2.8	0	0.0	1	2.0	
M4	3	8.3	3	21.4	6	12.0	0.589
M5	1	2.8	0	0.0	1	2.0	
M6	1	2.8	0	0.0	1	2.0	
M7	1	2.8	0	0.0	1	2.0	

MCP: P value based on Mont Carlo exact probability



FAB classification of AML patients

Fig. (9): Percentage of AML distribution according to FAB classification

Cytogenetic and molecular analysis of AML patients presented in figure 10:

Conventional cytogenetic done for 18 patients only, in other cases it was not applicable. Three cases had monosomy 7. Only one case had tisomy 21, other case (acute promyelocytic leukemia) had t (15, 17) by real-time PCR technique. Normal karyotyping was found in thirteen cases.

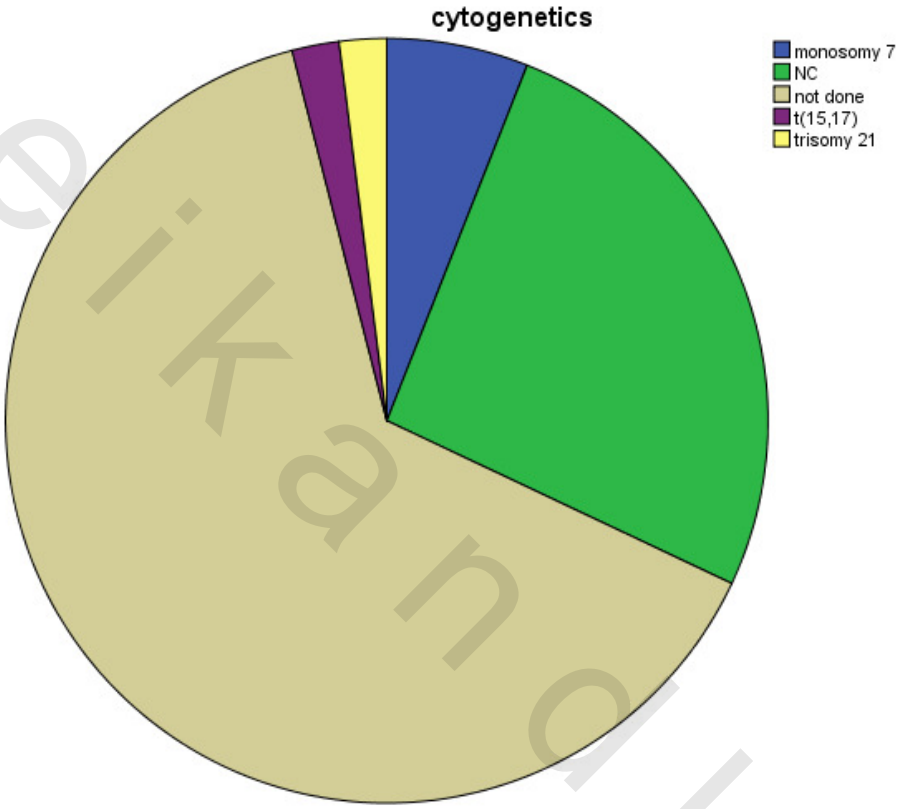


Fig. (10): Cytogenetic and molecular analysis of AML patients

Clinical outcome of AML patients illustrated in figure 11 and 12

The patients were treated according to the standard chemotherapy protocol for induction and they were followed up by bone marrow examination at day 28 to determine patient's response.

Nineteen cases achieved CR after induction therapy (38 %). Percentage of CR was higher in younger age group than in elderly (44.4 %, 21.4%) respectively. There was no statistically significant difference between elderly and adult AML patients.

Fifteen cases died during induction with higher percentage of deaths in the elderly group (35.7 vs 27.8) but also there was no statistically significant difference between elderly and adult AML patients.

Seven cases attained partial remission. Percentage of partial remission was higher in elderly but also no statistically significant difference between elderly and adult AML patients.

Nine cases achieved no response at all. Seven cases are younger than sixty and two cases are elderly but also no statistically significant difference between elderly and adult AML patients.

During follow up of cases nine cases who attained remission relapsed. seven cases are younger than sixty while two cases are elderly. Also no statistically significant difference between elderly and adult AML patients.

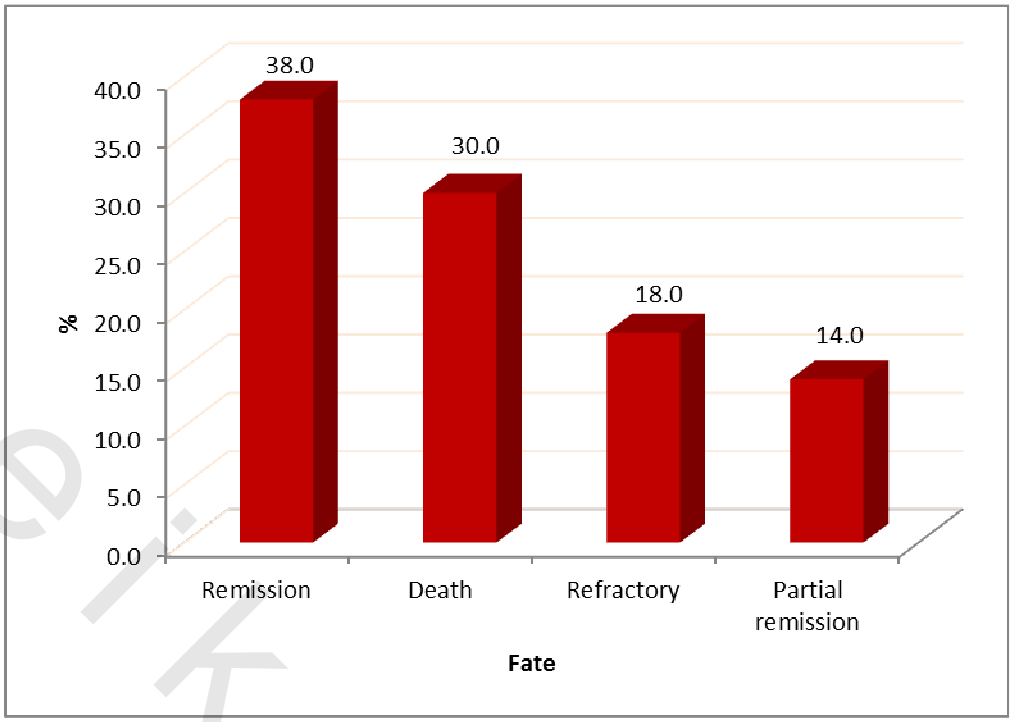
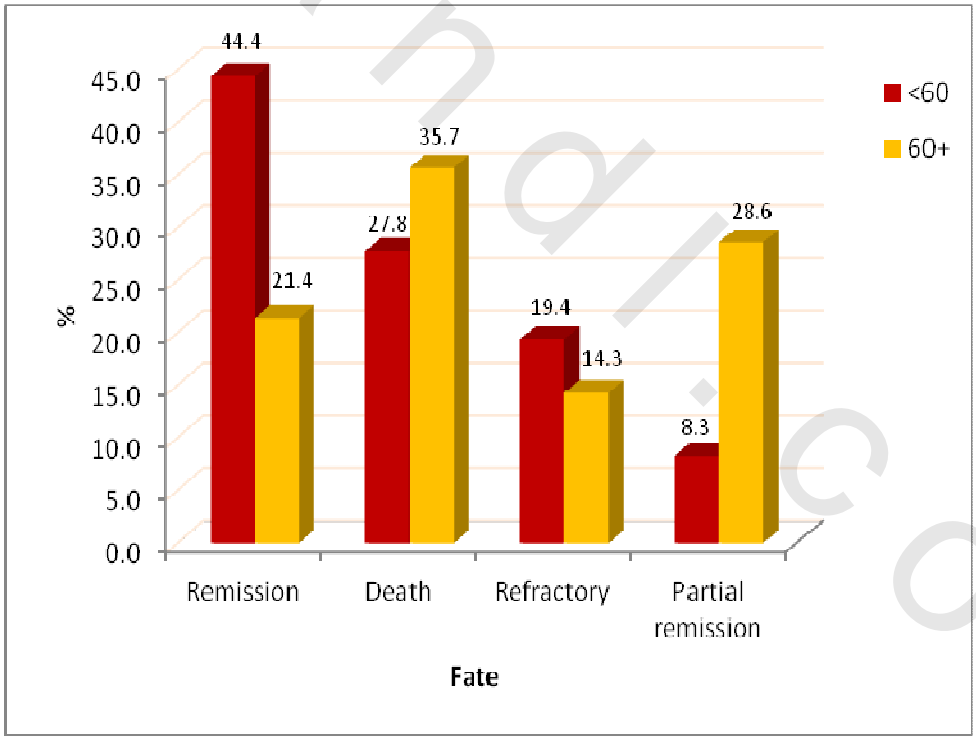


Fig. (11): Treatment outcome in all AML patients



Distribution of fate by age

Fig. (12): Distribution of treatment outcome by age in AML patients.

Relation between age and out-come illustrated in table XVII and XVIII:

Mean age in patients who achieved CR was 45 years while in patients who did not achieve CR it was 54 years. Mean age was statistically significant lower in patients who achieved remission (t= -2.592, p=0.013*).

Table XV: Mean age of AML patients in different out-comes.

		Age	
		Mean	**SD**
Remission	**No**	54	11
	Yes	45	13
Relapse	**No**	44	12
	Yes	47	15
Death	**No**	49	13
	Yes	55	11

Table XVI: Relation between age and different out-come (T-Test)

Independent Samples Test			
		t-test for Equality of Means	
		t	**P**
Age	**Remission**	-2.592	0.013*
Age	**Death**	-1.408	0.166
Age	**Relapse**	0.445	0.662

A- **Serum levels of hTERT in AML patients and control subjects illustrated in table XVII and figure 13**.

Serum hTERT concentrations were determined with a polyclonal sandwich ELISA kit. Level expressed in ng/ml using antibody to hTERT which was pre-coated onto plastic micro well strips. The intensity of the developed yellow color was directly proportional to the concentration of hTERT in the samples.

hTERT levels in the control ranged from 2.04 ng/ml to 11.11 ng/ml. Mean level was 5.06 ± 2.32 ng/ml. serum level in AML cases ranged from 4.9 ng/ml to 98 ng/ml. Mean level was 43.3 ± 25.4 ng/ml. There was statistically significant higher level of hTERT in patients than controls ($z=-6.107$, $p = 0.000$).

B- **Serum levels of hTERT in AML patients in both age groups illustrated in table XVIII and figure 14**.

Although the mean hTERT level in elderly was higher than that in adult cases (44 and 43.1 ng/ml respectively), there was no statistical significant difference between elderly and young adult.

C- **Serum levels of hTERT in AML patients in males and females illustrated in table XIX**.

There was no statistical significant difference in mean hTERT level between male and females ($p= 0.6$) it was higher in females than that in males (43.6 and 42.9 respectively).

Table XVII: Serum levels of hTERT in AML patients and control subjects

Items			Z	P
	case	control		
hTERTng/ml			-6.107	0.000
▪ Minimum	4.9	2.04		
▪ Maximum	98.0	11.11		
▪ Mean	43.3	5.05		
▪ SD	25.4	2.3		
▪ Median	55.3	4.48		

Z: Mann-Whitney test

Table XVIII: Serum levels of hTERT in AML patients in both age groups

Items	Age		Total	Z	P
	<60	60+			
hTERTng/ml				0.01	0.991
▪ Minimum	4.9	4.9	4.9		
▪ Maximum	98.0	80.5	98.0		
▪ Mean	43.1	44.0	43.3		
▪ SD	25.8	25.1	25.4		
▪ Median	55.3	54.1	55.3		

Z: Mann-Whitney test

Table XIX: Serum levels of hTERT in AML patients in males and females.

Items			Z	P
	male	female		
hTERT ng/ml			-0.525	0.6
▪ Mean	42.99	43.65		
▪ SD	26.89	24.39		

Z: Mann-Whitney test

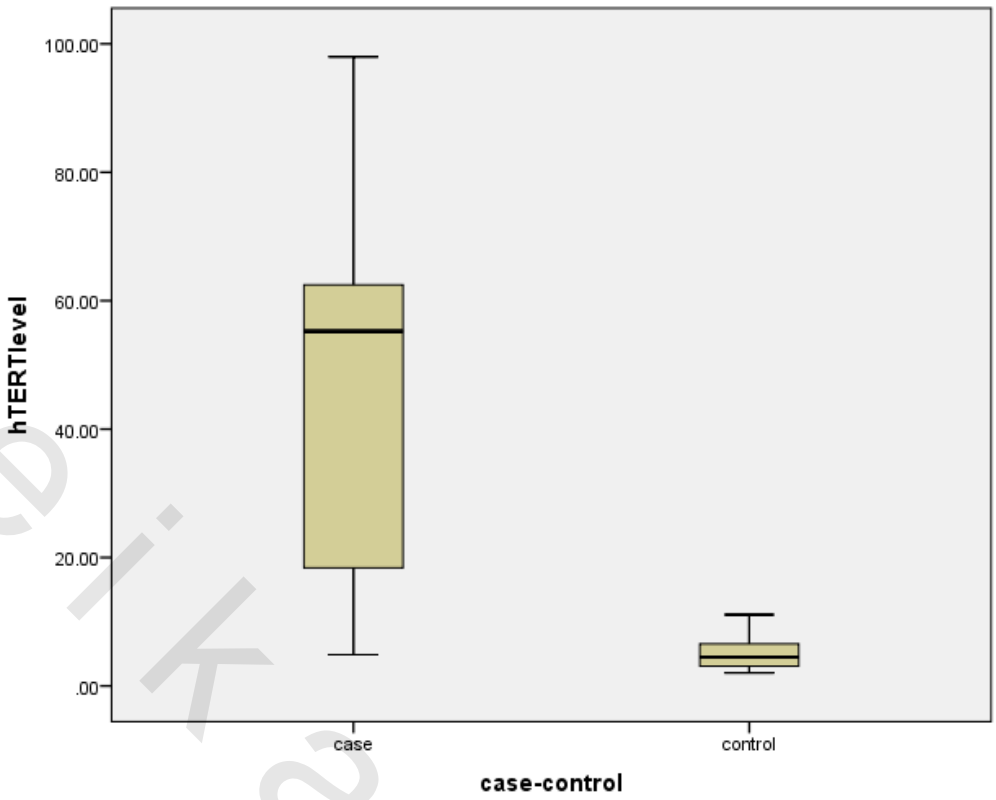
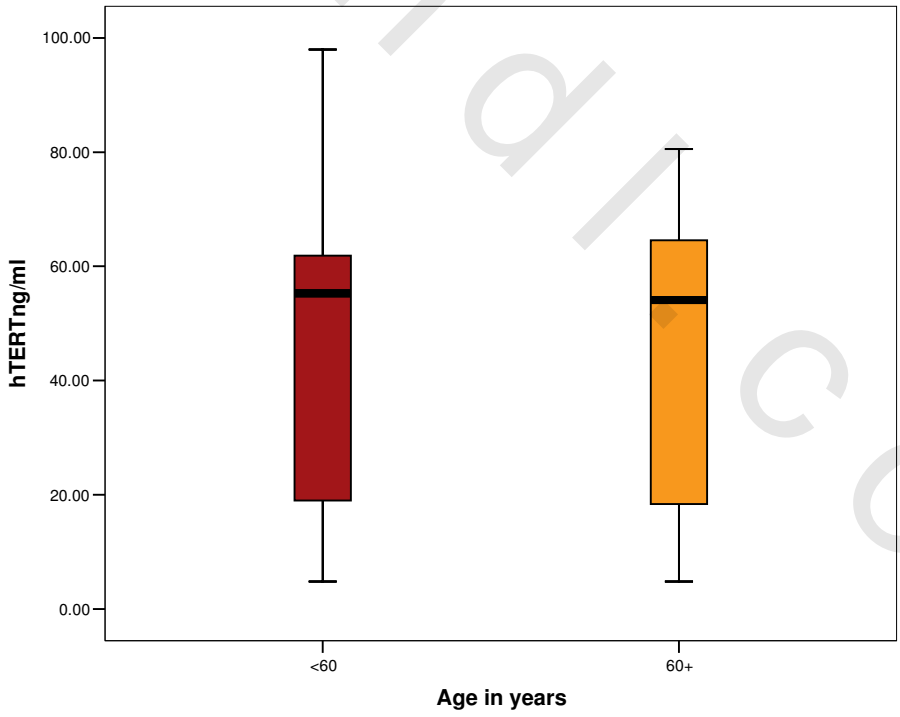


Fig. (13): Box plot for hTERT in both control group and AML patients



Box plot for hTERT

Fig. (14): Box plot for hTERT in both age groups in AML patients

1- Relative telomere length by real-time PCR in AML patients and control subjects illustrated in table XX and figure 15.

Relative telomere length (RTL) in AML patients ranged from 0.01 to 1.1. Mean level was 0.4 ± 0.3. Mean telomere length in normal subjects was 3.75. There was statistically significant lower RTL in patients than control (z=-6.384, p=0.000).

2- Relative telomere length in AML patients in both age groups illustrated in table XXI and figure 16.

Mean RTL in young adult was longer than that in elderly cases (0.5 and 0.4 respectively) but there was no statistical significant difference between elderly and young adult as regard mean RTL.

3- Relative telomere length in AML patients in males and females illustrated in table XXII.

There was no statistical significant difference as regard RTL level between male and females (p= 0.415) it was longer in males than that in females (0.5 and 0.4 respectively).

Table XX: Relative telomere length in AML patients and control subjects.

Items			Z	P
	patients	control		
Relative T/S ratio (RTL)			-6.384	0.000
▪ Minimum	0.0	0.85		
▪ Maximum	1.1	6.77		
▪ Mean	0.4	3.75		
▪ SD	0.3	1.7		
▪ Median	0.4	3.4		

Z: Mann-Whitney test

Table XXI: Relative telomere length in AML patients in both age groups

Items	Age		Total	Z	P
	<60	60+			
Relative T/S ratio (RTL)				0.77	0.443
▪ Minimum	0.0	0.0	0.0		
▪ Maximum	1.1	1.0	1.1		
▪ Mean	0.5	0.4	0.4		
▪ SD	0.4	0.3	0.3		
▪ Median	0.4	0.3	0.4		

Z: Mann-Whitney test

Table XXII: Relative telomere length in AML patients in males and females

Items			Z	P
	male	female		
Relative T/S ratio (RTL)			-.816	0.415
▪ Mean	0.5	0.4		
▪ SD	0.373	0.294		

Z: Mann-Whitney test

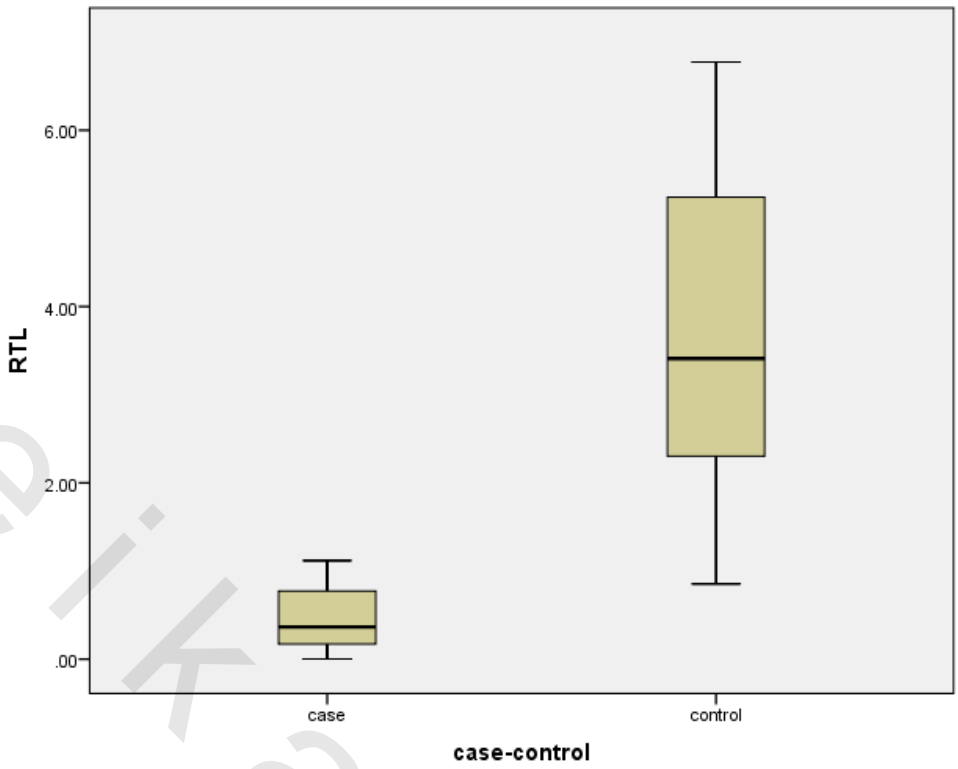
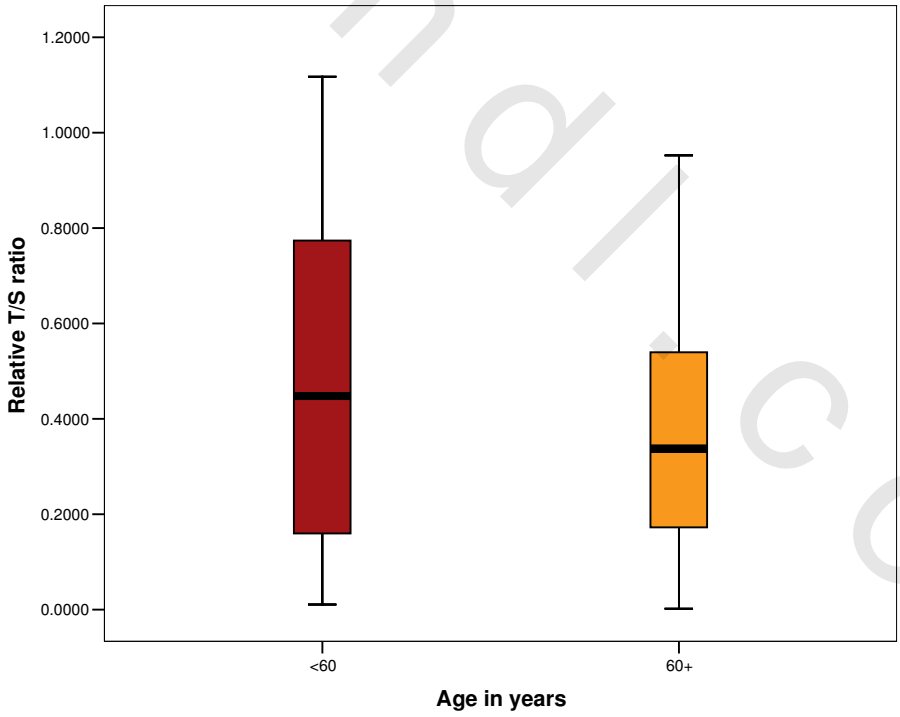


Fig. (15): Box Plot for RTL in control group and AML patients



Box Plot for RTL

Fig. (16): Box Plot for RTL in both age groups in AML patients

Correlation between hTERT and RTL illustrated in table XXIII and figure 17:

There was a negative correlation between serum level of hTERT and the relative telomere length (P=0.011). Level of correlation was intermediate as r_s level was 0.36 as Intermediate level is: (0.25-0.74).

Correlation between age and both hTERT and RTL illustrated in table XXIV and XXV:

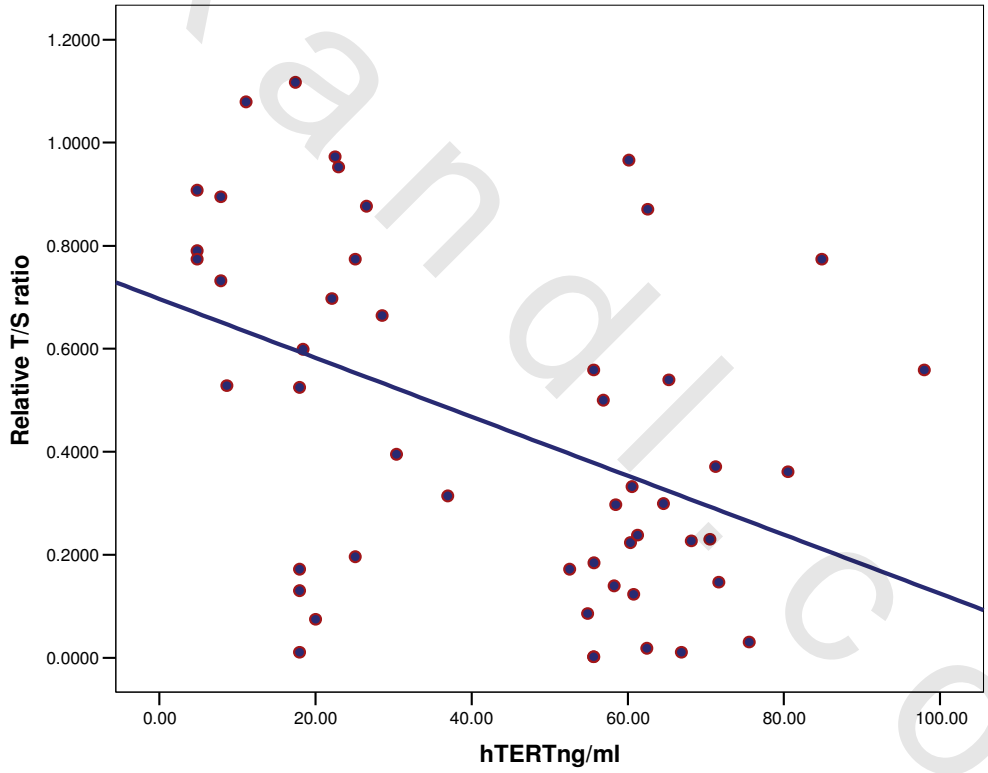
There was no statistically significant correlation between patient age and both hTERT and relative telomere length.

Relation between FAB subtypes and both hTERT and RTL illustrated in table XXVI and XXVII:

There was no statistically significant relation between FAB subtypes and both hTERT and relative telomere length.

Table XXIII: Correlation between hTERT and RTL in AML patients.

			hTERTng/ml	Relative telomere length
Spearman's rho	hTERTng/ml	Correlation Coefficient	1.000	-.357*
		Sig. (2-tailed)	.	.011
		N	50	50
	RTL	Correlation Coefficient	-.357*	1.000
		Sig. (2-tailed)	.011	.
		N	50	50
*. Correlation is significant at the 0.05 level (2-tailed).				



r_s=-0.36 (P=0.011)*

Interpretation of r_s

Weak (0.1-0.24)
Intermediate (0.25-0.74)
Strong (0.75-0.99

Fig. (17): Correlation between hTERT and RTL in AML patients

Table XXIV: Correlation between age and hTERT in AML patients

	hTERTng/ml	Age
Correlation Coefficient	1.000	-.042
Sig. (2-tailed)	.	0.771
N	50	50

Table XXV: Correlation between age and RTL in AML patients

	Age	RTL
Correlation Coefficient	1.000	-.138
Sig. (2-tailed)	.	0.339
N	50	50

Table XXVI: Relation between FAB subtypes and hTERT in AML patients

	hTERTng/ml	FAB classification
Correlation Coefficient	1.000	-.070
Sig. (2-tailed)	.	0.628
N	50	50

Table XXVII: Relation between FAB subtypes and RTL in AML patients.

	FAB classification	RTL
Correlation Coefficient	1.000	-.103
Sig. (2-tailed)	.	0.475
N	50	50

Table XXVIII: Mean serum level of hTERT (ng/ml) and RTL in AML patients of different FAB subtypes.

FAB classification	hTERT ng/ml	Relative Telomere Length
M1 (9 cases) Mean±SD	56.0292±21.7	0.350118±0.197
M2 (31 cases) Mean±SD	36.3629±25.6	0.538901±0.361
M3 (1 case) Mean±SD	61.2540	0.238160
M4 (6 cases) Mean±SD	52.1483±17.8	0.124784±0.141
M5 (1 case) Mean±SD	58.4500	0.297302
M6 (1 case) Mean±SD	84.8550	0.773782
M7 (1 case) Mean±SD	17.9700	0.524858

Comparison of hTERT level and RTL in AML patients with more and less differentiated FAB subtypes illustrated in table XXIX:

Within FAB subtypes, AML patients with monocytic subtypes (M4 and M5) had statistically significant shorter telomere length than AML patients with granulocytic subtypes (M1, M2). Relative telomere length was (0.149 and 0.49 respectively) (p = 0.011). But no statistical significant difference between both groups as regard hTERT level.

Table XXIX: Comparison of hTERT level and RTL in AML patients with monocytic and granulocytic FAB subtypes.

Item	Granulocytic subtypes	Monocytic subtypes	Z	P
hTERT ng/ml			-599	0.549
▪ Mean	41.29	53.05		
▪ SD	25.83	16.51		
Relative Telomere Length			-2.56	0.011*
▪ Mean	0.49	0.149		
▪ SD	0.337	0.144		

Z: Mann-Whitney test

Statistical analysis of hTERT level and relative telomere length in relation to induction mortality illustrated in table XXX:

The median level of hTERT in cases that died during induction was 55.7 ng/ml while in survived cases it was 54.9 ng/ml. Although cases with induction mortality show higher median level of hTERT than survived cases, there was no statistical significant difference as regard median level of hTERT (p = 0.169).

Median relative telomere length (RTL) was shorter in cases that died than in survived cases after induction therapy, (RTL was 0.2 and 0.5 respectively). There was a border line statistical significant difference as regard median level of RTL (p = 0.057).

Table XXX: Comparison of hTERT level and RTL in AML patients according to induction mortality.

	Induction Mortality		P
	No	**Yes**	
hTERTng/ml			0.169
▪ Minimum	4.9	18.0	
▪ Maximum	84.9	98.0	
▪ Median	54.9	55.7	
Relative Telomere Length			0.057
▪ Minimum	0.0	0.0	
▪ Maximum	1.1	1.0	
▪ Median	0.5	0.2	

P: Mann-Whitney test

Statistical analysis of hTERT level and relative telomere length in relation to remission state illustrated in table XXXI:

Mean hTERT level in patients who did not achieve haematological remission was statistically significant higher than that in patients who achieved haematological remission (48.87 and 34.32 respectively). (z= -1.98, p= 0.048).

Mean RTL in patients who achieved remission was longer than that in patients who did not achieve remission (0.56 and 0.37 respectively). There was no statistical significant difference between both groups.

Table XXXI: Comparison of hTERT level and RTL in AML patients according to remission state.

Items			Z	P
	Remission	No-remission		
hTERTng/ml				
▪ Mean	34.32	48.87	-1.98	0.048
▪ SD	23.6	25.16		
Relative Telomere Length				
▪ Mean	0.56	.37	-1.59	0.110
▪ SD	0.36	0.30		

Z: Mann-Whitney test

Statistical analysis of hTERT level and relative telomere length in censored and dead cases illustrated in table XXXII:

At the end of the study (after 30 ms) we found that mean hTERT level was statistically significant higher in patients who died during the study than mean level in censored cases (43.33 and 24.75 respectively). (z= -3.188, p= 0.001).

Mean RTL was statistically significant longer in censored cases than in dead cases (0.725 and 0.341 respectively). (z= -3.393, p= 0.001).

Table XXXII: Comparison of hTERT level and RTL in censored and dead cases.

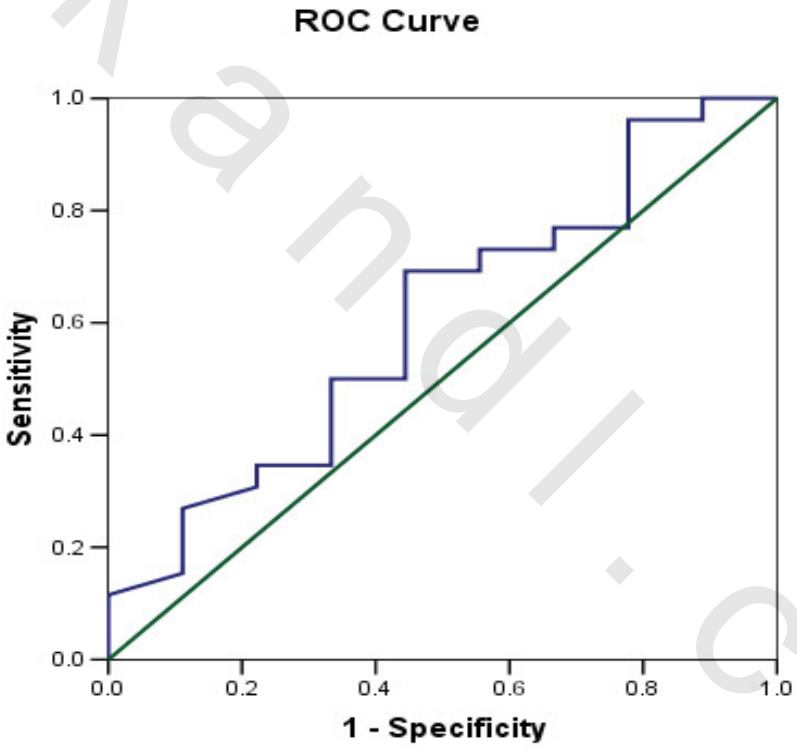
Items			Z	P
	censored	dead		
hTERT ng/ml			-3.188	0.001
▪ Mean	24.75	43.33		
▪ SD	19.79	23.73		
Relative Telomere Length			-3.393	0.001
▪ Mean	0.725	0.341		
▪ SD	0.282	0.291		

Z: Mann-Whitney test

Receiver operating characteristics (ROC) curve for hTERT illustrated in figure 18:

ROC curve showed that certain hTERT level (57.57 ng/ml) would be considered as a level that could predict response in AML patients. (Area under the curve (AUC) = 0.603, represents the overall accuracy of this value in predicting good response (complete remission). This means that, in AML patients with value less or equal to 57.57 ng/ml, 60.3% of patients will achieve complete remission.

Sensitivity of hTERT cut-off value (57.57 ng/ml) as a predictive value for response was **69.2 %** (sensitivity = the ability of the test to identify correctly those cases that truly have the criteria under the study; complete remission), and **specificity** was **56 %** (the ability of the test to identify correctly those cases that truly do not have the criteria under the study; complete remission).



Criterion values and coordinates of the ROC curve:

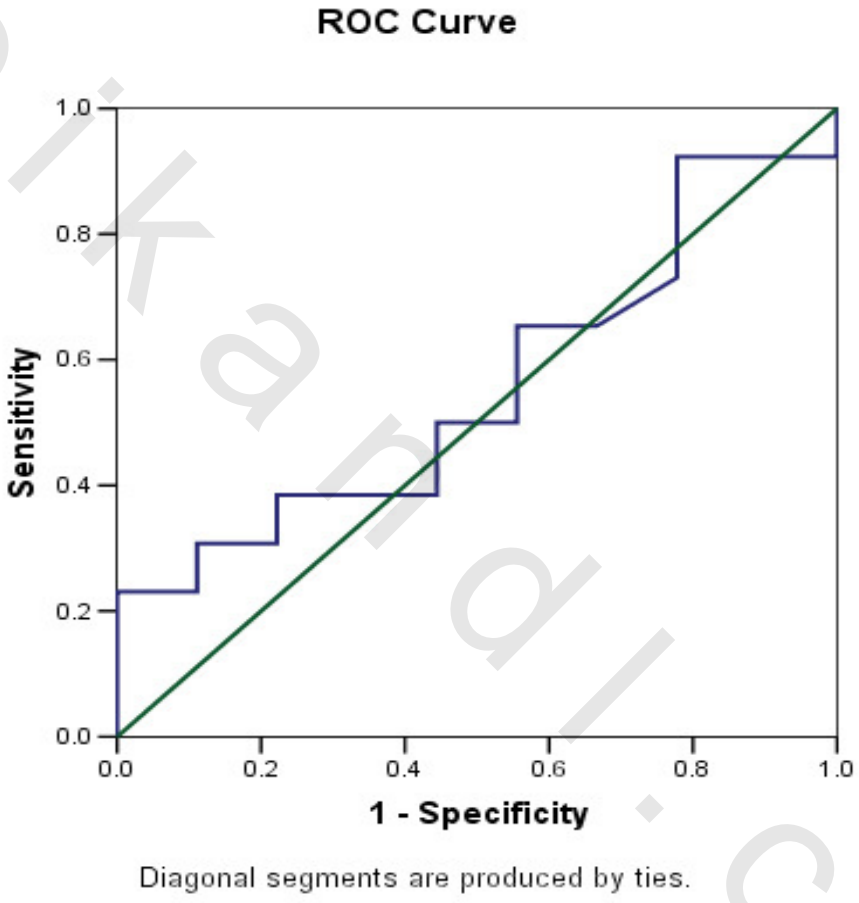
Area under the ROC curve (AUC) = 0.603 (overall accuracy = 60.3%)
Cut-off value: less than or equal to 57.57 ng/ml
Sensitivity = 69.2 %
Specificity =56 %

Fig. (18): ROC curve for hTERT showing the sensitivity, specificity and overall accuracy of 57.57 ng/ml cut-off value as a predictive value for AML outcome.

ROC curve for relative telomere length illustrated in figure 19:

Also ROC curve showed that certain RTL value (0.5) would be considered as a factor that could predict response in AML patients. (Area under the curve (AUC) = 0.556, represents the overall accuracy of this value in predicting good response (complete remission). This means that, in AML patients with value equal or more than 0.5, 55.6% of patients will achieve complete remission.

Sensitivity of RTL cut-off value (0.5) as a predictive value for response was **50** %, and **specificity** was **56 %**.



Criterion values and coordinates of the ROC curve:

Area under the ROC curve (AUC) = 0.556 (overall accuracy = 55.6%)
Cut-off value: less than or equal to 0.5
Sensitivity = 50 % Specificity =56 %

Fig. (19): ROC curve for RTL showing the sensitivity, specificity and overall accuracy of 0.5 cut-off value as a predictive value for AML outcome

Survival analysis

Kaplan-Meier estimate was used to measure the fraction of subjects living for a certain amount of time after induction therapy. Also it was calculated for two groups of subjects (young adult and elderly AML) also their statistical difference in the survivals was estimated.

For the construction of survival time probabilities and curves, the serial times for individual subjects are arranged from the shortest to the longest, without regard to when they entered the study.

By All subjects within the study begin the analysis at the same point and all are surviving until something happens to one of them. The two things that can happen are:

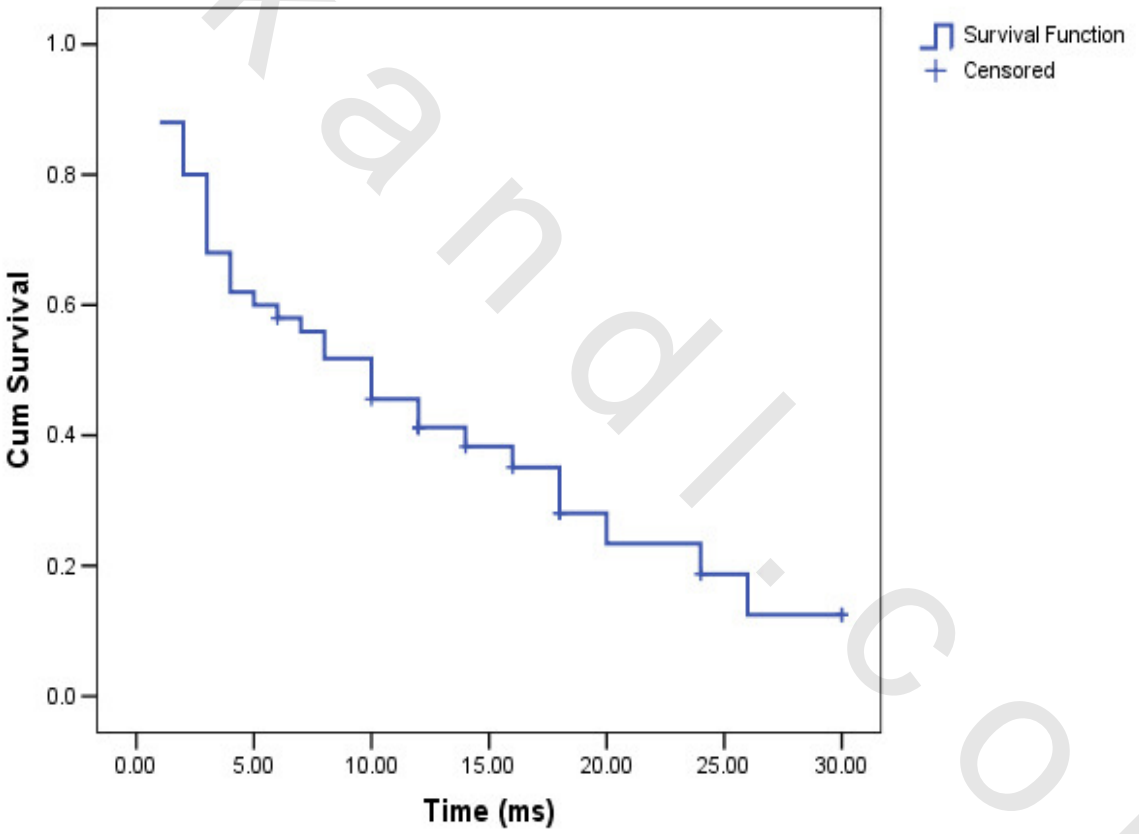
(1) A subject can have the event of interest (**death**).

(2) They are **censored** (means end of the study).

During duration of our study (30 months), number of events was 36 and censored cases were 14 (total number of our cases = 50). Median survival time for all AML patients in the study was 10 months. 95% Confidence Interval: 5.271-14.729 months (table XXXIII, Figure 20)

Table XXXIII: The mean and median survival for all AML patients

Mean				Median			
Estimate	Std. Error	95% Confidence Interval		Estimate	Std. Error	95% Confidence Interval	
		Lower Bound	Upper Bound			Lower Bound	Upper Bound
12.330	1.530	9.331	15.328	10.000	2.413	5.271	14.729



Case Processing Summary:

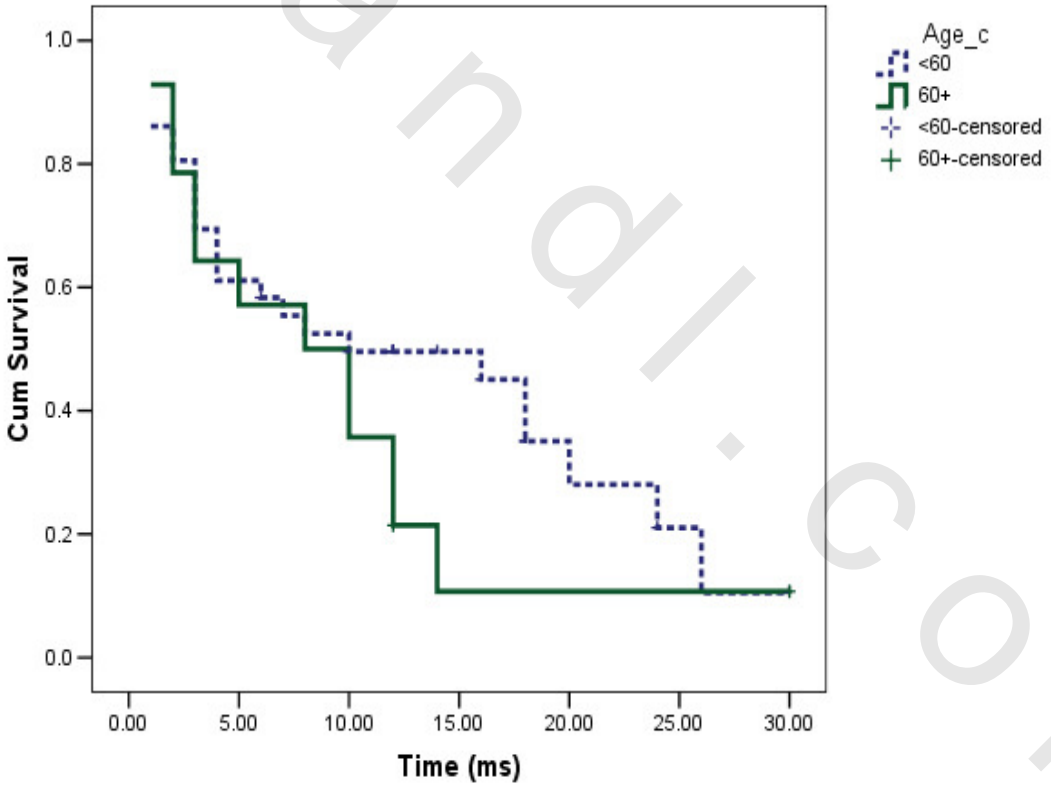
Total number: 50
Number of events: 36
Censored number: 14

Fig. (20): The median survival of all AML patients.

Median survival time in elderly patients was shorter than that in younger patients (8 ms and 10 ms respectively), but there was no statistical significant difference as regard mean survival time between both age groups (Log Rank (Mantel-Cox) test: Chi-Square = 1.272, p= 0.259). (Table XXXIV, figure 21)

Table XXXIV: Survival comparison between young adult and elderly AML patients

Age_c	Mean				Median			
	Estimate	Std. Error	95% Confidence Interval		Estimate	Std. Error	95% Confidence Interval	
			Lower Bound	Upper Bound			Lower Bound	Upper Bound
<60	13.312	1.861	9.663	16.960	10.000	4.536	1.109	18.891
60+	9.571	2.327	5.010	14.133	8.000	3.118	1.889	14.111
Overall	12.330	1.530	9.331	15.328	10.000	2.413	5.271	14.729



Case Processing Summary:

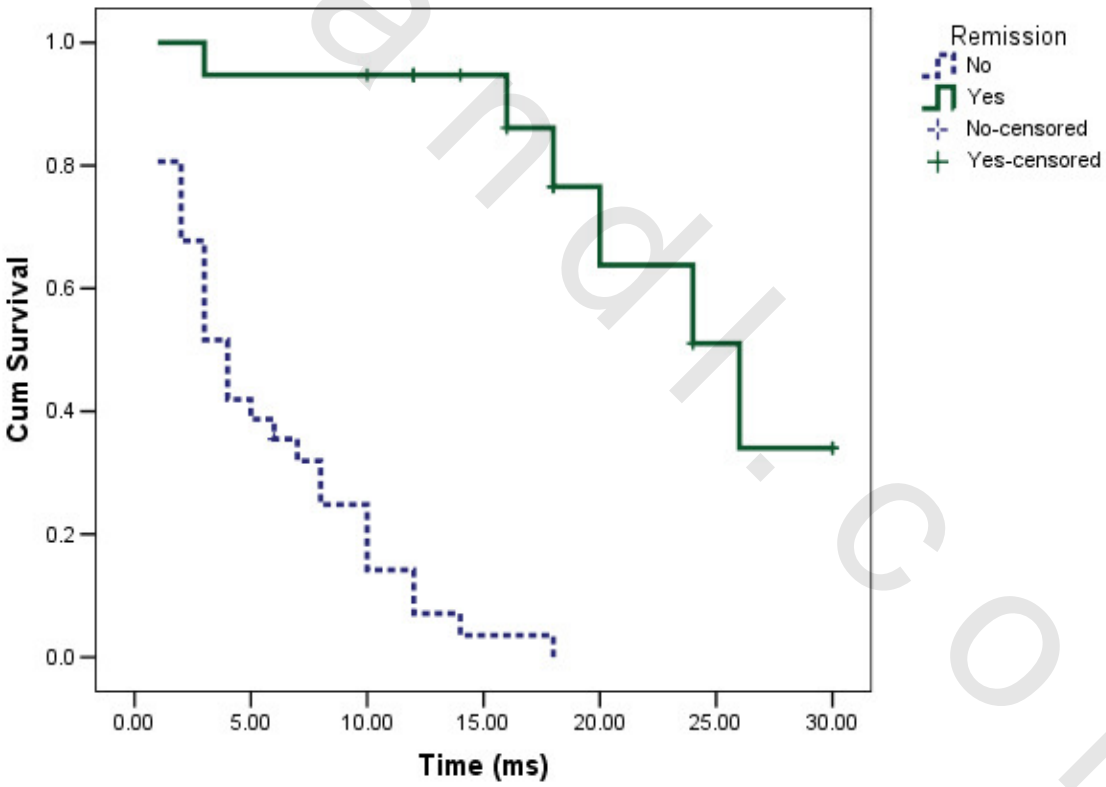
In adult AML patients: events number=24, censored number=12
In elderly AML patients: events number=12, censored number=2

Fig. (21): Kaplan-Meier analysis of overall survival according to age groups.

Median survival time in patients who achieved remission was 26 months while in those who did not achieve remission was 4 months. There was a highly statistical significant difference in median survival time in both groups (Log Rank (Mantel-Cox) test: Chi-Square = 38.952, p= 0.000). (Table XXXV, figure 22)

Table XXXV: Survival comparison in AML patients according to remission state.

Remission	Mean				Median			
	Estimate	Std. Error	95% Confidence Interval		Estimate	Std. Error	95% Confidence Interval	
			Lower Bound	Upper Bound			Lower Bound	Upper Bound
No	5.545	.835	3.910	7.181	4.000	.687	2.654	5.346
Yes	23.503	1.955	19.671	27.335	26.000	3.602	18.940	33.060
Overall	12.330	1.530	9.331	15.328	10.000	2.413	5.271	14.729



Case Processing Summary:

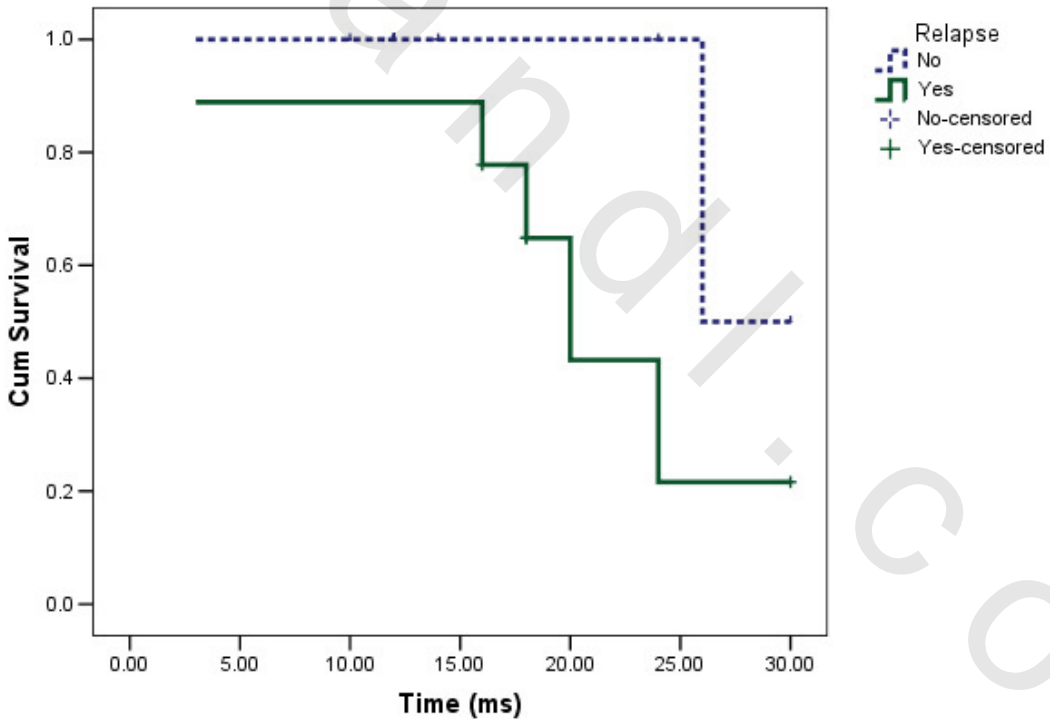
AML patients in remission: events number = 6, censored number = 13
AML patients not in remission: events number = 30, censored number = 1

Fig. (22): Survival comparison in AML patients according to remission state.

Median survival time in relapsed patients was shorter than that in non relapsed patients (20 ms, 26 ms respectively). There was a statistical significant difference in median survival time in both groups (Log Rank (Mantel-Cox) test: Chi-Square = 2.609, p= 0.050). (Table XXXVI, figure 23)

Table XXXVI: Survival comparison between relapsed and non relapsed AML patients.

Relapse	Mean				Median			
	Estimate	Std. Error	95% Confidence Interval		Estimate	Std. Error	95% Confidence Interval	
			Lower Bound	Upper Bound			Lower Bound	Upper Bound
No	28.000	1.414	25.228	30.772	26.000	.	.	.
Yes	20.432	2.797	14.950	25.915	20.000	1.926	16.226	23.774
Overall	23.503	1.955	19.671	27.335	26.000	3.602	18.940	33.060



Case Processing Summary:

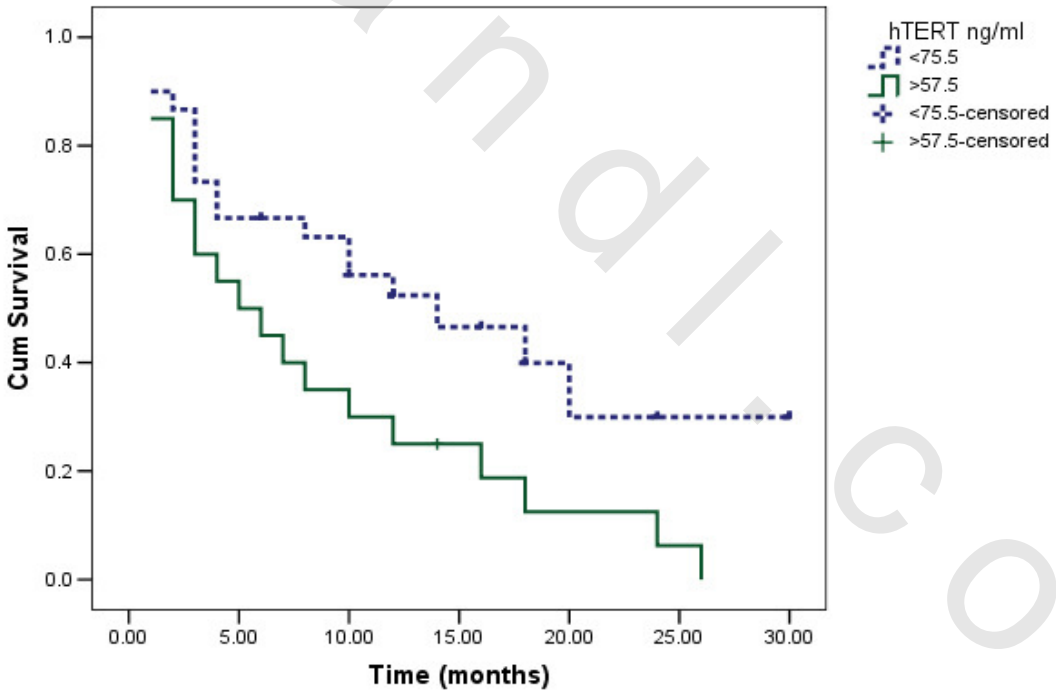
Relapsed AML patients: events number = 5, censored number = 4
Non-Relapsed AML patients: events number = 1, censored number = 9

Fig. (23): Survival comparison between relapsed and non relapsed AML patients.

Median survival time in patients with hTERT level higher than cut-off level was shorter than median survival time in patients with lower value, (5 ms, and 14 ms respectively). There was a statistical significant difference in median survival time in both groups (Log Rank (Mantel-Cox) test: Chi-Square = 5.653, p= 0.017). (Table XXXX, figure 24)

Table XXXVII: Survival comparison according to hTERT level (cut-off level) in AML patients

hTERT_c	Mean				Median			
	Estimate	Std. Error	95% Confidence Interval		Estimate	Std. Error	95% Confidence Interval	
			Lower Bound	Upper Bound			Lower Bound	Upper Bound
<57.5 ng/ml	15.256	2.267	10.812	19.700	14.000	4.884	4.428	23.572
>57.5 ng/ml	8.600	1.851	4.971	12.229	5.000	2.236	.617	9.383
Overall	12.330	1.530	9.331	15.328	10.000	2.413	5.271	14.729



Case Processing Summary

AML patients with higher level than cut-off: events number = 19, censored number = 1

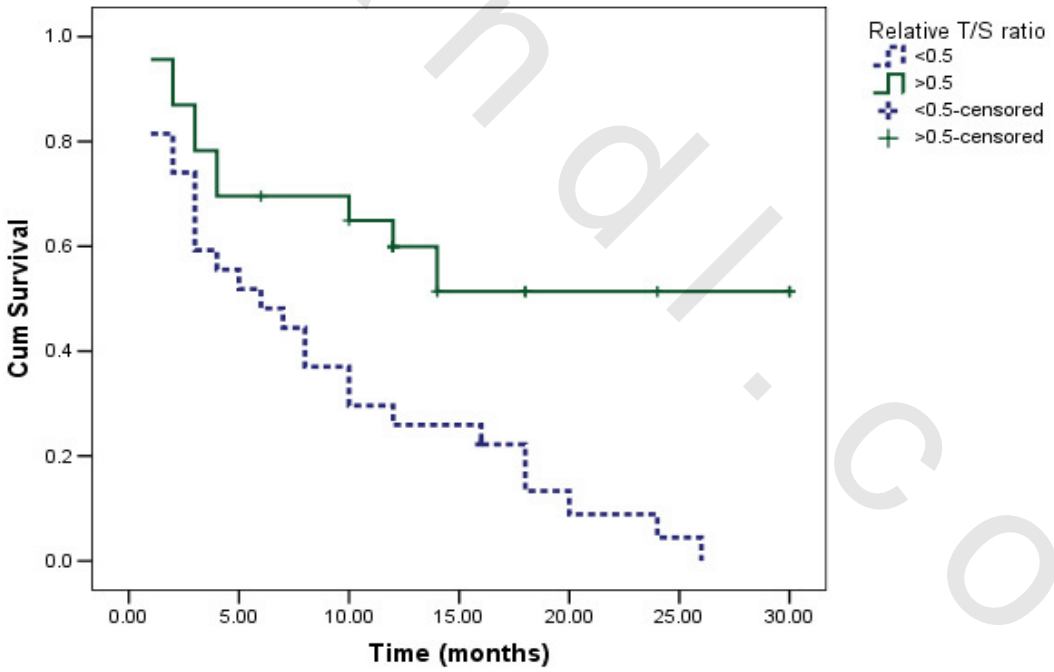
AML patients with lower level than cut-off: events number = 17, censored number = 13

Fig. (24): Survival comparison according to hTERT level (cut-off level) in AML patients.

Median survival time in patients with relative telomere length lower than cut-off value was shorter than median survival time in patients with higher value, (6 ms, and 14 ms respectively). There was a statistical significant difference in median survival time in both groups (p= 0.002). There was a statistical significant difference in median survival time in both groups (Log Rank (Mantel-Cox) test: Chi-Square = 9.305, p= 0.002). (Table XXXXI, figure 25)

Table XXXVIII: Survival comparison according to RTL (cut-off level) in AML patients

T_S.ratio_c	Mean				Median			
	Estimate	Std. Error	95% Confidence Interval		Estimate	Std. Error	95% Confidence Interval	
			Lower Bound	Upper Bound			Lower Bound	Upper Bound
<0.5	8.674	1.532	5.672	11.676	6.000	2.596	.911	11.089
>0.5	18.499	2.735	13.140	23.859	14	.	.	.
Overall	12.330	1.530	9.331	15.328	10.000	2.413	5.271	14.729



Case Processing Summary

AML patients with higher RTL than cut-off: events number = 10, censored number = 13

AML patients with lower RTL than cut-off: events number = 26, censored number = 1

Fig. (25): Survival comparison according to RTL (cut-off level) in AML patients.