RESULTS

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In the present study, the increase in telomerase activity was estimated in the peripheral blood mononuclear cells (PBMCs) of 25 de novo AML cases and 15 normal age matched subjects as a control group using PCR-ELISA technique (TRAP) assay.

Control group:

The studied control group included 9 males and 6 females of normal age matched subjects, their age ranged from 20-60 years with a mean value of 37.94±12.62 years. The individual and laboratory data of control group is shown in (table 11).

Results of the control group showed that haemoglobin concentration ranged from 9-13 g /dL with a mean value of 11.24±1.32 g /dL. TLC ranged from 5.3-9.2 x $10^3/mm^3$ with a mean value of 7.28±1.3 and platelet count ranged from (150-250) x $10^3/mm^3$ with a mean value of 201.18±30.83.

Descriptive individual and clinical data of AML cases:

The studied AML cases included 13 (52%) males and 12 (48%) females of de novo AML cases. Their age ranged from 15-73 years with a mean of 35.16±15.816, (table 3, fig. 3).

The clinical data of AML cases is shown in (table 1, figure 1). Hepatomegaly was detected in 16 cases (64%), splenomegaly was detected in 18 cases (72%) while lymphadenopathy was detected in 12 cases (48%).

Descriptive laboratory data of AML cases:

Laboratory data of AML cases is shown in (table 12) as:

- Haemoglobin concentration ranged from 4.1-10 g /dL with mean value of 6.98±1.57g /dL.
- TLC ranged from 2.1-268.5 x $10^3/mm^3$ with a mean value of 52.39±66.30, and SEM = 13.26.
- Platelet count ranged from 16-584 x $10^3/mm^3$ with a mean value of 86.36±122.31, SEM= 24.462.
- Blasts in PB ranged from 9-96% with a mean value of 54.9±27.2.
- Blast cells in bone marrow ranged from 30-100% with a mean value of 65.3±28.4.
- According to FAB classification, 8 cases (32%) were M1, 9 cases (36%) were M2, 3 cases (12%) were M3, 3 cases (12%) were M4 and 2 cases (8%) were M5 (table 2,fig. 2).
- As regards immunophenotyping, all cases expressed CD13 and CD33, 5 cases expressed CD7, 5 cases expressed CD14, one case CD9, one case CD22 and one case CD19.

Comparison between laboratory data of AML cases and control group is shown in (table 4, fig. 4). Where as, predicted, there was statistically high significant difference between AML cases and control group in respect to:

- Haemoglobin concentration in AML cases, it ranged from 4.1-10.0 g/dL with a mean value and SD was 6.98±1.570 while in control group it ranged from 9-13g/dL with mean and SD of 11.24±1.318 and P value of 0.0001 and t-test 8.84.
- TLC ranged from 2.1-268.5 x $10^3/mm^3$ with a mean value and SD of 52.392±66.3 in AML cases while it ranges from 5.3-9.2 x $10^3/mm^3$ with mean and SD of 7.28±1.301 in control group. P value was 0.02 and t-test=2.7.
- Platelet ranged from 16.0-584.0 x $10^3/mm^3$ and a mean, SD was 86.36±122.31 in AML cases while it ranged from (150-250) x $10^3/mm^3$ and mean, SD was 201.33±30.828 in control group, P value was 0.01 and t = 3.56.
- Blast cells in peripheral blood ranged from 9-96% and mean, SD was 54.9±27.2 in AML cases while it was zero in control group.

  It was difficult to get bone marrow from normal persons so we could not do bone marrow examination for the control group.

Telomerase activity in the studied groups:

1. **Control group:**

The mean value of RTA was 1.010±0.606 and ranged from 0.2-2.20 and from this result we calculated the cut off value:

Cut off value = mean + 2 x SD = 2.2 RTA

As shown in (table 5).

2. AML cases:

RTA ranged from 1.6-456.0 with a mean value of 67.92±122.36, (table 5). Comparison between RTA in AML cases and control is shown in (table 5 and figure5). We predicted that statistically, there is high significant difference in RTA between AML cases and control group P value was 0.00001 and t-test = 12.9. Four cases (16%) of AML exhibited RTA within normal i.e., up to cut off point (2.2), while 14 cases (56%) exhibited low and intermediate RTA (less than 10 folds elevation of cut-off value) i.e., less than 10 x 2.2 = 22; and 7 cases (28%) showed high RTA (equal or more than 10 folds elevation of cut-off value) i.e., equal or more than 22, (table 6).

Correlation between RTA and TLC:

There was statistically significant correlation between RTA and TLC in AML cases where r-value = 0.619 and P-value = 0.001 (table 7).

Correlation between RTA and blasts in bone marrow:

There was statistically significant correlation between RTA and blast cells percentage in bone marrow of AML cases where P value = 0.012 and r-value = 0.494, (table 7).

Correlation between RTA and CD34+ blast cells:

There was no statistically significant correlation between RTA and CD34+ blast cells in AML cases where r-value = 0.316 and P-value = 0.458 (table 7).

Relation between RTA and FAB classification:

Table 8 and figure 6 showed that there was statistically significant difference between the level of RTA in FAB subtypes of AML cases i.e., M1 cases were 8 in number, their RTA ranged from 10.40-202.7 with mean value of 67.7±79.91. As regards M2 cases, they were 9 in number with RTA ranged from 2.10-391.0 with mean value of 59.07±128.38. But M3 cases were 3 with RTA ranged 1.60-3.20 with mean value of 2.30±0.818. For M4 cases, they were 3 in number. Their RTA ranged 2.10-456.0 with mean value of 202.23±231.654. While M5 cases were 2, their RTA ranged from 3.5-7.7 with mean value of 5.60±2.969. This data showed that there is statistically high significant difference between the level of RTA and different AML FAB subtypes as
P-value = 0.0001 and F-test = 128.36. All M3 cases expressed low or even normal values of RTA.

There was no statistically significant difference between the level of RTA whether high or low and intermediate and FAB subtypes of AML cases where 3 cases (12%) of 8 M1 cases expressed high RTA, while 5 cases (20%) showed low and intermediate levels. M2 cases were 9, 2 of them (8%) exhibits high RTA while 5 (24%) exhibit low and intermediate RTA. M3 cases were 3, no one case showed high RTA and only one case showed low and intermediate RTA. M4 cases were 3, 2 of them showed high RTA and no low and intermediate values were recorded. M5 cases were 2 and both of them exhibited low to intermediate RTA. This is shown in (table 9 and figure 7) where Chi square test $X^2 = 5.81$ and P-value = 0.21.

Comparison between AML cases with high RTA and AML cases with low and intermediate RTA:

As shown in (table 10), there was no statistically significant difference between cases with high RTA and those with low and intermediate RTA in respect to:

- Age where P-value = 0.24.
- Sex where P-value = 0.87.
- Organomegaly where P-value = 0.79.
- Platelets where P-value = 0.068 with mean value and SD of high RTA cases were 147.29±212.94 while mean and SD of low and intermediate RTA cases were 53.86±48.39.
- CD34+ blast cells where P-value = 0.208 with mean value and SD of high RTA cases were 52.50±25.43 while mean and SD of low and intermediate cases were 42.97±22.68.

Table 10 also showed that there was statistically significant difference in

- haemoglobin concentration (Hb) between high and low and intermediate RTA cases where P-value = 0.05 with mean value and SD of high RTA cases were 7.814±1.65 and mean value and SD of low and intermediate RTA cases were 6.71±1.77.
- TLC where P-value = 0.0001 with mean value and SD of high RTA cases were 130.36±77.92 and mean and SD of low and intermediate RTA cases were 19.79±23.43.
- Blasts in P.B. where P-value = 0.0035 with mean and SD of high RTA cases were 79.14±13.36 and mean and SD of low and intermediate RTA cases were 48.57±24.82.

- Blasts in BM where P-value = 0.0002 with mean and SD of high RTA cases were 95.57±5.22 and mean and SD of low and intermediate RTA cases were 50.57±22.54.

Statistical analysis:

Data was analyzed using statistical package for science "SPSS version 9" software *(Norusis, 1999)*. Data were presented as number and percentage for qualitative variables but were presented as mean and standard deviation for quantitative continuous variables. The significance of difference between mean values of paired observation was performed using paired t-test.

Chi square (X^2) is used for comparison between distributions of patients according to different items.

$$X^2 = \sum \frac{(O-E)^2}{E}$$

O = Observed results

E = Expected results

$(O - E)^2$ = *differance* squared

Where $E = \frac{\text{Total row x total column}}{\text{Grand total}}$

Table (1): Clinical data of AML cases

Clinical	No = 25	%
1. Liver		
Normal	9	36.0
Hepatomegaly	16	64.0
2. Spleen		
Normal	7	28
Splenomegaly	18	72
3. Lymphonode		
Normal	13	52
Enlarged	12	48

Table (2): FAB characteristics of AML cases.

FAB	No. 25	%
M1	8	32
M2	9	36
M3	3	12
M4	3	12
M5	2	8

This table shows that according to FAB classification 8 cases (32%) were M1, 9 cases (36%) were M2, 3 cases (12%) were M3, 3 cases (12%) were M4 and 2 cases (8%) were M5.

Table (3): Statistical comparison of age and sex between AML cases and control group.

	Patients n = 25		Control n = 15		Statistics
	No	%	No	%	
Sex					
Female	12	48	6	40	$X^2 = 0.62$
Male	13	52	9	60	P = 0.41 N.S.
Age					
Range	15-73		20-60		t = 0.58
Mean±SD	35.16±15.816		37.94±12.62		p= 0.38 N.S.

S. : Significant (P<0.05)

NS. : Not significant (P> 0.05)

This table shows that statistically there was no significant difference in either age or sex between AML cases and control group.

Table (4): Statistical comparison of laboratory data between AML cases and control group

Items	AML cases (n = 25)	Control (n = 15)	Statistics
Hb (g/dl)			
Range	4.1-10.0	9.0-13.0	t=8.84
Mean±SD	6.92±1.590	11.24±1.318	p=0.0001*
TLC ($X10^3mm^3$)			
Range	2.1-268.5	5.30-9.20	t=2.7
Mean±SD	53.39±66.3	7.28±1.301	p=0.02*
Platelets ($X10^3mm^3$)			
Range	16.0-584.0	150-250	t=3.56
Mean±SD	86.36±122.31	201.33±30.828	p=0.01*
Blasts In P.B (%)			
Range	9.0-96.0	0.00	
Mean±SD	54.48±25.31	0.00	-

This table shows that statistically there was significant difference between the laboratory data (Hb, TLC, platelets and blasts in P.B) in AML cases and control group.

Table (5): Statistical comparison of RTA between AML cases and control group.

RTA	AML cases (n=25)	Control group (n=15)	Statistics
Range	1.6-456.0	0.2-2.20	t=12.9
Mean± SD	67.92±122.36	1.01±0.606	p=0.00001* HS

Cutoff value for RTA = 2.2 (mean±2SD of the control group)

HS. = Highly significant

This table shows that statistically there was highly significant difference in RTA between AML cases and control group.

Table (7): Statistical correlation between RTA and TLC, blasts in B.M. and CD34 +ve blast cells in AML cases

Items	Correlation coefficient r-value	P-value	Significance
TLC	0.619	0.001	Significant
Blasts in B.M	0.494	0.012	Significant
CD34 +ve blast cells	0.136	0.458	N.S.

This table shows that statistically there was significant correlation between RTA and TLC and blasts in BM, while there was no significant correlation between RTA and CD 34+ blast cells in AML cases.

Table (8): Statistical comparison of RTA in FAB subtypes in AML cases.

	n	Range	Mean ± S.D.
M1	8	10.40-202.7	67.7±79.908
M2	9	2.10-391.0	59.06±128.35
M3	3	1.60-3.20	2.30±0.818
M4	3	2.10-456.0	202.23±231.654
M5	2	3.5-7.7	5.60±2.969
F		128.36	
p		0.0001*	

This table shows that statistically there was significant difference between RTA in FAB subtypes of AML cases.

Table (9): Statistical relation between the level of RTA & FAB subtypes in AML cases.

	Low and intermediate RTA		High RTA	
	No	**%**	**No**	**%**
M1	5	20	3	12
M2	6	24	2	8
M3	1	4	0	0
M4	0	0	2	8
M5	2	8	0	0
Total	14	56	7	28
X2	5.81			
P	0.21			

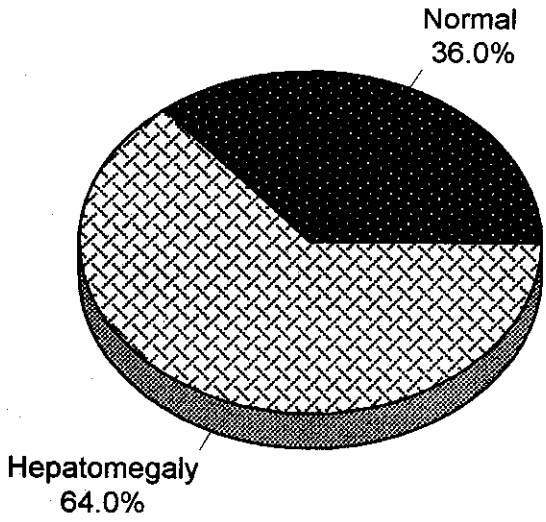
This table shows that statistically there was no significant difference between the level of RTA (high or low and intermediate) in FAB subtypes of AML cases.

Table (10): Comparison between high RTA and low and intermediate RTA in respect to some variables.

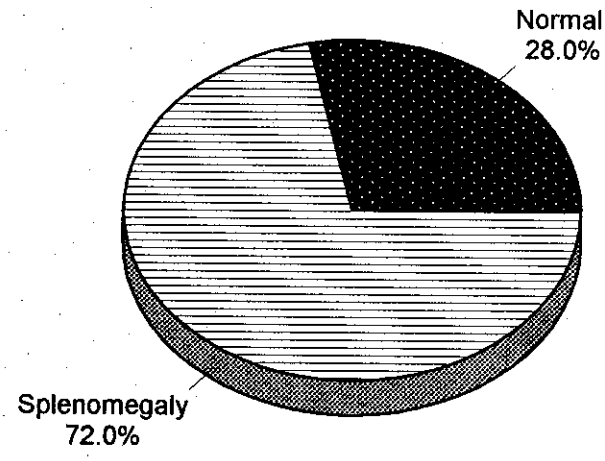
	Low& intermediate "n= 14"	High "n= 7"	P	Sig.
Age	37.07±17.91	31.57±13.78	0.24	N.S.
Sex				
Male	8 (57.1%)	4 (57.1%)	0.87	N.S.
Female	6 (42.9%)	3 (42.9%)		
Organomegally				
Hepatomegally	7	6	0.79	N.S.
Splenomegaly	9	5		
Lymphadenopathy	6	3		
Hb	6.71±1.77	7.814±1.65	0.05	Sig.
Platelet	53.86±48.39	147.29±212.94	0.068	N.S.
TLC	19.79±23.43	130.36±77.92	0.0001	Sig.
Blasts in P.B	48.57±24.82	79.14±13.36	0.0035	Sig.
Blasts in BM	50.57±22.54	95.57±5.22	0.0002	Sig.
CD34 + blast cells	42.97±22.68	52.50±25.43	0.208	N.S.

This table shows that statistically significant, there was significant difference between the cases with high RTA and those with low and intermediate RTA as regards (Hb, TLC, Blasts in both P.B and BM.), while no significant difference was recorded as regards age, sex, organomegaly, platelets and CD34 + blast cells.

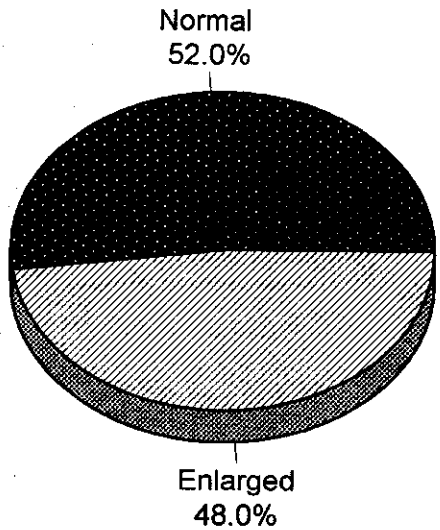
Figure (1): Clinical data of AML cases



Liver



Spleen



Lymph node

Figure (2): FAB characteristics of AML cases

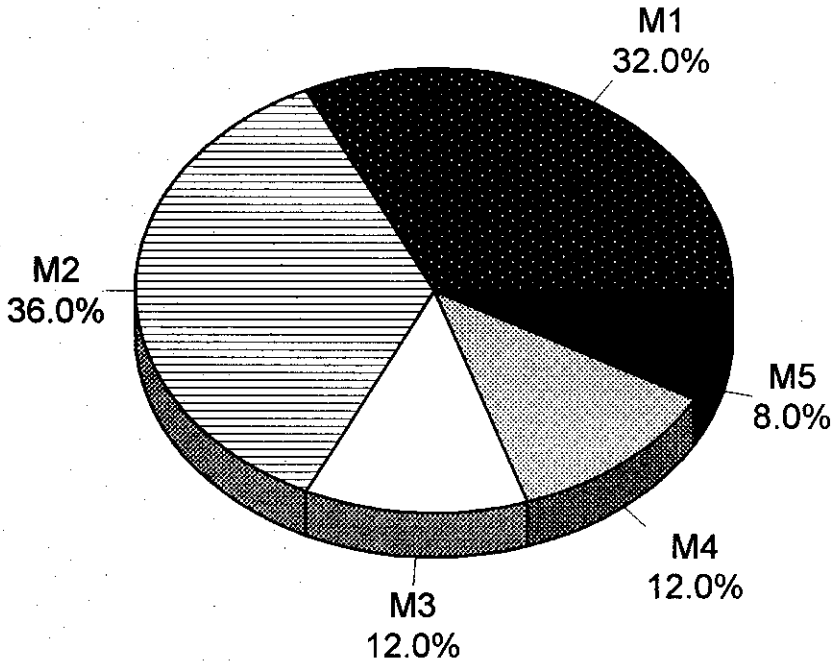
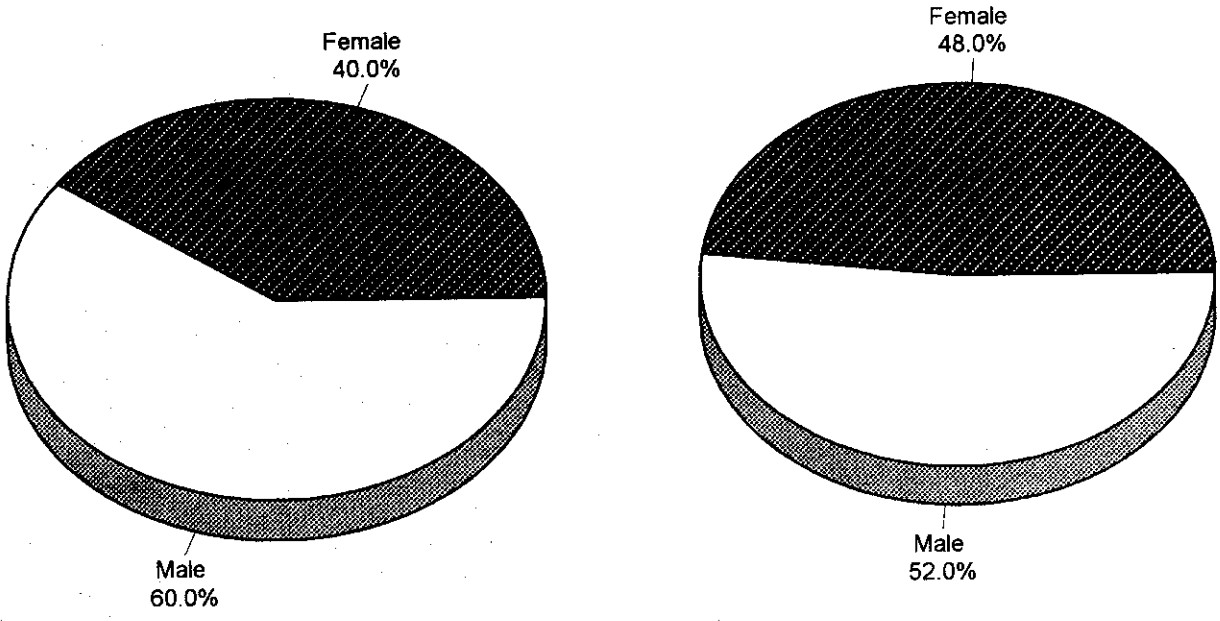


Figure (3): Statistical comparison of age and sex between AML cases and control group.



Control n = 15 **Patients n = 25**

Sex

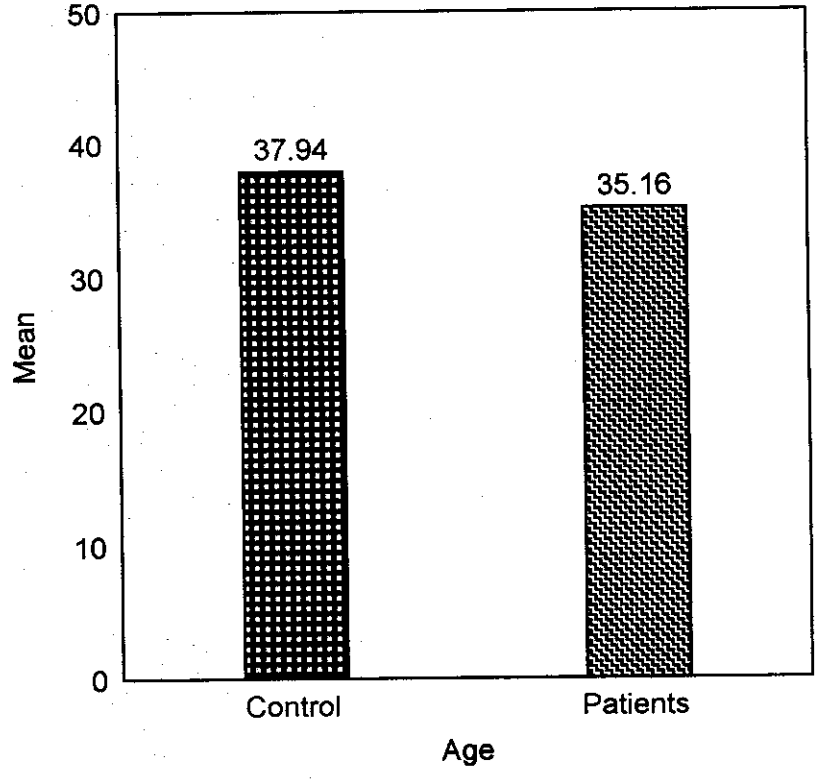
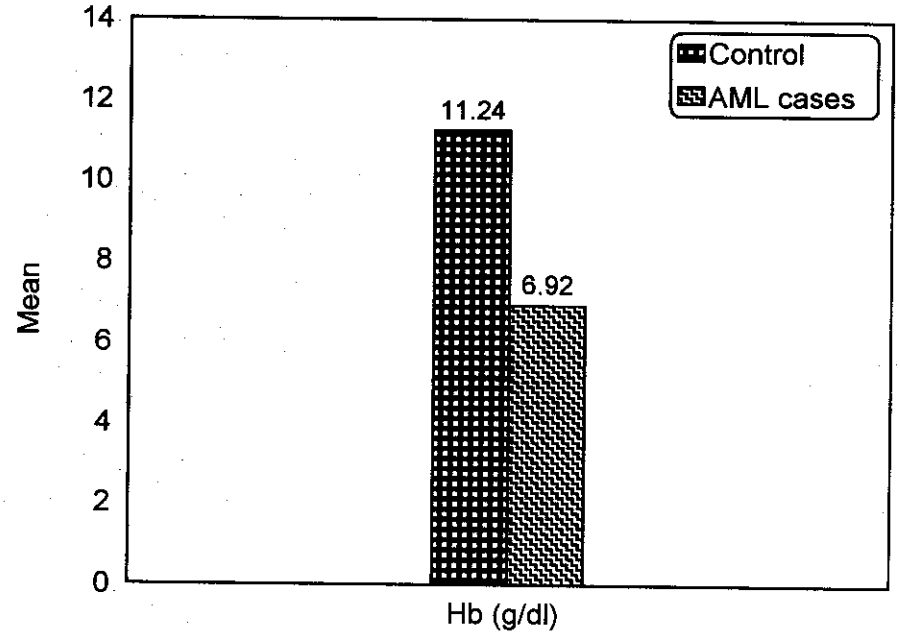


Figure (4): Statistical comparison of laboratory data between AML cases and control group



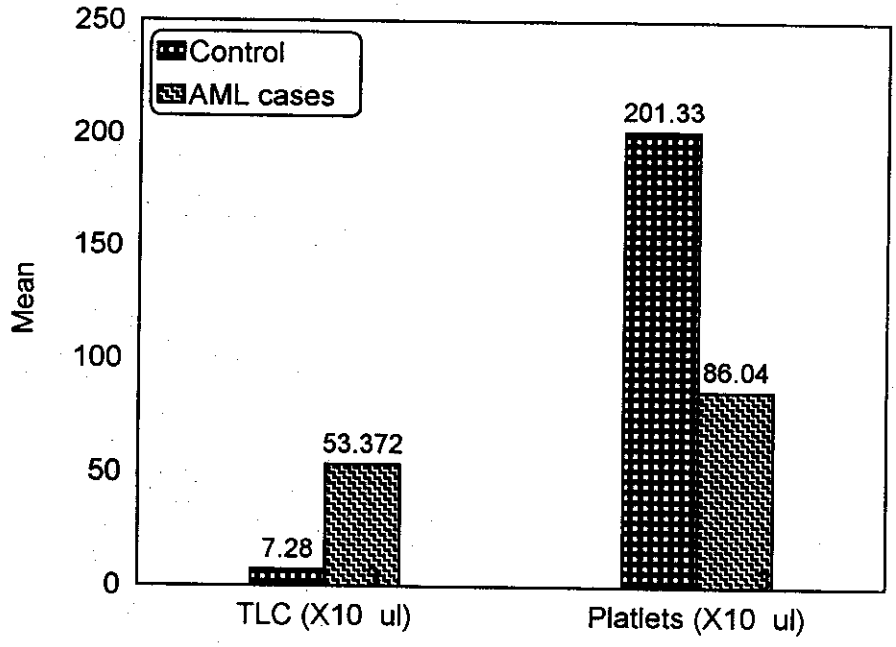


Figure (5): Statistical analysis of RTA in AML cases and control group.

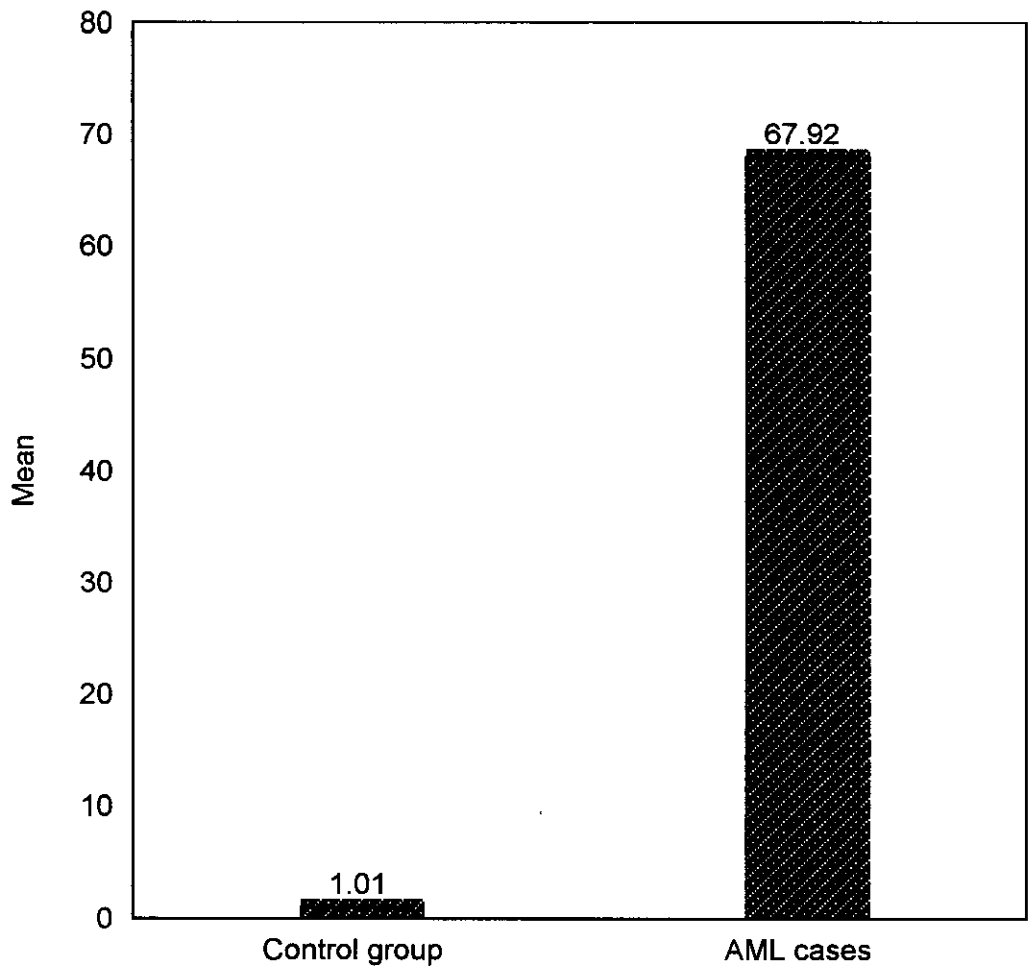


Figure (6): Descriptive statistical analysis of RTA in AML cases

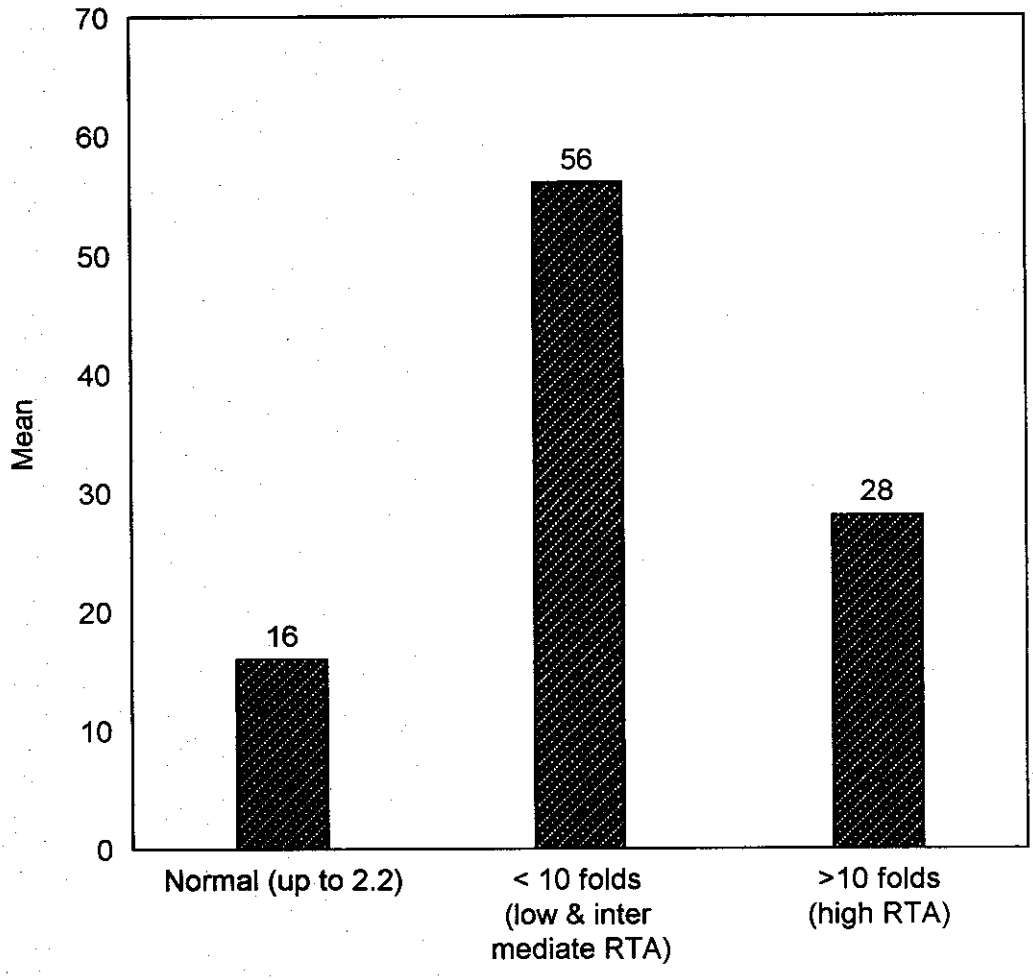


Figure (7): Statistical comparison of RTA in FAB subtypes in AML cases

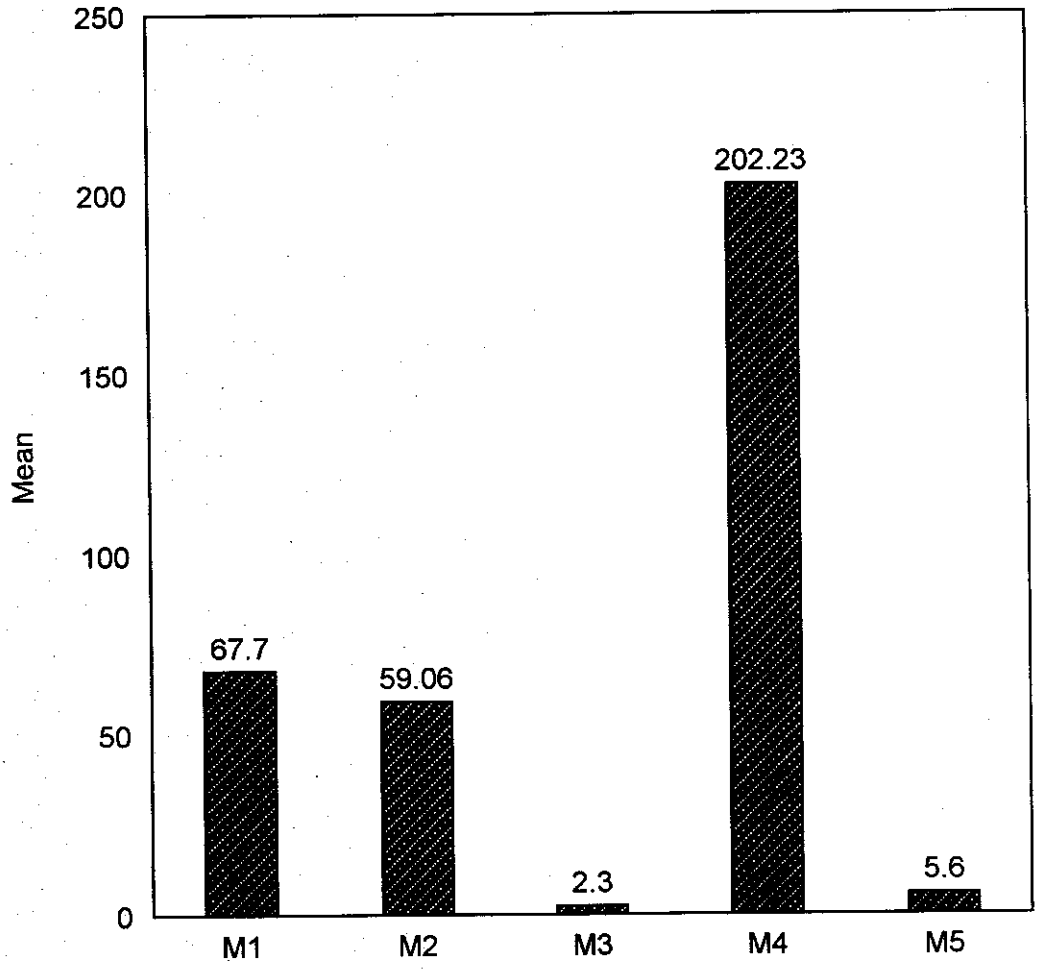


Fig. (8): Comparison of RTA in FAB subtypes of AML cases.