RESULTS

RESULTS

- 40 newly diagnosed patients with acute myloid leukemia were included in the present study. The individual clinical and laboratory data of all patients are shown in the appendix.

The study included 25 male patients and 15 female patients. They were adults (age ranged from 23 to 66 years).

♦ Presenting symptoms:

The main presenting symptoms of the patients are summarized in Table (R1).

Table(R1): **Presenting symptoms of the study group**

Symptom	No.	%
Fatigue	36	90
Pallor	36	90
Purpura	22	55
Abdominal swelling	15	37.5
Boneaches	36	90
Epistaxis	19	47.5
Neck's swelling	20	50

♦ Clinical findings:

The clinical signs found in the patients are summarized in the following table (R2).

Table (R2): **Clinical signs of the study group**

Sign	No.	%
Fever	29	72.5
Lymphoadenopathy	15	37.5
Hepatomegally	19	47.5
Splenomegally	19	47.5

♦ Hematological data:

Table R3 and (Fig.1) shows the hematological data of patients at diagnosis. It includes Hb (hemoglobin concentration), TLC (Total leucocytic count), Plt (platelet count) as well as percentage of blast cells in both peripheral blood and bone marrow.

Table (R3): **Hematological data of the study group at diagnosis.**

Item	Range	Mean	+SD
Hb (g/dL)	2.7– 9.5	6.25	1.929
WBCs (x10^3/μL)	3.3– 187	55	53.519
Plt (x10^3/μL)	5.5– 90	40	23.582
Blasts in PB (%)	22– 98	57.5	23.57
Blasts in BM (%)	45– 100	81	17.47

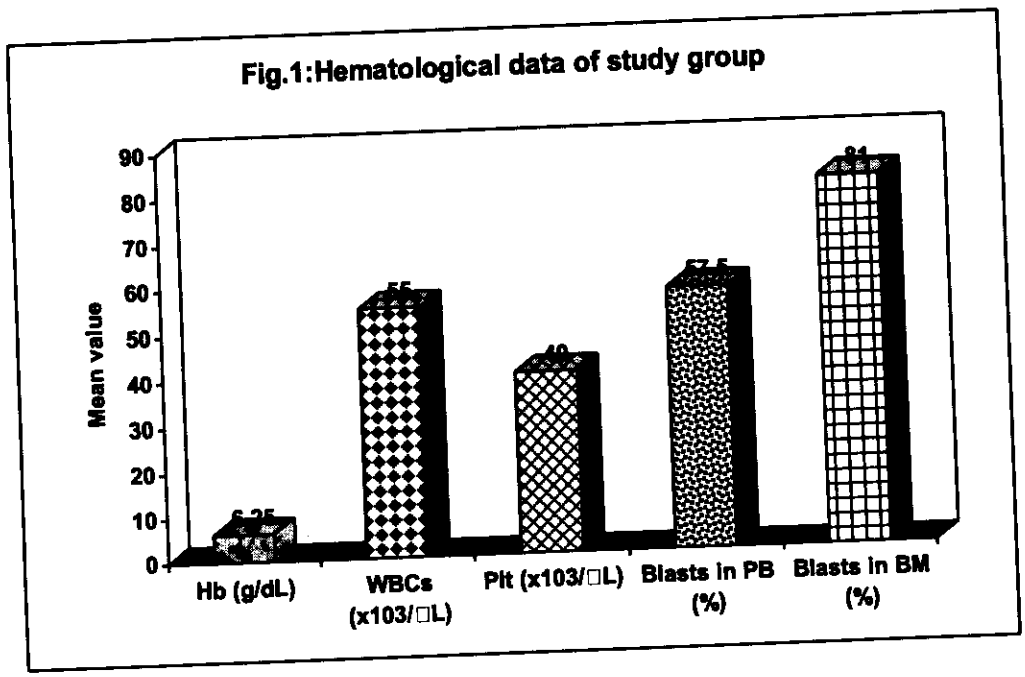


Table R4 and (Fig.2) summarizes the hemogram abnormalities in the studied patients, 90% of them showed hemoglobin level below 9 gm/dL, and only 10% showed a higher hemoglobin level. 40% of them showed leucocytosis from 11-50 x 10^3 /μL, 35% showed leucocytosis above 50 x 10^3 /μL, 15% showed a normal leucocytic count (4-11 x 10^3 /μL) while the remaining 10% showed leucopenia (below 4 x 10^3 /μL).

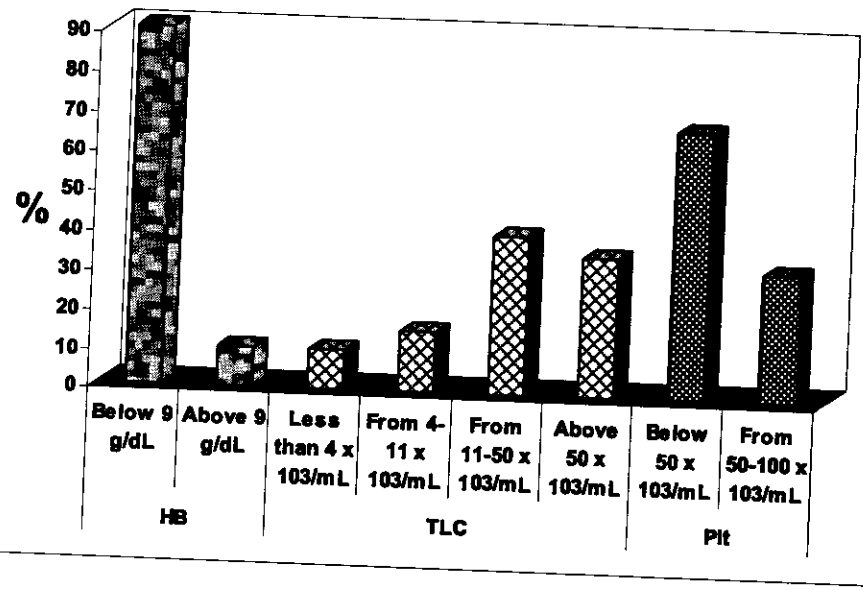
67.5% of patients were presented with marked thrombocytopenia (below 50 x 10^3/μL) while 32.5% of them suffered from moderate thrombocytopenia (50-100 x 10^3/μL).

Table (R4): **The hemogram abnormalities of the study group**

Item	No	%	X^2	P.value
Hb:				
Below 9 g/dL	36	90	9.351	0.001*
Above 9 g/dL	4	10		
TLC:				
Less than 4 x 10^3/µL	4	10	5.321	0.05*
From 4-11 x 10^3/µL	6	15		
From 11-50 x 10^3/µL	16	40		
Above 50 x 10^3/µL	14	35		
Plt:				
Below 50 x 10^3/µL	27	67.5	6.327	0.001*
From 50-100 x 10^3/µL	13	32.5		

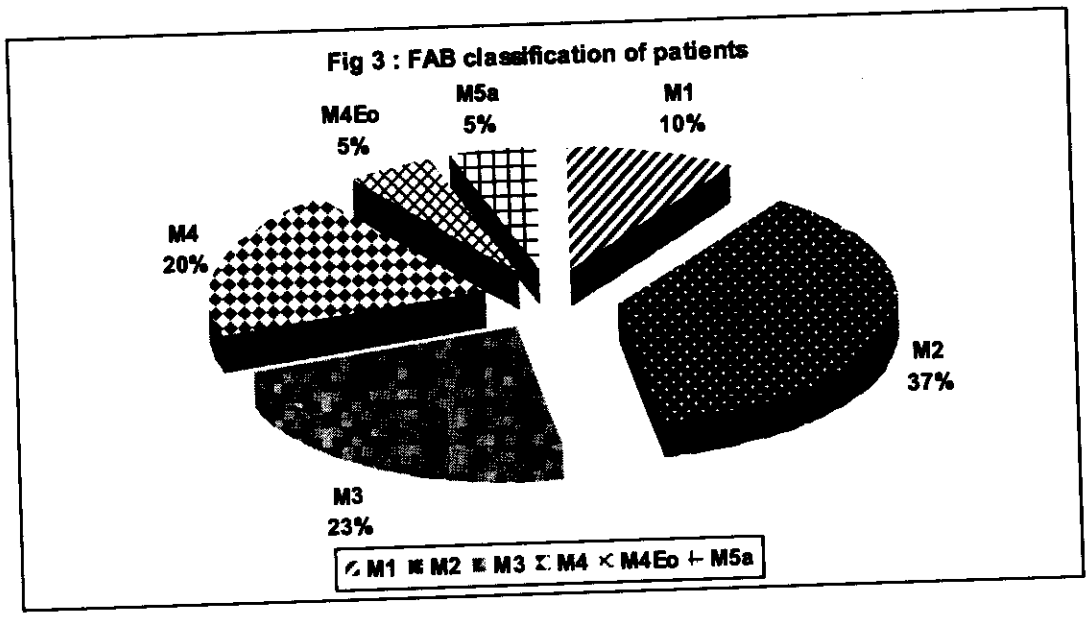
Significant P<0.05

Fig.2:The Hemogram abnormalities of the study group



Morphological characteristics (FAB classification):

Grouping of patients according to FAB classification revealed : 4 patients (10%) showed FAB M1 morphology, 15 of them (37.5%) showed FAB M2 morphology, 9 of them (22.5%) showed FAB M3, 8 of them (20%) showed FAB M4, 2 of them (5%) showed FAB M4Eo while the remaining 2 (5%) showed FAB M5a.(Fig.3)



Results of t(8;21), t(15;17) and inv(16):

Multiplex as well as FISH performed for t(8;21), t(15;17) and inv(16) on the 40 newly diagnosed cases with AML revealed that there is a complete agreement between the multiplex and FISH results as regard the positive and negative cases.

I. Results of translocation (8;21):

Out of the analyzed samples (40), there were 5 cases positive for t(8;21) (table R5 and Fig.4). This emphasizes an incidence of 12.5% positivity.

Table(R5): **Incidence of t(8;21)**

t(8;21) results	No. (40)	Frequency %	X^2	P. value
Positive	5	12.5	8.325	0.001*
Negative	35	87.5		

Significant P<0.05

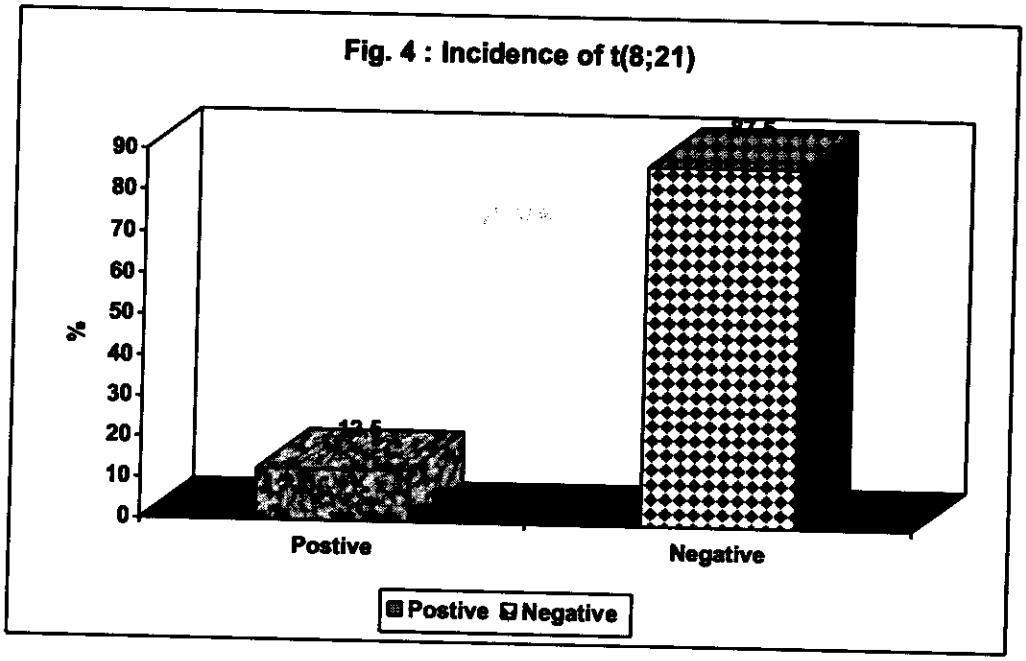


Table R6 shows the clinical, hematological and immunological data of the 5 positive cases for t(8;21)

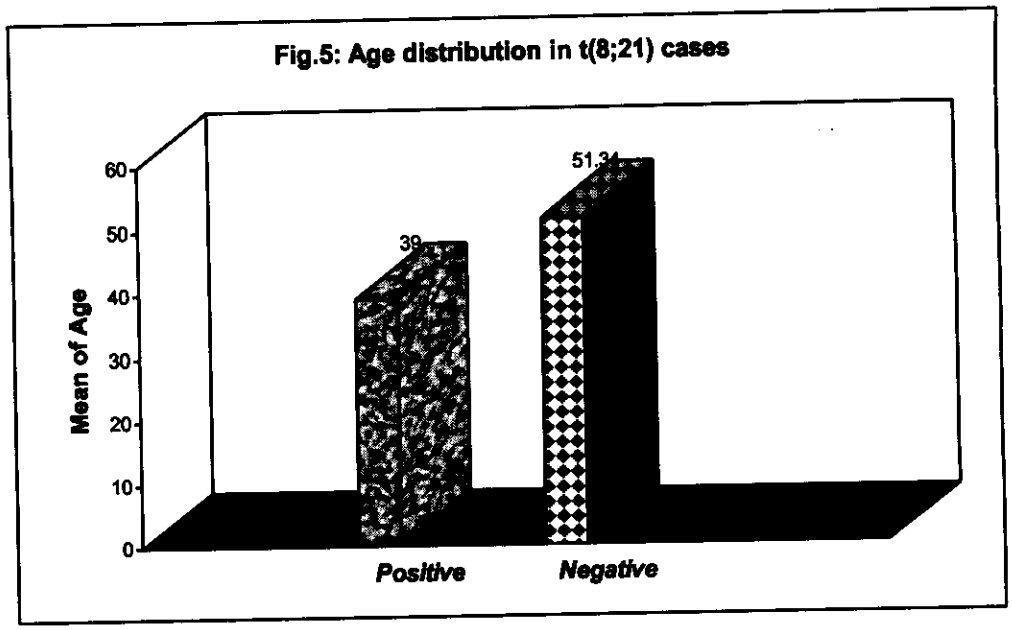
Table (R6): **Clinical and hematological data of t(8;21) positive cases**

Patient No.	FAB Diagnosis	Age	Sex	Hb	TLC	Plt	PB BL%	BM BL%	PCR	Fate	A/D
1.	M2	34	Male	3.5	73	28	73	100	t(8;21)	C.R.	Alive
3.	M2	40	Male	2.7	38.7	15	85	100	t(8;21)	C.R.	Alive
13.	M2	45	Female	9.4	7.8	5.5	46	72	t(8;21)	Relapse	Died
19.	M2	31	Male	7.1	3.3	90	80	91	t(8;21)	C.R.	Alive
23.	M2	45	Male	9.1	31	28	32	54	t(8;21)	C.R.	Alive

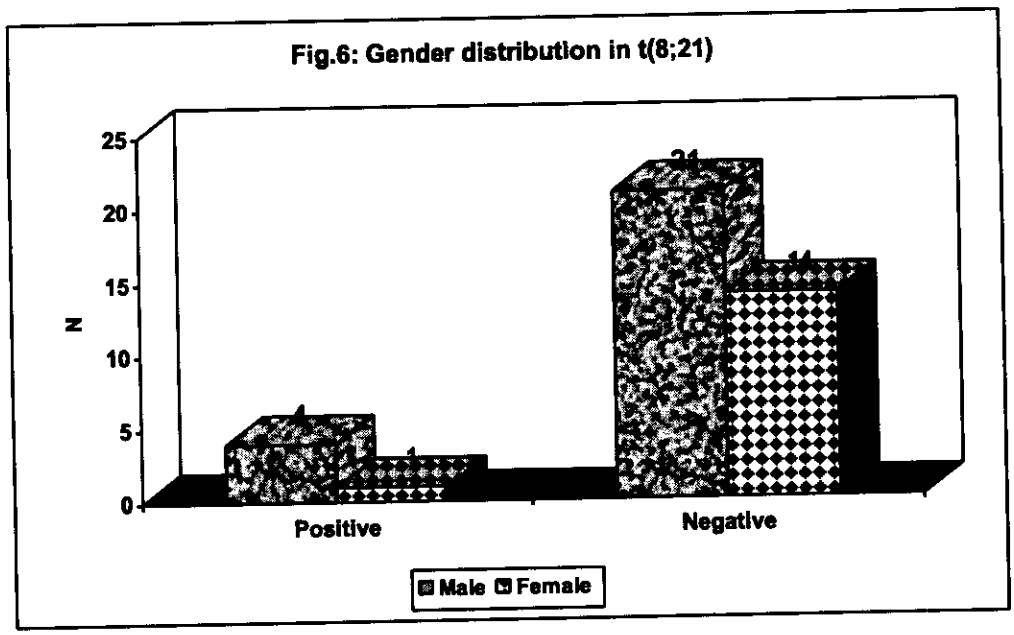
◊ Clinical characteristics of t(8;21) positive cases:

Age and gender:

The t(8;21) positive cases were 5 adults (age ranged from 31-45 years), the mean age of the whole group was 39 years. In the negative cases the age ranges between 23 and 66 years with a mean of 51.34 years (Fig.5). There was a statistically significant difference between both groups (p=0.020*).



The t(8;21) positive cases were 4 males and 1 female, while the distribution among the negative group was 21 males and 14 females with a ratio of 3 : 2 (Fig 6). There is a statistically significant difference between both groups (p=0.038*-)



◊ Hematological findings:

1. Hemoglobin level:

The Hb level of the t(8;21) positive cases ranges between 2.7-9.4 g/dL with a mean value of 6 g /dL. The negative group patients acquired a range between 2.7 – 9.5 g /dL for the hemoglobin level with a mean of 6.4 g /dL.

2. Total leucocytic count (T.L.C.):

The initial T.L.C. of patients with t(8;21) ranges between 3.3 - 73 x $10^3/\mu L$ with a mean of 30.76 x $10^3/\mu L$. For the negative cases its range is 3.5 - 187 x $10^3/\mu L$ at diagnosis with a mean of 58.46 x $10^3/\mu L$.

3. Platelet count:

The platelet count in the positive group patients ranged between 5.5 and 90 x $10^3/\mu L$ with a mean of 33.3 x $10^3/\mu L$. The range among the negative group was 8.5 - 90 x $10^3/\mu L$ with a mean of 40.95 x $10^3/\mu L$.

4. Number of blast cells:

In the peripheral blood, blast cells ranged between 32 – 85 % with a mean of 63.2 % in the positive cases while the t(8;21) negative patients showed a range of 22 - 98 % with a mean value of 56.68 %.

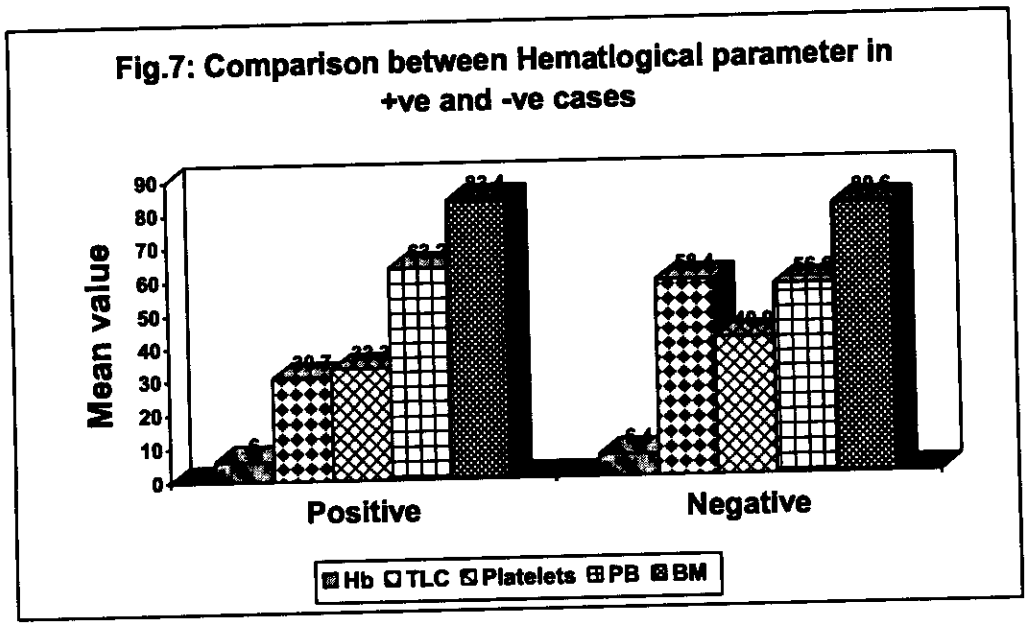
In the bone marrow, percentage of blast cells ranged between 54-100 % in the positive group (mean value of 83.4 %) compared to 45-100% in the negative group (mean value of 80.65 %).

Table R7 and (Fig.7) shows comparative study between t(8;21) positive and negative cases regarding the hematological parameters.

Table (R7): **Comparison between t(8;21) positive and negative cases regarding the hematological parameters.**

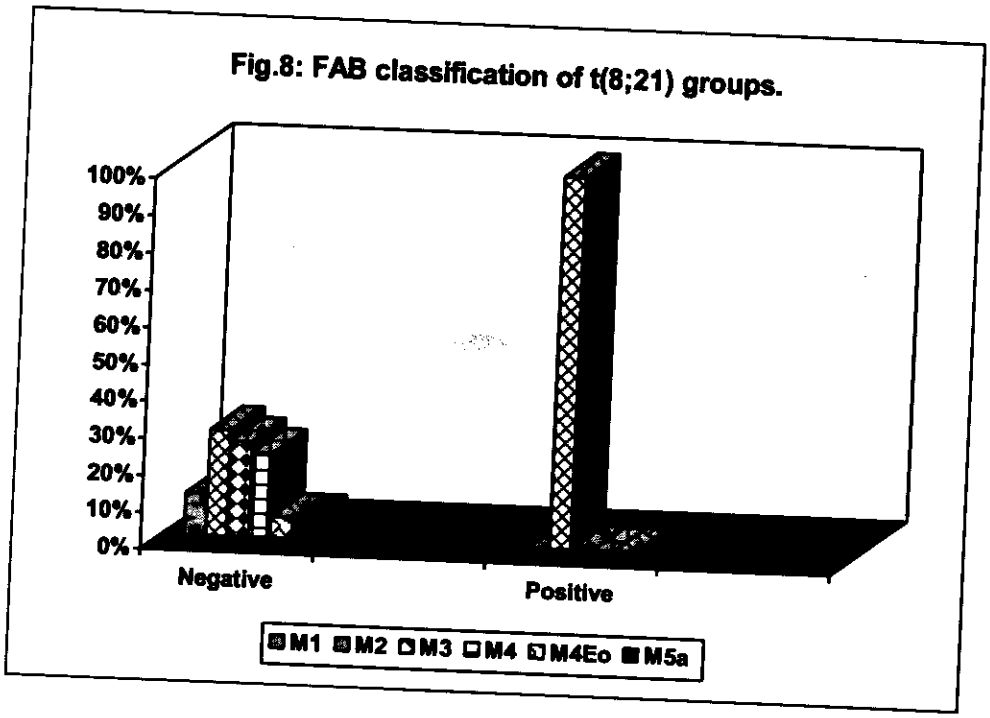
Parameter	Positive		Negative		p. value	Sig
	Range	*Mean*	*Range*	*Mean*		
Hb(g/dL)	2.7-9.4	6±1.23	2.7-9.5	6.4±1.23	**0.557**	**>0.05**
TLCx10^3/μL	3.3-73	30.76±4.32	3.5-187	58.4±9.35	**0.000**	**<0.001***
Platelets	5.5-90	33.3±4.36	8.5-90	40.95±14.11	**0.043**	**<0.05***
PB blast%	32-85	63.2±12.48	22-98	56.68±11.09	**0.051**	**>0.05**
BM blast%	54-100	83.4±24.57	45-100	80.65±19.39	**0.770**	**>0.05**

Significant P<0.05



◊ FAB classification:

All the t(8;21) positive cases showed a FAB M2 morphology. The negative cases were grouped into 10 cases (28.57%) with FAB M2 morphology, 4 cases (11.42%) with FAB M1 morphology,9 cases (25.7%) with FAB M3 morphology, 8 cases (22.85%) with FAB M4, 2 cases (5.7%) with FAB M4Eo, 2 cases (5.7%) with FAB M5a (Fig.8). There is statistically significant difference between both groups as regards the FAB subtype (p=-0.0.041*).



◊ Prognosis:

Regarding the fate of the t(8;21) positive cases, 4 out of 5 patients (80%) achieved a complete remission and 1 patient (20%) relapsed during two years of follow up, while among the negative cases 5 patients (14.3%) achieved a complete remission, 12 patients (34.3%) no remission, 16 patients (45.7%) relapsed and 2 patients(5.7%) delayed remission during two years of follow up (Table R8, Fig.9). Statistical analysis reveals a significant difference between both groups regarding the fate of the patients *(p=0.001)*

Table (R8): **Fate of t(8;21) positive and negative cases.**

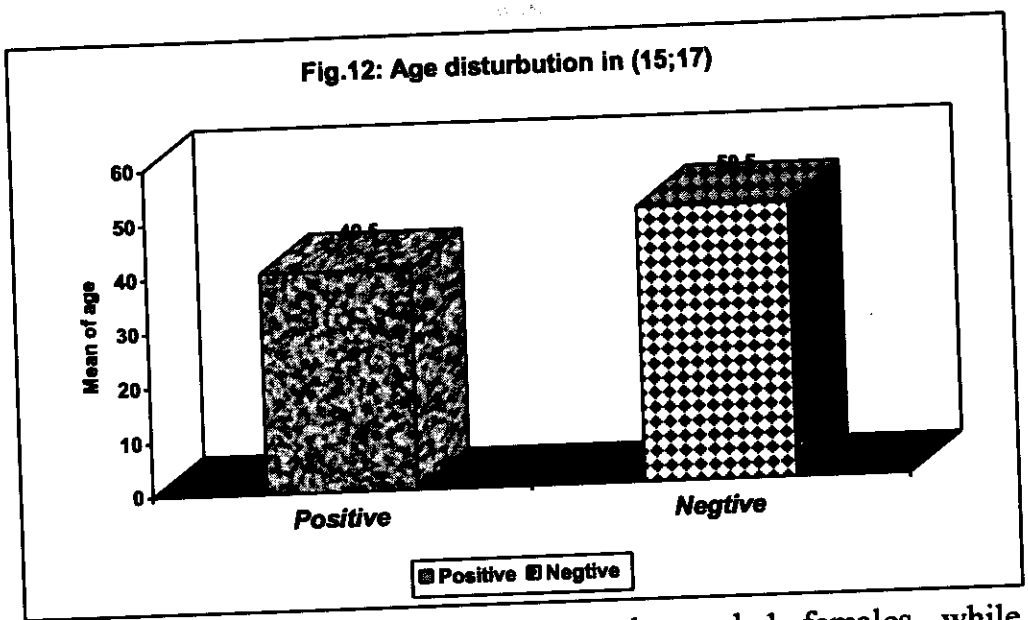
Fate	t(8;21) positive(5)		t(8;21) negative (35)	
	No.	%	No.	%
C.R.	4	80	5	14.3
No Rem	0	0	12	34.3
Relapse	1	20	16	45.7
Del Rem	0	0	2	5.7
X^2	11.081			
P. value	0.001*			

Significant P<0.05

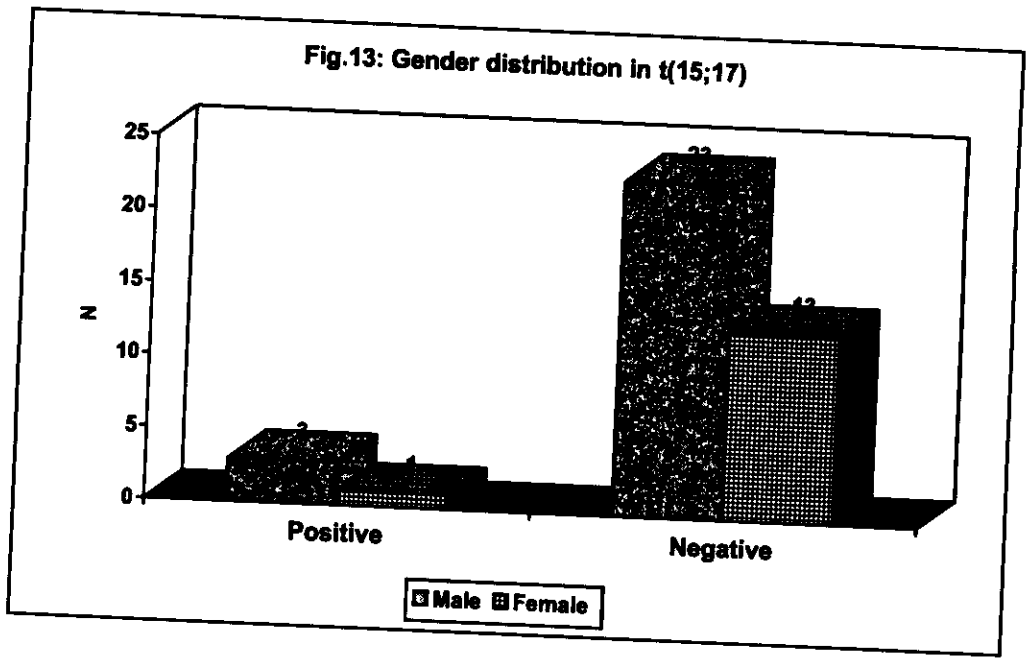
◊ Clinical characteristics of t(15;17) positive cases:

Age and gender:

The t(15;17) positive cases ranged in age between 30-52 years with a mean of 40.5 years. In the negative cases the age range was 23- 66 years with a mean of 50.8 years (Fig 12). There was no statistically significant difference between both groups (p=0.051).



The t(15;17) positive cases were 3 males and 1 females, while the distribution among the negative cases were 23 males and 13 females (Fig.13).Statistical analysis revealed significant difference between both groups(p=0.049*)



◊ Hematological findings:

1. Hemoglobin level:

The Hb level of the t(15;17) positive cases ranges between 5.8-8.2 g /dL, with a mean value of 7.1 g /dL. The negative group has a range of 2.7-9.5 g /dL hemoglobin level with a mean of 6.16 g /dL.

2. Total leucocytic count (T.L.C.):

The initial T.L.C. of patients with t(15;17) ranges between 43-89 x $10^3/\mu L$ with a mean of 54.87 x $10^3/\mu L$. The negative cases lies in the range of T.L.C. between 3.3 - 187 x $10^3/\mu L$ at diagnosis, with a mean of 57.68 x $10^3/\mu L$.

3. Platelet count:

The platelet count in the positive group patients ranges between 33-85 x $10^3/\mu L$ with a mean of 62 x $10^3/\mu L$. The range among the negative group was 5.5-90 x $10^3/\mu L$ with a mean of 37.5 x $10^3/\mu L$.

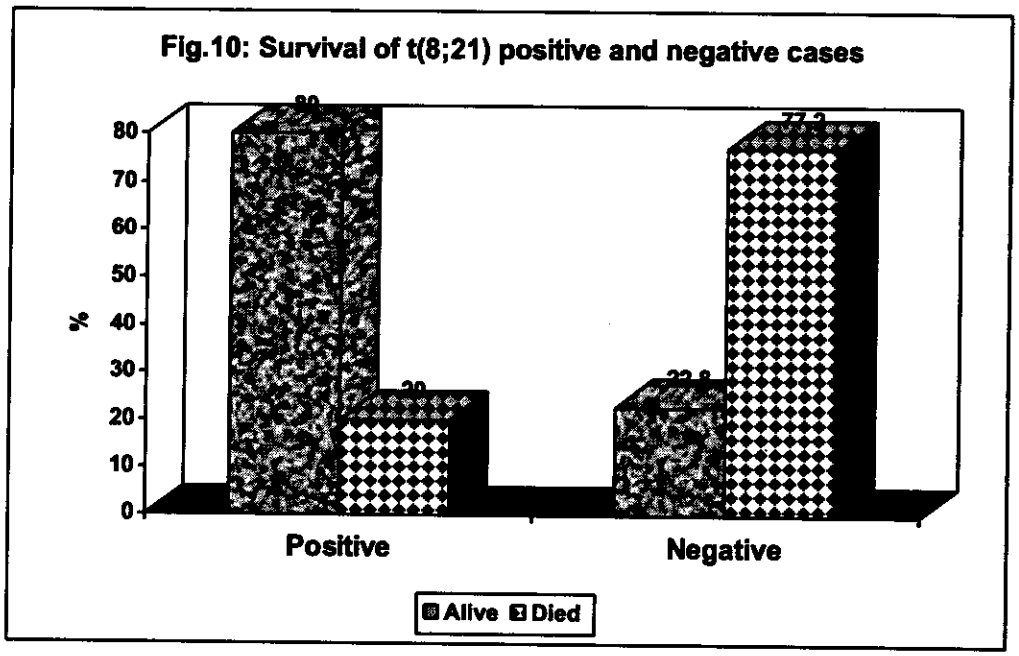
4. Number of blast cells:

In the peripheral blood, blast cells ranged between 28-90 % with a mean of 54.5 % in the positive cases, while the t(15;17)

Table (R9): **Survival of t(8;21) positive and negative cases.**

Alive/Died	t(8;21) positive (5)		t(8;21) negative (35)	
	No.	%	No.	%
Alive	4	80%	8	22.8%
Died	1	20%	27	77.2%
X2	6.80			
P. value	0.009*			

Significant P<0.05



Results of translocation(15;17):

Out of the 40 analyzed samples , there were 4 positive cases for t(15;17) (table R10 and Fig.11). This reveals an incidence of 10% positivity.

Table(R10): **Incidence of t(15;17)**

t(15;17) results	No.(out of 40)	Frequency%
Positive	4	10%
Negative	36	90%

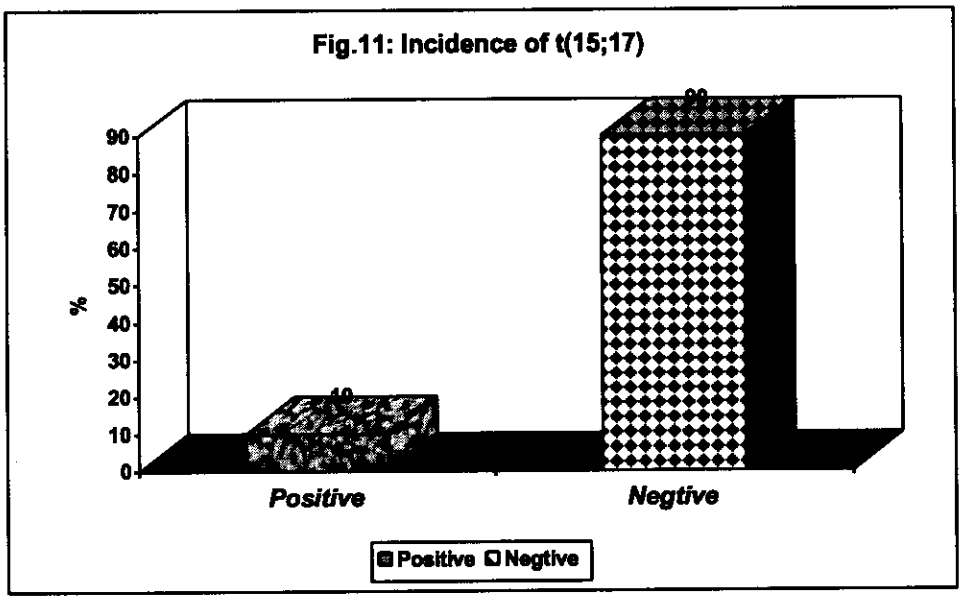


Table R11 shows the clinical, hematological and immunological data of the 4 positive cases for t(15;17)

Table (R11): **Clinical and hematological data of t(15;17) positive cases**

Patient No.	FAB Diagnosis	Age	Sex	Hb	TLC	Plt	PB BL%	BM BL%	PCR	Fate	A/D
8.	M3	30	Male	8.1	52	85	40	78	t(15;17)	C.R.	Alive
17.	M3	52	Male	8.2	47.5	71	28	58	t(15;17)	Relapse	Died
27.	M3	39	Female	6.1	89	33	90	100	t(15;17)	C.R.	Alive
31.	M3	41	Female	5.8	43	59	60	96	t(15;17)	C.R.	Alive

negative patients have a range of 22-98 % with a mean value of 57.8 %.

In the bone marrow, percentage of blast cells ranges between 58-100% for the positive group (mean value of 83 %) compared to 45-100 % in the negative group (mean value of 80.7%).

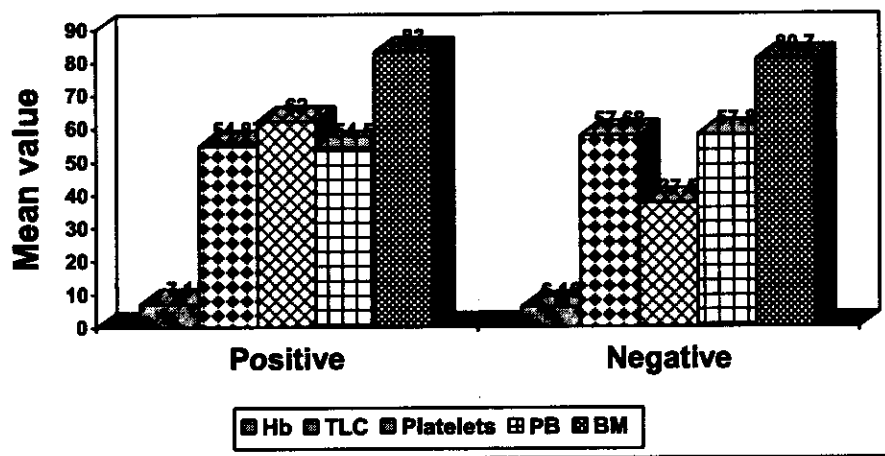
Table R12 and Fig.14 shows comparative study between t(15;17) positive and negative cases regarding the hematological parameters.

Table (R12): **Comparison between t(15;17) positive and negative cases regarding the hematological parameters.**

Parameter	Positive		Negative		P value	Sig.
	Range	*Mean*	*Range*	*Mean*		
Hb(g/dL)	5.8-8.2	7.1	2.7-9.5	6.16	0.089	>0.05
TLCx10^3/μL	43-89	54.87	3.3-187	57.68	0.057	>0.05
Platelets	33-85	62	5.5-90	37.5	0.042*	<0.05*
PB blast%	28-90	54.5	22-98	57.8	0.068	>0.05
BM blast%	58-100	83	45-100	80.7	0.236	>0.05

Significant P<0.05

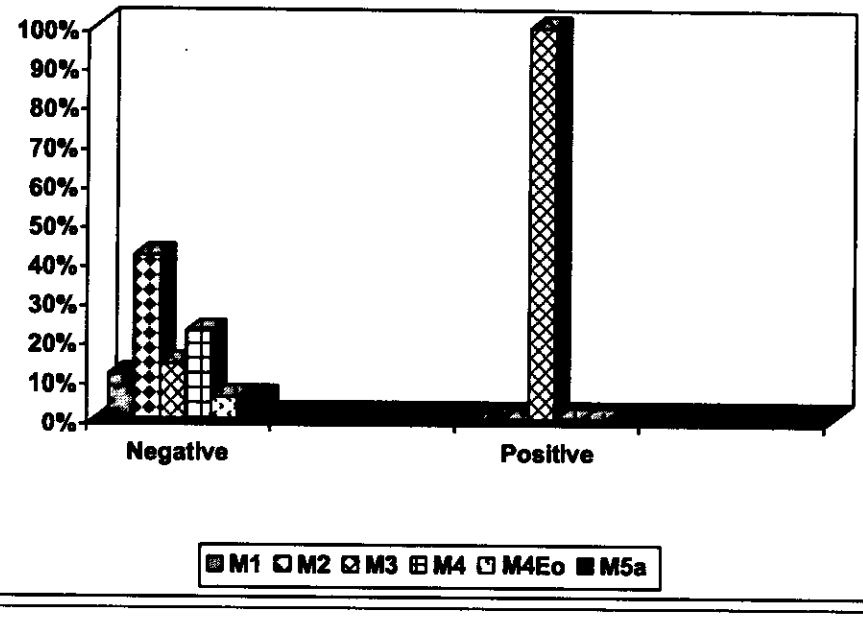
Fig.14: Comparison between hematological parameter In +ve and -ve cases



◊ FAB classification:

All the t(15;17) positive cases showed a FAB M3 morphology. The negative cases were grouped into 4 cases (11.1%) with FAB M1 morphology , 15 cases (41.7%) with FAB M2 morphology, 5 cases (13.9%) with FAB M3 morphology, 8 cases (22.3%) with FAB M4 morphology, 2 cases (5.5%) with FAB M4Eo morphology, 2 cases (5.5%) with FAB M5a morphology (Fig. 15). There is statistically significant difference between both groups as regards the FAB subtype (p=0.043*).

Fig.15: FAB classification of t(15;17) groups.



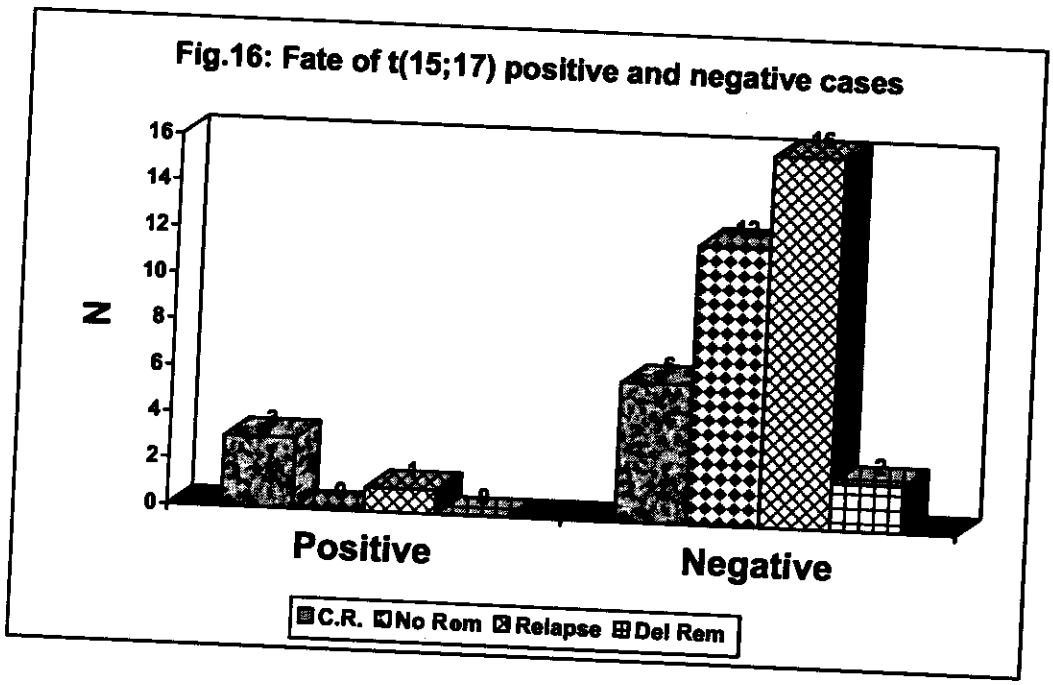
◊ Prognosis:

Regarding the fate of the t(15;17) positive cases, 3 out of 4 patients (75%) achieved a complete remission and 1 patient (25%) relapsed during two years of follow up, while among the negative cases 6 patients (16.6%) achieved a complete remission, 12 patients (33.3%) no remission, 16 patients (44.4%) relapsed and 2 patients(5.5%) delayed remission during two years of follow up (Table R13, Fig.16). Statistical analysis reveals a significant difference between both groups regarding the fate of the patients *(p=0.043)*

Table (R13): **Fate of t(15;17) positive and negative cases.**

Fate	t(15;17) positive(4)		t(15;17) negative (36)	
	No.	%	No.	%
C.R.	3	75%	6	16.6%
No Rem.	0	0	12	33.3%
Relapse	1	25%	16	44.4%
Del Rem.	0	0	2	5.5%
X^2	6.320			
P.value	0.043*			

Significant P<0.05

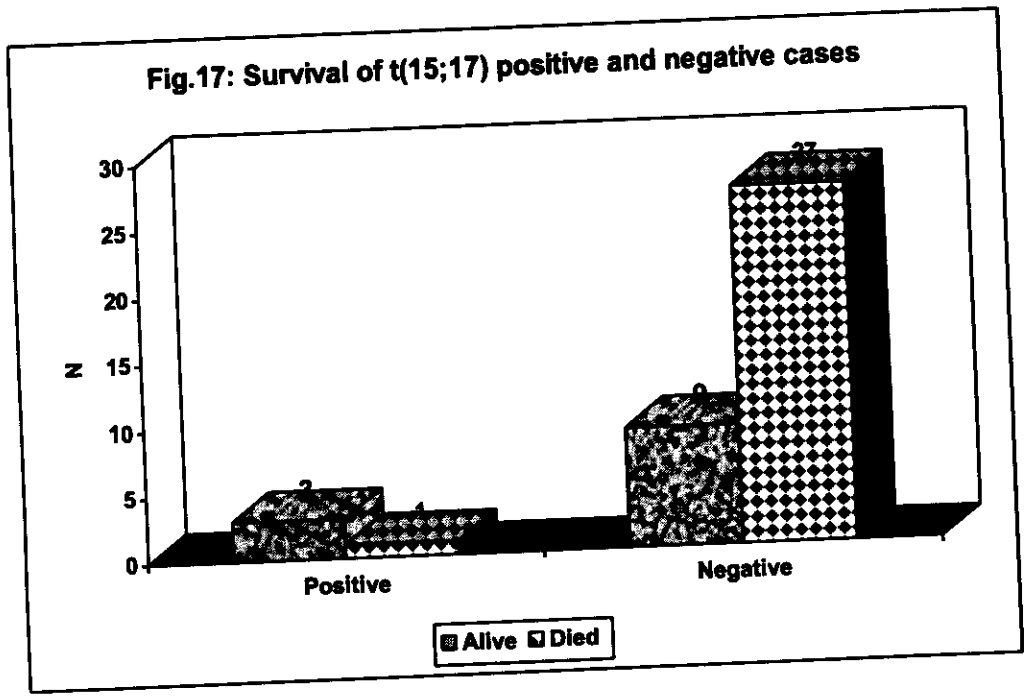


Regarding the Survival of the t(15;17) positive cases, 3 out of 4 patients (75%) alive and 1 patient (25%) died during two years of follow up, while among the negative cases 9 patients (25%) alive and 27 patients(75%) died during two years of follow up (Table R14, Fig.17). Statistical analysis reveals a significant difference between both groups regarding the fate of the patients (*p=0.012)*

Table (R14): **Survival of t(5;17) positive and negative cases.**

Alive/Died	t(5;17) positive (4)		t(5;17) negative (36)	
	No.	%	No.	%
Alive	3	75%	9	25%
Died	1	25%	27	75%
X^2	4.326			
P.value	0.012*			

Significant P<0.05



II. Results of the inversion(16):

Out of the 40 analyzed samples, there were 2 positive cases for inv(16) (table R15 and Fig.18). This reveals an incidence of 5% positivity.

Table(R15): **Incidence of inv(16)**

Inv(16) results	No.(out of 40)	Frequency%
Positive	2	5%
Negative	38	95%

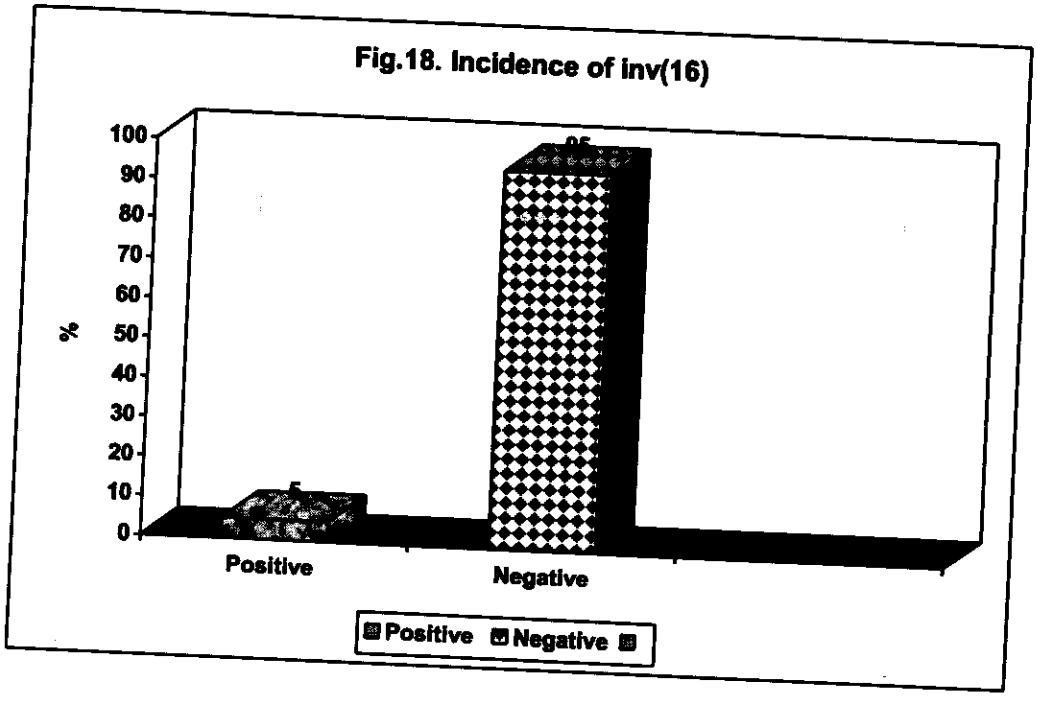


Table R16 shows the clinical, hematological and immunological data of the 2 positive cases for inv(16)

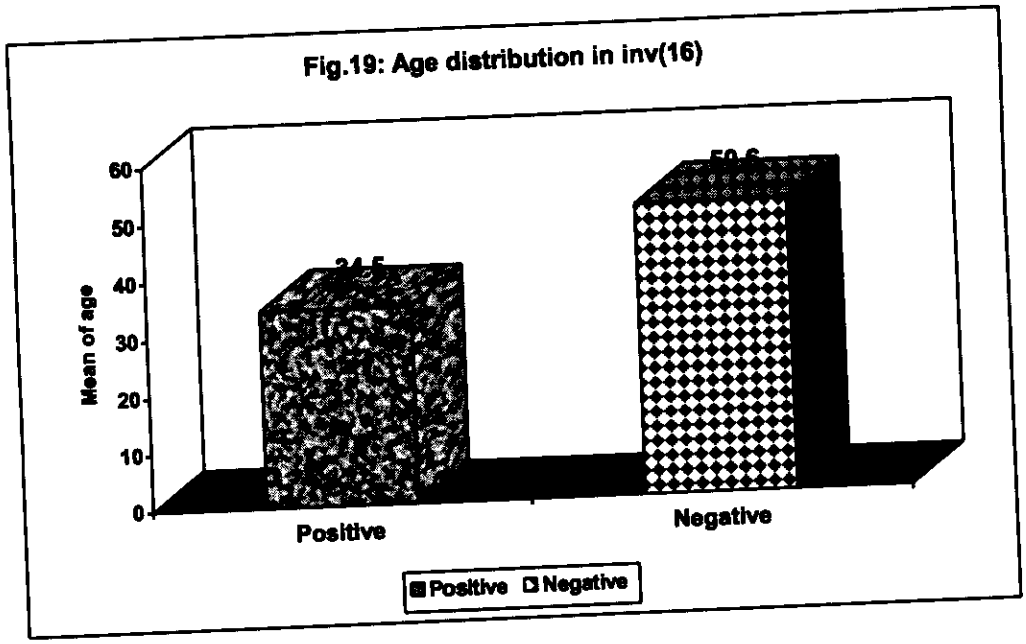
Table (R16): **Clinical, hematological and immunological data of inv(16) positive cases**

Patient No.	FAB Diagnosis	Age	Sex	Hb	TLC	Plt	PB BL%	BM BL%	PCR	Fate	A/D
4.	M4Eo	23	Male	5.2	46	12	98	100	inv(16)	C.R.	Alive
15.	M4Eo	46	Male	6.5	44	13	53	100	inv(16)	C.R.	Alive

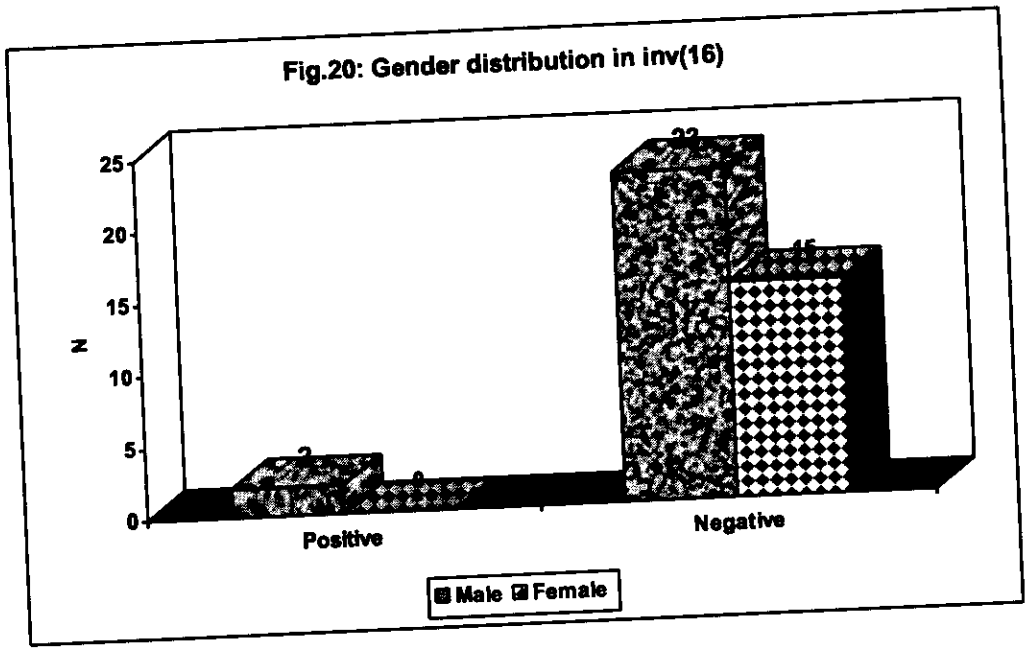
◊ Clinical characteristics of inv(16) positive cases:

Age and gender:

The inv(16) positive cases ranged in age between 23-46 years with a mean of 34.5 years. In the negative cases the age range was 30-66 years with a mean of 50.6 years (Fig 19). There was statistically significant difference between both groups (p=0.010*).



The inv(16) positive cases were 2 males, while the distribution among the negative cases were 23 males and 15 females (Fig 20).Statistical analysis revealed significant difference between both groups(p=0.049*)



Hematological findings:

1. Hemoglobin level:

The Hb level of the inv(16) positive cases ranges between 5.2 - 6.5 g /dL, with a mean value of 5.85 g /dL. The negative group has a range of 2.7 - 9.5 g /dL hemoglobin level with a mean of 6.27 g /dL.

2. Total leucocytic count (T.L.C.):

The initial T.L.C. of patients with inv(16) ranges between 44 - 46 x $10^3/\mu L$ with a mean of 45 x $10^3/\mu L$. The negative cases lies in the range of T.L.C. between 3.3-187 x $10^3/\mu L$ at diagnosis, with a mean of 55.5 x $10^3/\mu L$.

3. Platelet count:

The platelet count in the positive group patients ranges between 12- 13 x $10^3/\mu L$ with a mean of 12.5 x $10^3/\mu L$. The range among the negative group was 5.5-90 x $10^3/\mu L$ with a mean of 41.4 x $10^3/\mu L$.

4. Number of blast cells:

In the peripheral blood, blast cells ranged between 53-98 % with a mean of 75.5 % in the positive cases, while the inv(16) negative patients have a range of 22 - 91 % with a mean value of 56.5 %.

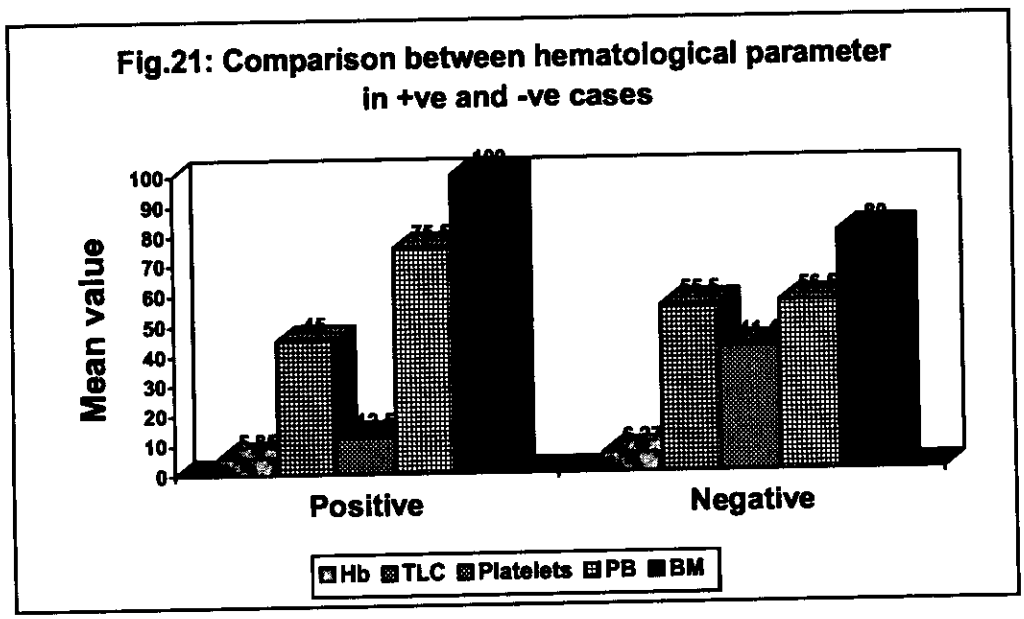
In the bone marrow, percentage of blast cells is 100 % for the positive group (mean value of 100 %) compared to 45 – 100 % in the negative group (mean value of 80 %).

Table R17 and (Fig.21) shows comparative study between inv(16) positive and negative cases regarding the hematological parameters.

Table (R17): **Comparison between inv(16) positive and negative cases regarding the hematological parameters.**

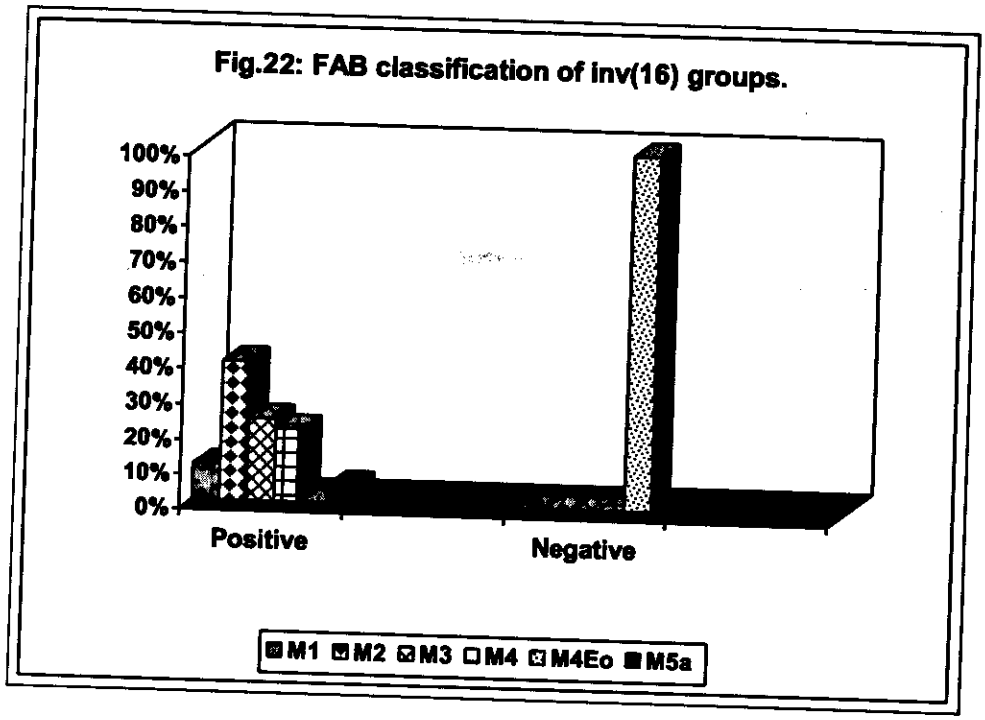
Parameter	Positive		Negative		P value	Sig.
	Range	*Mean*	*Range*	*Mean*		
Hb(g/dL)	5.2-6.5	5.85	2.7-9.5	6.27	0.936	>0.05
TLCx10^3/µL	44-46	45	3.3-187	55.5	0.235	>0.05
Platelets	12-13	12.5	5.5-90	41.4	0.032*	<0.05*
PB blast%	53-98	75.5	22-91	56.5	0.041*	<0.05*
BM blast%	100-100	100	45-100	80	0.017*	<0.05*

Significant P<0.05



FAB classification:

All the inv(16) positive cases showed a FAB M4Eo morphology. The negative cases were grouped into 4 cases (10.5 %) with FAB M1 morphology, 15 cases (39.5%) with FAB M2 morphology, 9 cases (23.7%) with FAB M3 morphology, 8 cases (21%) with FAB M4 morphology, 2 cases (5.3%) with FAB M5a morphology (Fig.22). There is statistically significant difference between both groups as regards the FAB subtype (p=0.008*-).



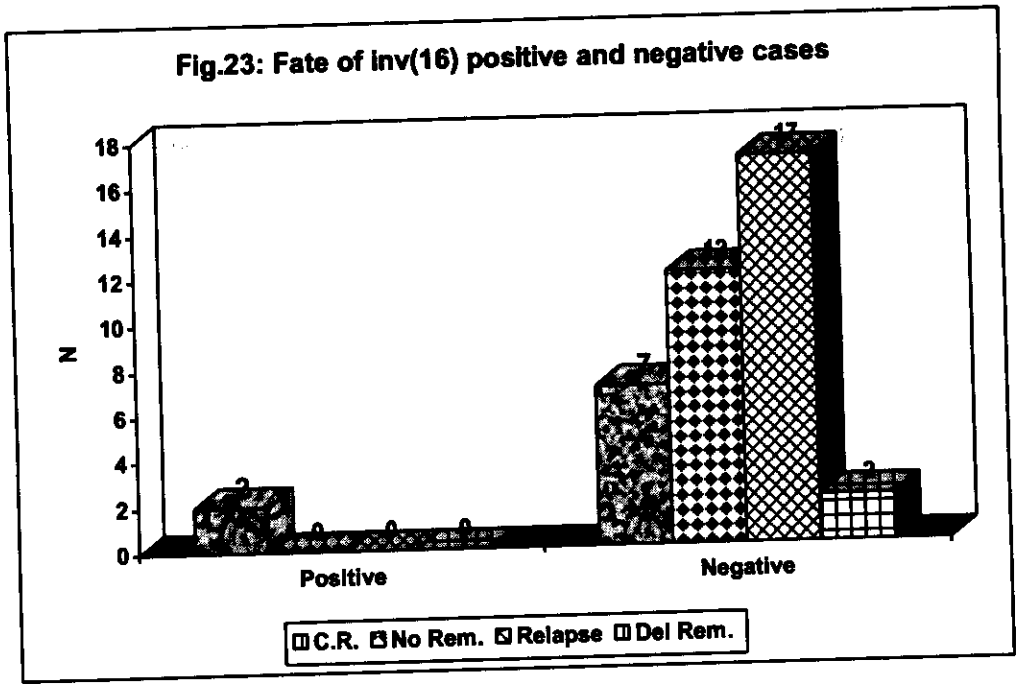
Prognosis:

Regarding the fate of the inv(16) positive cases, 2 patients (100%) achieved a complete remission during two years of follow up, while among the negative cases 7 patients (18.5%) achieved a complete remission, 12 patients (31.5%) no remission, 17 patients (44.7%) relapsed and 2 patients(5.3%) delayed remission during two years of follow up (Table R18, Fig.23). Statistical analysis reveals a significant difference between both groups regarding the fate of the patients (*p=0.011*)

Table (R18): **Fate of inv(16) positive and negative cases.**

Fate	Inv(16) positive(2)		Inv(16) negative (38)	
	No.	%	No.	%
C.R.	2	100%	7	18.5%
No Rem.	0	0	12	31.5%
Relapse	0	0	17	44.7%
Del Rem.	0	0	2	5.3%
X^2	7.236			
P. value	0.011*			

Significant P<0.05

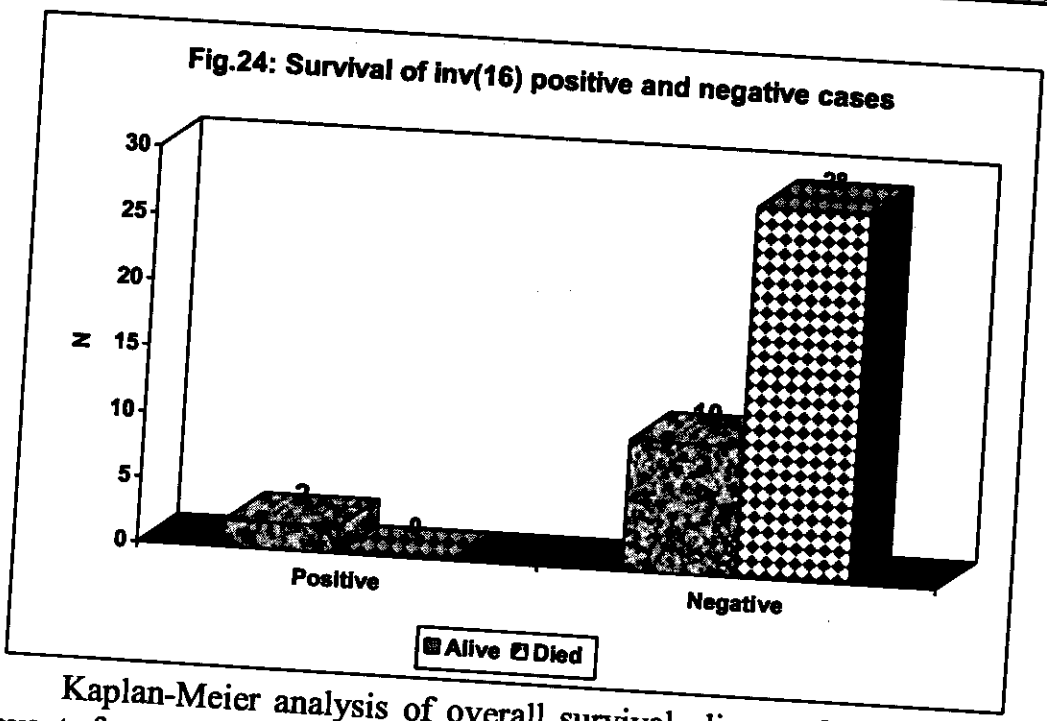


Regarding the Survival of the inv(16) positive cases, 2 patients (100%) alive during two years of follow up, while among the negative cases 10 patients (26.3%) alive and 28 patients(73.7%) died during two years of follow up (Table R9, Fig.24). Statistical analysis reveals a significant difference between both groups regarding the fate of the patients (*p=0.047)*

Table (R19): **Survival of inv(16) positive and negative cases.**

Alive/Died	Inv(16) positive (2)		Inv(16) negative (38)	
	No.	%	No.	%
Alive	2	100%	10	26.3%
Died	0	0	28	73.7%
X^2	5.328			
P. value	0.047*			

Significant P<0.05



Kaplan-Meier analysis of overall survival, disease free survival and event free survival was used as prognostic test and showed a high statistically significant difference in prognosis between patients with and without FISH detected chromosomal abnormalities in t(8;21),t(15;17) and inv(16).Good prognosis observed in patients with FISH detected abnormalities in t(8;21),t(15;17) and inv(16).

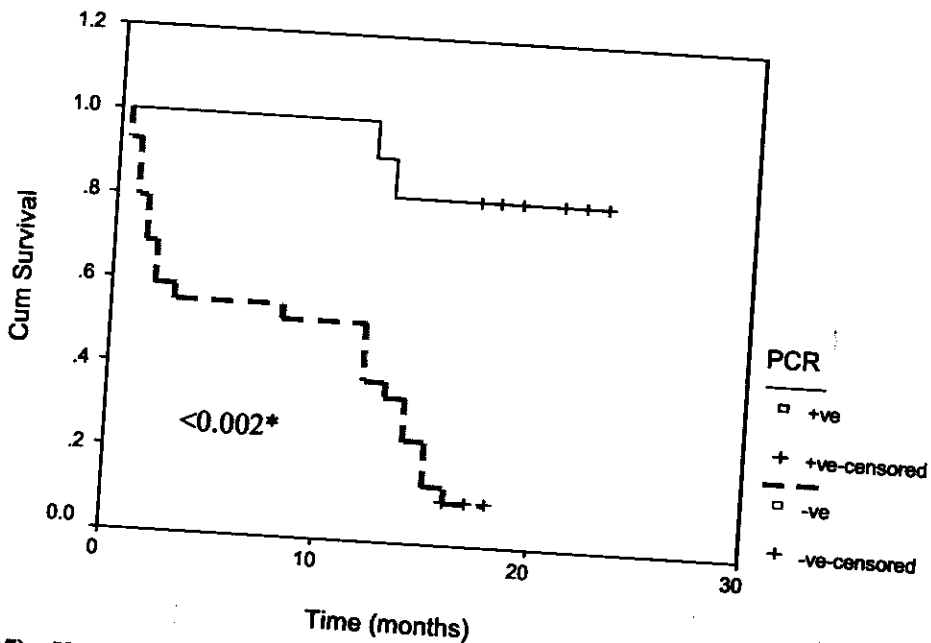


Fig (25): Kaplan-Meier analysis of overall survival in cases with abnormalities in t(8;21),t(15;17) and inv(16) in studied groups showed significant increase in survival in these patients.(P<0.002)

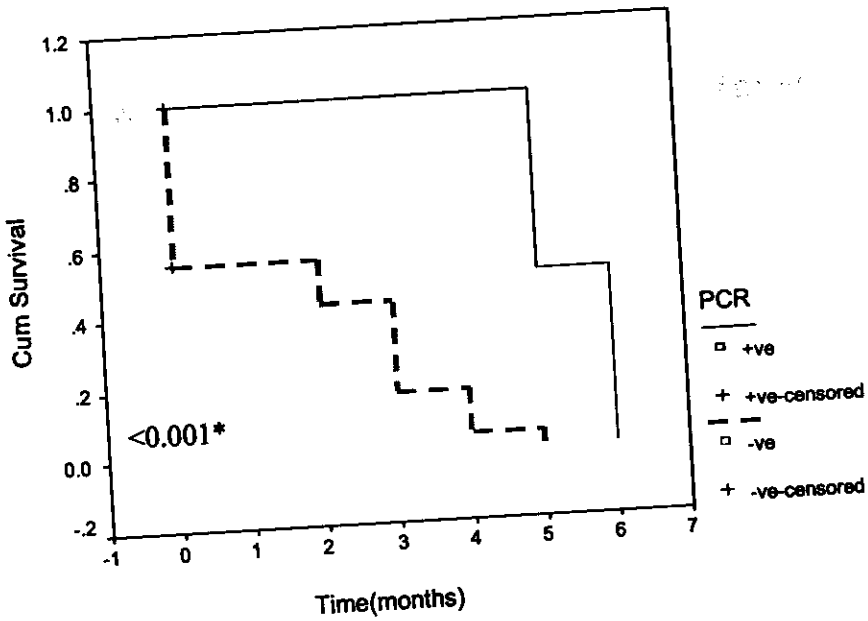


Fig (26): Kaplan-Meier analysis of disease free survival in cases with abnormalities in t(8;21),t(15;17) and inv(16) in studied groups showed significant increase in DFS in these patients (P<0.001)

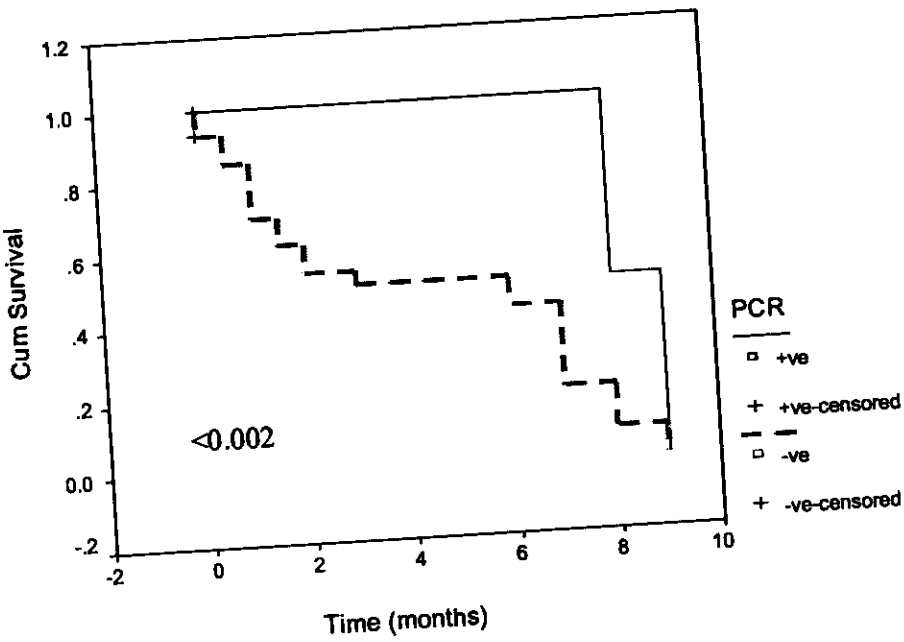
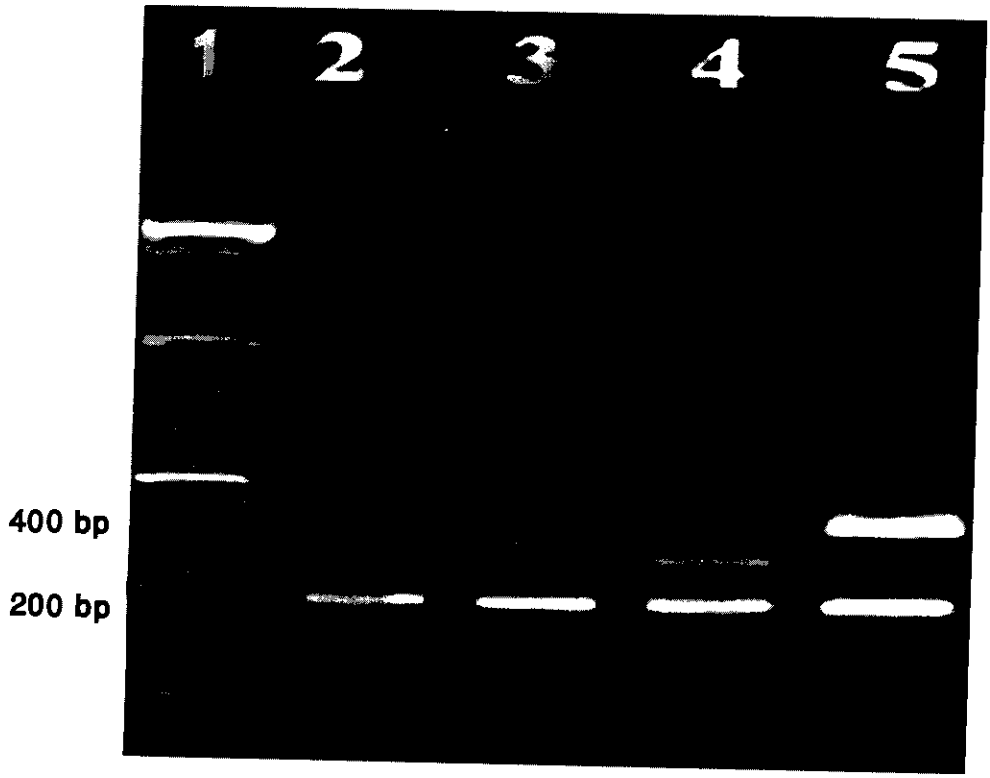
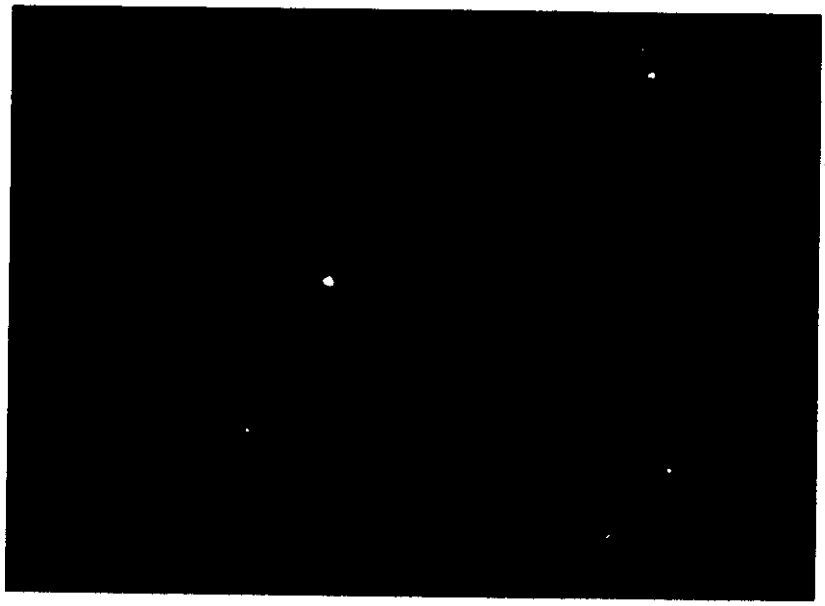


Fig.27: Kaplan-Meier analysis of event free survival in cases with abnormalities of t(8;21),t(15;17) and inv(16) in studied groups showed significant increase in EFS in these patients (P<0.002)



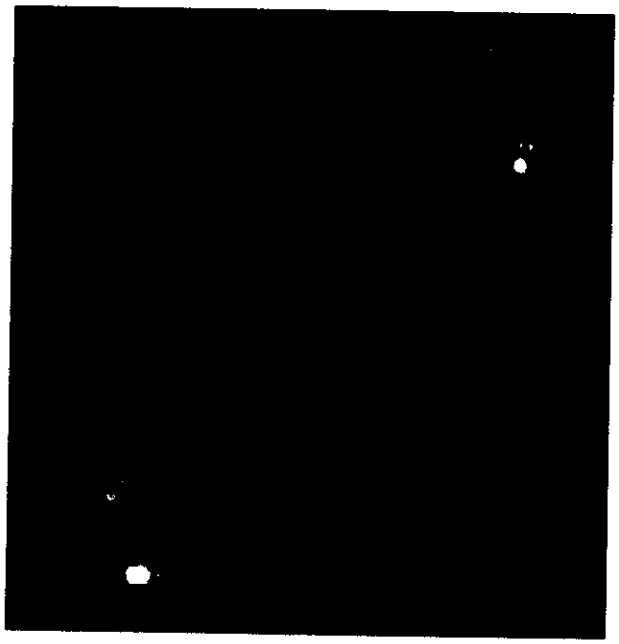
- ***Photograph 1.*Agarose gel analysis of fusion transcripts detected by the multiplex RT-PCR. In the Panel:**

- *Lane 1* is 100-bp DNA marker.
- *Lane 2* is known negative control from healthy individual.
- *Lane 3, 4, and 5* are positive for AML-ETO (395 bp), PML-RARA (381 bp), and CBFB-MYH11 (418 bp), from bone marrow samples respectivley.
- Internal positive controls GAPDH (226 bp) are demonstrated in all lanes

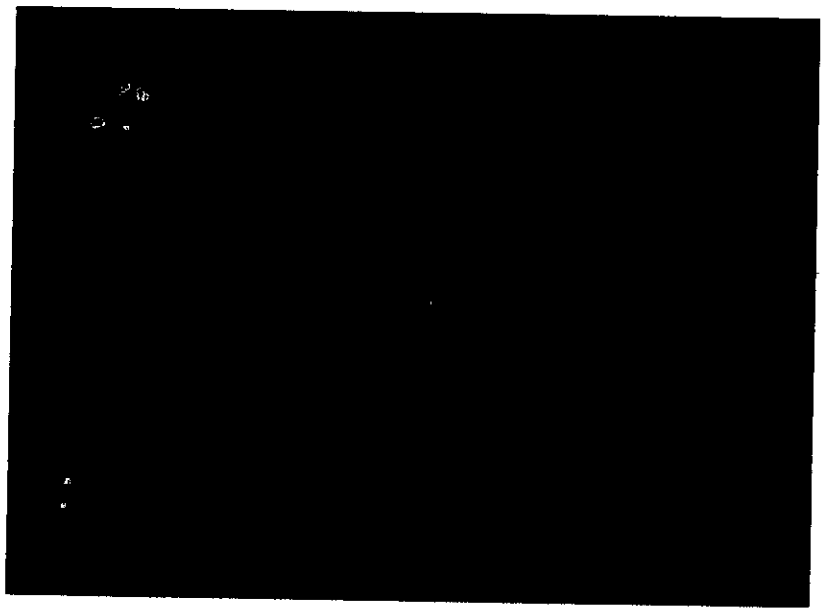


Photograph 2: Fluorescence in situ hybridization (FISH) analysis of bone marrow cells using the AML1/ETO Dual Color, Dual Fusion Translocation Probe showing cell containing the AML1/ETO fusion gene, one orange (ETO), one green (AML1), and two orange/green (yellow) fusion signals are observed.

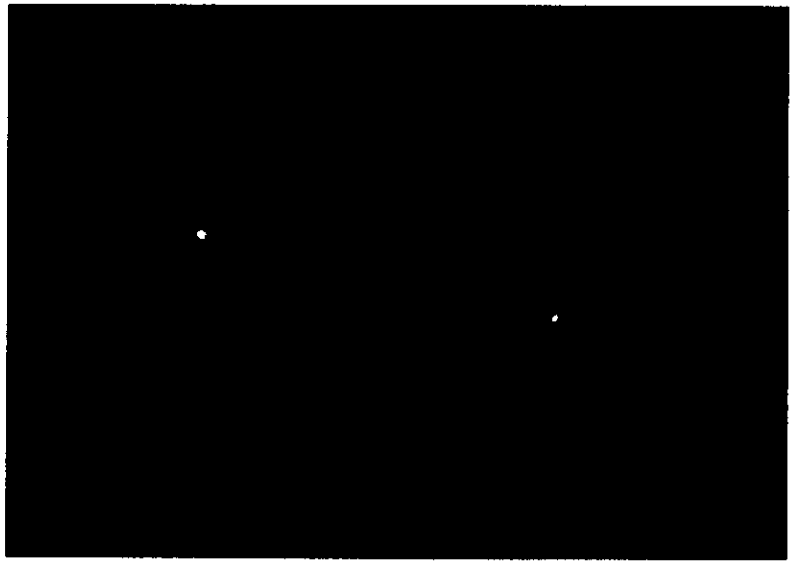
Photograph 3: Fluorescence in situ hybridization (FISH) analysis of bone marrow cells using the AML1/ETO Dual Color, Dual Fusion Translocation Probe showing a normal interphase and metaphase cells, two orange signals representing two normal copies of ETO genes and two green signals representing two normal copies of AML1 genes are observed.



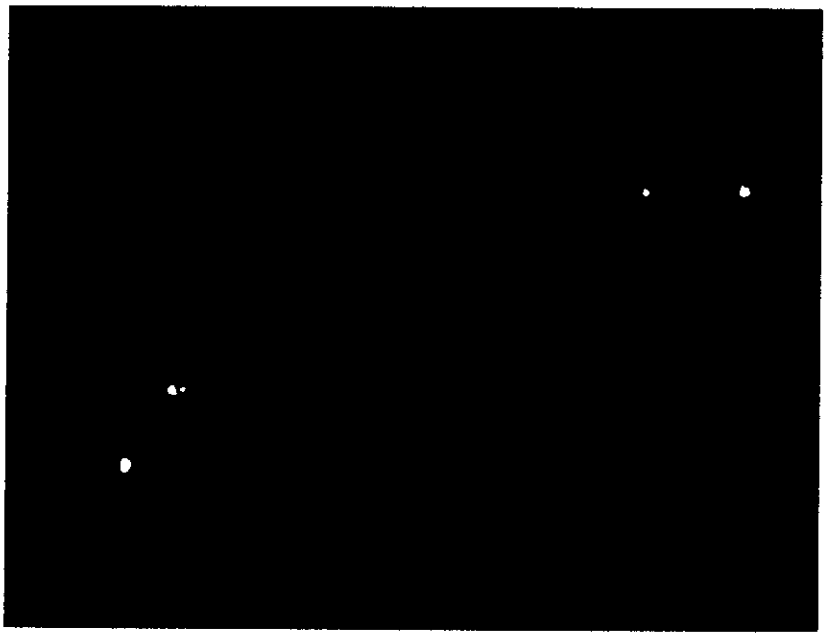
Photograph 4: Fluorescence in situ hybridization (FISH) analysis of bone marrow cells using the PML/RARA Dual Color Translocation Probe showing an abnormal cell containing a PML/RARA fusion, the one green (RARA), one orange (PML), and fused green/orange (yellow) signal pattern (1O1G1F) is observed.



Photograph 5: Fluorescence in situ hybridization (FISH) analysis of bone marrow cells using the PML/RARA Dual Color Translocation Probe showing a normal interphase cells with two orange and two green (2O2G) signal pattern.



Photograph 6: Fluorescence in situ hybridization (FISH) analysis of bone marrow cells using the CBFB Dual Color, Break Apart Probe, breakapart probe showing a interphase nuclei containing an inv(16) results in separate red and green signals appearing on opposite arms of the inverted 16 chromosome, and one fusion signal.



Photograph 7: Fluorescence in situ hybridization (FISH) analysis of bone marrow cells using the CBFB Dual Color, Break Apart Probe, breakapart probe showing interphase nuclei lacking inv(16) with two fused red/green (yellow) signals (2F).