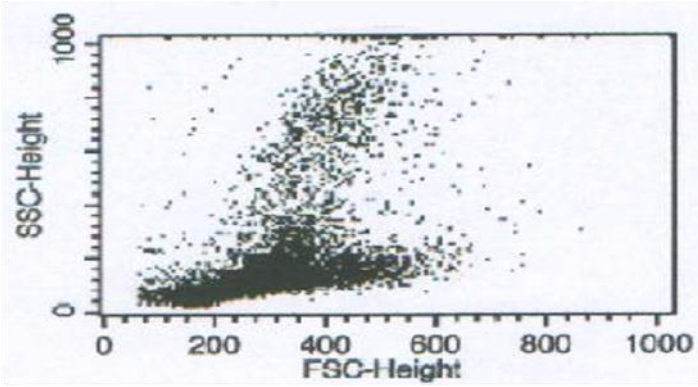
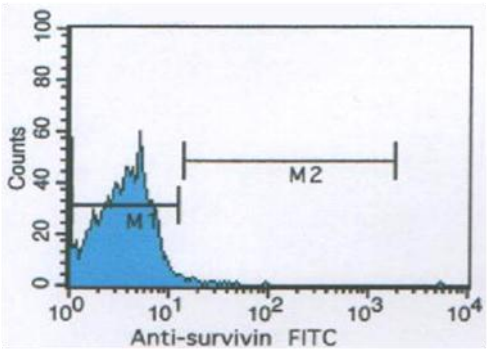
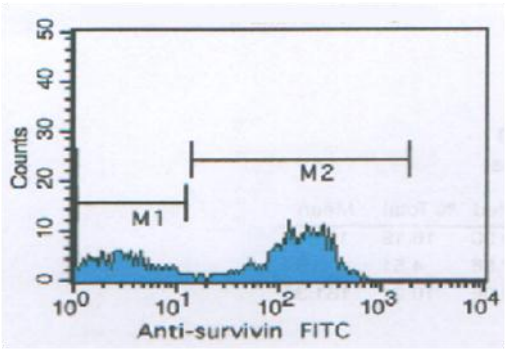


Histogram I

Figure (16): shows side scatter versus forward scatter of the mononuclear cells (Blasts) of a case of ALL; the blast cells reside in the lower and left side of the Chart and extend slightly to the right as the blast cells were small and a granular.



Survivin expression Positive



Survivin expressiom Negative

Histogram II

Figure (17):1- shows histograms of the expression of the anti-survivin of two cases of ALL; in the first case, the survivin expression was positive and in the second case, the survivin expression was negative.

2-M1 refers to survivin MFI control.

3- M2: refers to survivin MFI of the measured sample.

Statistical Analysis

Data were entered, checked and analyzed using SPSS version 10.0 (Statistical Package for the Social Sciences, Chicago, IL). Data were expressed as number and percentage for qualitative variables, mean (standard deviation (SD) for quantitative ones. For normal distributed data, student't' test was used for calculating the difference between two groups only and ANOVA (F) test were used for comparing three or more groups. The Mann-Whitney (U) test was used for statistical comparisons of survivin expression and MFI between two groups of patients and Kruskal-Wallis test (K) used between more than three groups of patients with non-parametric data.

Chi-square test $\chi 2$ tests, Fisher exact tests were used in the comparison of the groups for the categorical variables. Sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV) and accuracy were calculated.

Spearman rank correlation was used as appropriate for analysis of results. The accepted level of significance in this study was 0.05 i.e ($P<0.05$).

- Sensitivity: Proportion of persons with condition who test positive.

$$\text{Sensitivity} = = \frac{\text{True positive}}{\text{True positive+ false negative}} \times 100$$

- **Specificity:** Proportion of persons without condition who test negative.

$$\text{Specificity} = \frac{\text{True negatives}}{\text{True negatives + false positives}} \times 100$$

- **Positive Predictive Value (PPV):** Proportion of persons with positive test who have condition.

$$PPV = \frac{\text{True positives}}{\text{True positives + false positives}} \times 100$$

- **Negative Predictive Value (NPV):** Proportion of persons with negative test who do not have condition.

$$NPV = \frac{\text{True negatives}}{\text{True negatives + false negatives}} \times 100$$

Results

Survivin is a unique member of IAP family, plays role in regulating both apoptosis and cell division. Over expression of survivin is associated with increased risk of recurrence and poor outcome in cancer patients **(Zhongguo et al., 2006)**.

The aim of this study was to assess survivin protein expression levels with regard to the potential role of this protein as a prognostic marker in precursor B-ALL, the most common childhood malignancy .

The present study involved 42 children with Acute Lymphoblastic Leukemia grouped as the following:

Group I: (Denovo group) 35 newly diagnosed children with ALL. Their age ranged from (3-16) years.

Group II: (Relapsed group) 7 unrelated children with acute lymphoblastic leukemia were in relapse. Their age ranged from (5-13) years.

Group III: (Control group**)** 15 normal children served as a control group. Their age ranged from (1.5-12) years.

All individuals were investigated to estimate survivin level for assessment of the prognostic significance of survivin in pediatric Acute Lymphoblastic Leukemia. The results were summarized in the following tables (from table 7 to table 18) and figures (from figure 18 to figure 26).

Table (7): Demographic data (age &sex) of the studied groups:

Demographic data	**Group I (Denovo) (35)**		**Group II (Relapsed) (7)**		**Group III (Control) (15)**		**Test of Significance (F) test**	**P value**
Age/y(mean±SD)	6.3±3.8		8.1±2.7		6.7±3.8		0.7	>0.05
Demographic data	**Group I (Denovo) (35)**		**Group II (Relapsed) (7)**		**Group III (Control) (15)**		**Test of Significance (X^2) test**	**P value**
Gender	**No**	**%**	**No**	**%**	**No**	**%**		
Male	22	63%	4	57%	8	53%	0.4	>0.05
Female	13	37%	3	43%	7	47%		

P > 0.05: not significant

This table shows no significant difference between all groups (de novo, Relapsed and control groups) as regard of age and gender. But we noticed that ALL is higher in males than females.

Table (8): Clinical data of the patients groups.

Clinical data	Group I (DeNovo)		Group II (Relapsed)		P value	Test of Significance (Z) test
	No	%	No	%		
Pallor + ve	35	100%	7	100%	----	----
Fever + ve	28	80%	5	71%	>0.05	0.5
Bleeding tendency + ve	21	60%	6	86%	>0.05	1.3
Lymph nodes + ve	27	77%	5	71%	>0.05	0.3
Hepatosplenomegaly + ve	26	74%	5	71%	>0.05	0.2
CNS manifestation + ve	1	3%	0	0%	>0.05	0.5
Mediastinal mass + ve	4	11%	0	0%	>0.05	0.9
Bone pain + ve	16	46%	2	29%	>0.05	0.8

P>0.05= not significant

This table shows no significant difference between both de novo and relapsed groups as regard the absence or presence of clinical data (pallor, fever**,** bleeding tendency, lymph nodes, hepatosplenomegaly, CNS manifestation, mediastinal masses and bone pain).

Table (9): Laboratory findings of the patients groups:

Laboratory data	Group I (Denovo) (35)	Group II (Relapsed) (7)	Group III (Control) (15)	Test of significance (F) test	p value
TLC ($\times 10^9$/l)	47.4±34.9	40.1±37.5	6.6±1.3	9.5	<0.001**
Hb (g/dl)	7.5±1.8	8.1±1.8	12.2±0.9	44.5	<0.001**
Platelets ($\times 10^9$/l)	34.3±30.8	32.7±16.4	227.8±111.7	174.4	<0.001**
Laboratory data	**Group I (Denovo) (35)**	**Group II (Relapsed) (7)**	**Group III (Control) (15)**	**Test of significance (t) test**	**p value**
% of Blasts in PB	68.8±17.6	70±16.4	-	0.2	>0.05
% of Blasts in BM	79.1±20.9	87.3±10.2	-	1.01	>0.05

****= Highly Significant PB: Peripheral Blood BM: Bone Marrow**

LSD: Least significance difference of ANOVA:-

From the above table, it can be detected the least significant difference among those 3 groups.

	Denovo-group	Relapsed-group	Control-group
Denovo-group	---------	>0.05	<0.001**
Relapsed-group	>0.05	----------	<0.001**
Control-group	<0.001**	<0.001**	----------

**= Highly Significant

♦ This table is obtained from the previous table to show LSD among studied groups.

♦ There is highly significant difference between (denovo and control group), also between relapsed and control group regard as (TLC, Hb, platelets). TLC is highly elevated in both denovo and relapsed group than control group; hemoglobin and platelets are significantly decreased in both denovo and Relapsed groups than control group.

♦ But there is no significant difference between both denovo and relapsed group as regard as % of blast in both peripheral blood and bone marrow.

Table (10): Frequency of FAB and immunophenotyping subtypes in patient groups.

		Group I (Denovo ALL)	%	Group II (Relapsed ALL)	%
FAB	**L1**	19	54.3	5	71.4
	L2	14	40	2	28.6
	L3	2	5.7	0	0
Immunophenotyping					
Pro B-ALL		2	5.7	1	14.3
C-ALL		21	60	2	28.6
Pre B-ALL		7	20	3	42.8
MatureALL(B-ALL)		5	14.3	1	14.3

This table shows that:

♦ According to FAB subtypes, it shows that L1 is highly frequent in de novo and relapsed groups.

♦ According to immunophenotyping subtypes, it shows that c-ALL is highly frequent in de novo group. But a pre-B subtype is highly frequent in relapsed group.

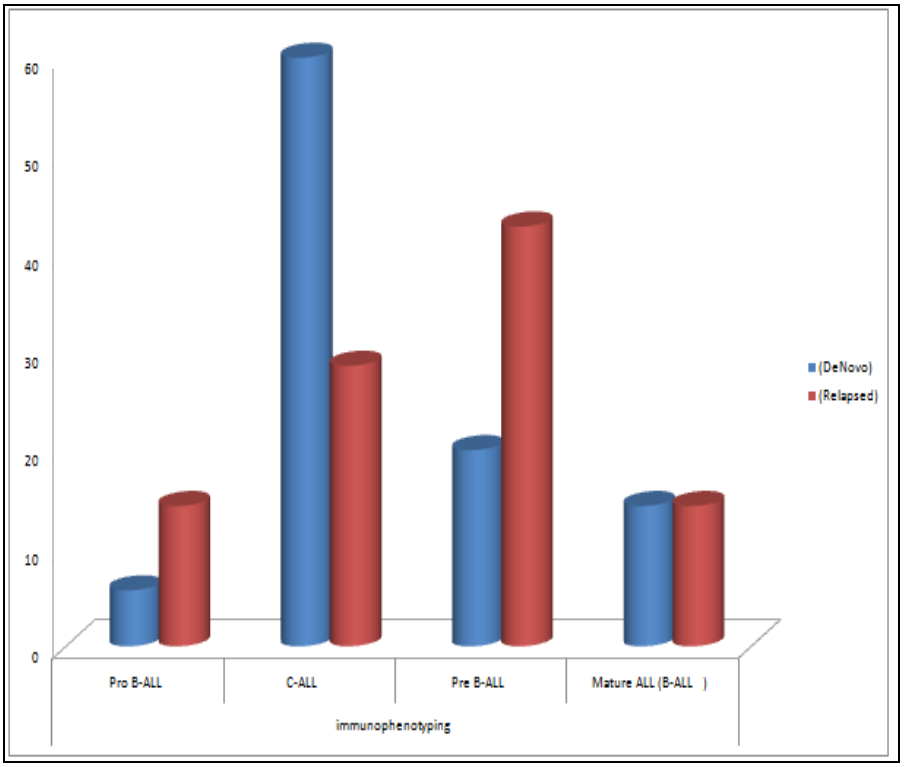


Figure (18): Immunophenotyping classification in both de-novo and relapsed groups.

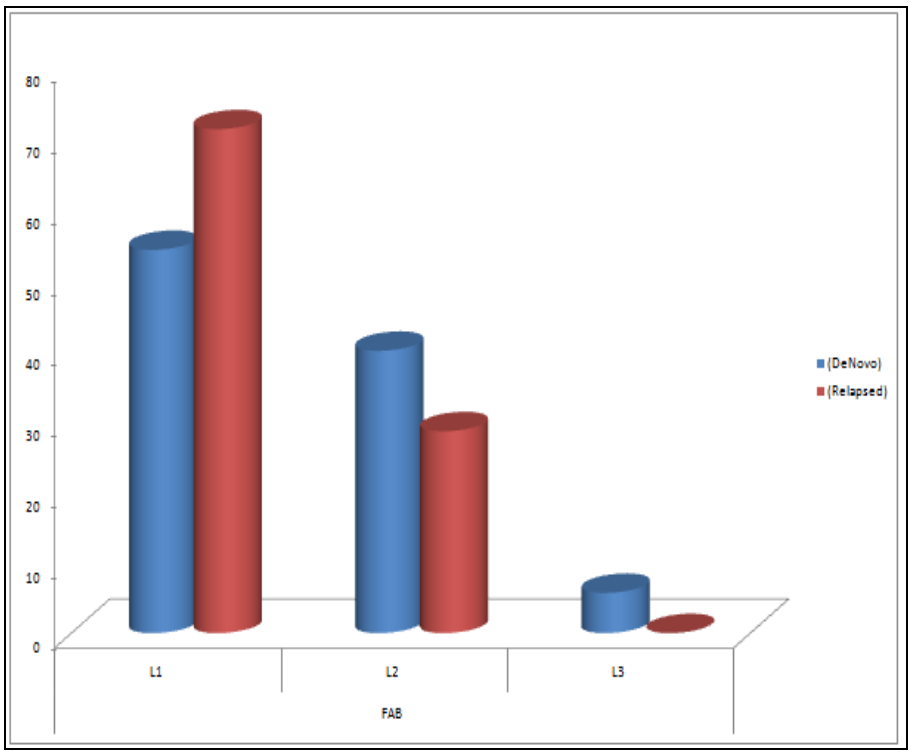


Figure (19): FAB classification in both de novo and relapsed ALL group.

Table (11): Survivin MFI in the studied groups:

Clinical data	Group I (DeNovo) (35)	Group II (Relapsed) (7)	Group III (Control) (15)	Test of significance (F) test	P value
MFI mean ±SD	119.82±192.7	275.4±93.8	19.7±8.1	6.3	<0.05*

***= Significant P<0.05= significant**

LSD: Least significance difference of ANOVA:-

	Denovo-group	Relapsed-group	Control-group
Denovo-group	---------	>0.05	<0.05*
Relapsed-group	>0.05	----------	<0.05*
Control-group	<0.05*	<0.05*	----------

*= Significant

This table is obtained from the previous table to show LSD among studied groups.

♦ This table shows that there is significant difference of survivin MFI between denovo group and control group.

♦ It shows that there is significant difference of survivin MFI of relapsed group and control group.

♦ It shows that there is no significant difference of survivin MFI of relapsed group and denovo group.

Table (12): Survivin expression in the studied groups.

Survivin expression	Group I (DeNovo) (35)		Group II (Relapsed) (7)		Group III (Control) (15)		Test of Significance (Z) test	P value
	No	%	No	%	No	%		
Positive cases (16)	11	31%	5	71%	0	0%	Z1=1. 98 Z2=2.5 Z3=3.7	0.002**
Negative cases (41)	24	69%	2	29%	15	100%		

Positive ≥Cut off **Cut off= mean +2SD=12.7** ****= Highly Significance**

Z1 between Group I & Group II
Z2 between Group I & Group III
Z3 between Group II & Group III

This table shows highly significant difference among all groups. As survivin expression is higher in relapsed group than denovo group and completely absent in control group.

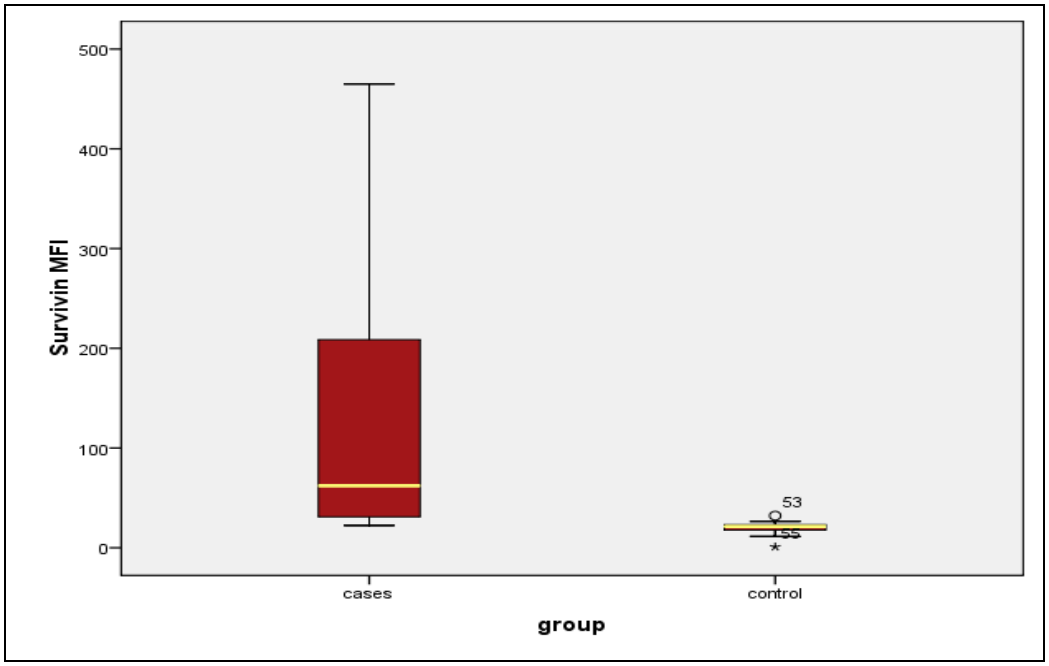


Figure (20): shows survivin MFI among ALL patients (denovo and relapse) in comparison to control.

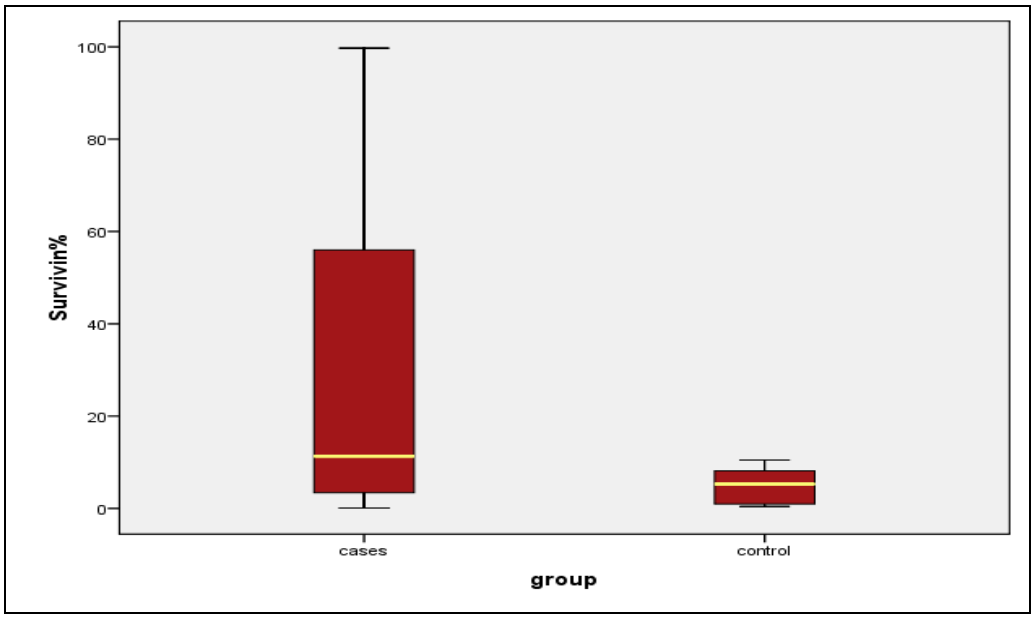


Figure (21): shows of survivin expression among ALL patients (denovo and relapse) in comparison to control.

Table (13): Distribution of Survivin expression in the patient groups (denovo and relapsed) as regard as age and sex.

		No.	Survivin expression		Test of significance (Z)test
			+ve	-ve	
Age	≤ 10/y	35	12	23	<0.05*
	>10/y	7	3	4	>0.05
sex	Male	26	11	15	>0.05
	Female	16	4	12	>0.05

Positive ≥Cut off **Cut off= mean +2SD=12.6** ***= Significance**

♦ This table shows significant decrease in survivin expression in patients under the age of ten years. But there is no significant difference between Survivin expression and older patients.

♦ This table shows no significant difference between survivin expression and sex.

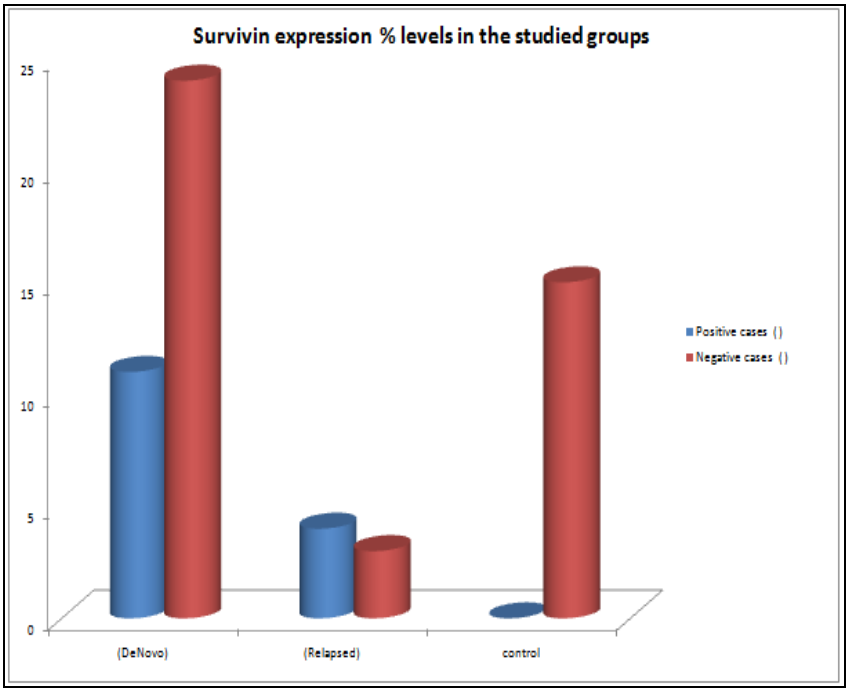


Figure (22): Comparisons between positive and negative survivin expression studied groups.

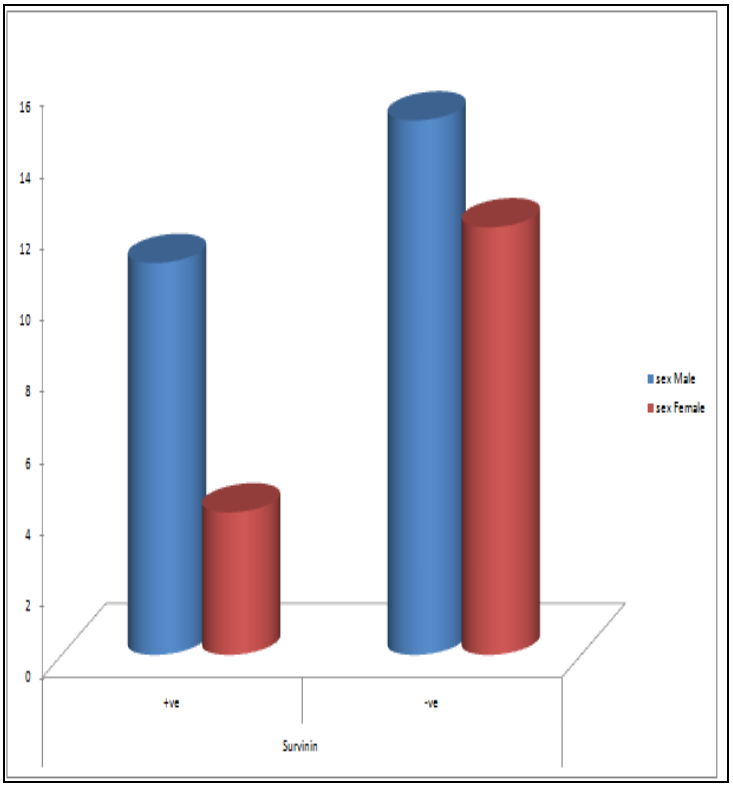


Figure (23): Illustrates the difference between positive and negative survivin expression regard the sex of patients (denovo and relapse cases).

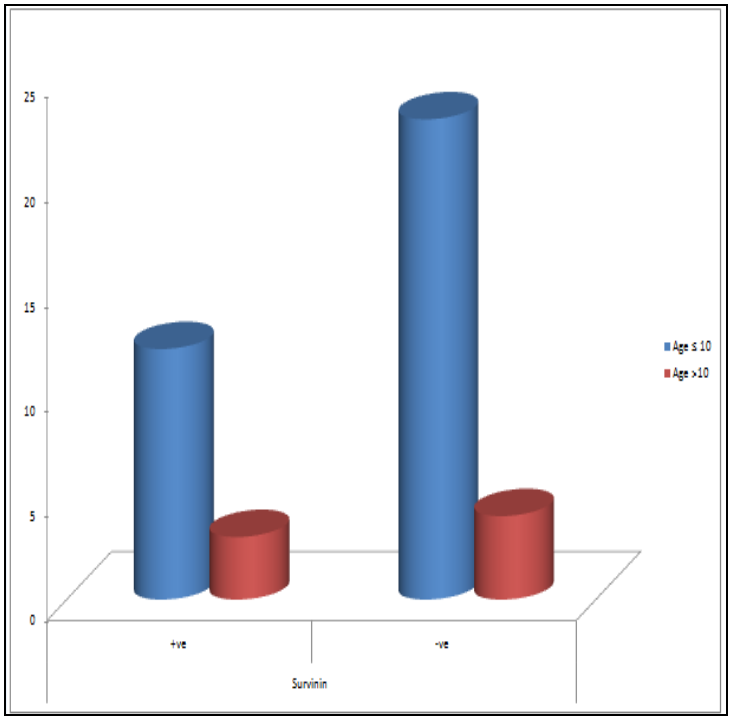


Figure (24): Illustrates the difference between positive and negative expression and ages of the ALL patients (denovo and relapsecases).

Table (14): Comparison of survivin expression and laboratory data in Denovo group:

Parameters	Survivin expression			mean±SD	Test of significance (U)	P value
	No	+ve	-ve			
TLC ($\times10^9$/l) >50	14	3	11	31.23±38.79	143	>0.05
<50	21	8	13	21.27±31.7		
Hb(g/dl) <10	30	8	22	23.26±32.7	152	>0.05
>10	5	3	2	53.12±49.04		
Platelets ($\times10^9$/l) <30	16	6	10	37.5±39.9	131	>0.05
>30	19	4	15	19.2±31.2		
FAB	**No**	**+ve**	**-ve**	**Test of significance (X^2)**		**P value**
L1	19	4	15	0.3		>0.05
L2	14	6	8			
L3	2	1	1			
Immunophenotyping	**No**	**+ve**	**-ve**	**Test of significance (X^2)**		**P value**
Pro-B ALL	2	>0.05	1	3.3		>0.05
C-ALL	21		15			
Pre-B ALL	7	3	4			
Mature ALL	5	1	4			

P>0.05= not significant

This table shows that there is no significant association between the survivin expression and laboratory data (TLC, Hb, FAB, immunophenotyping and platelets) in de-novo group.

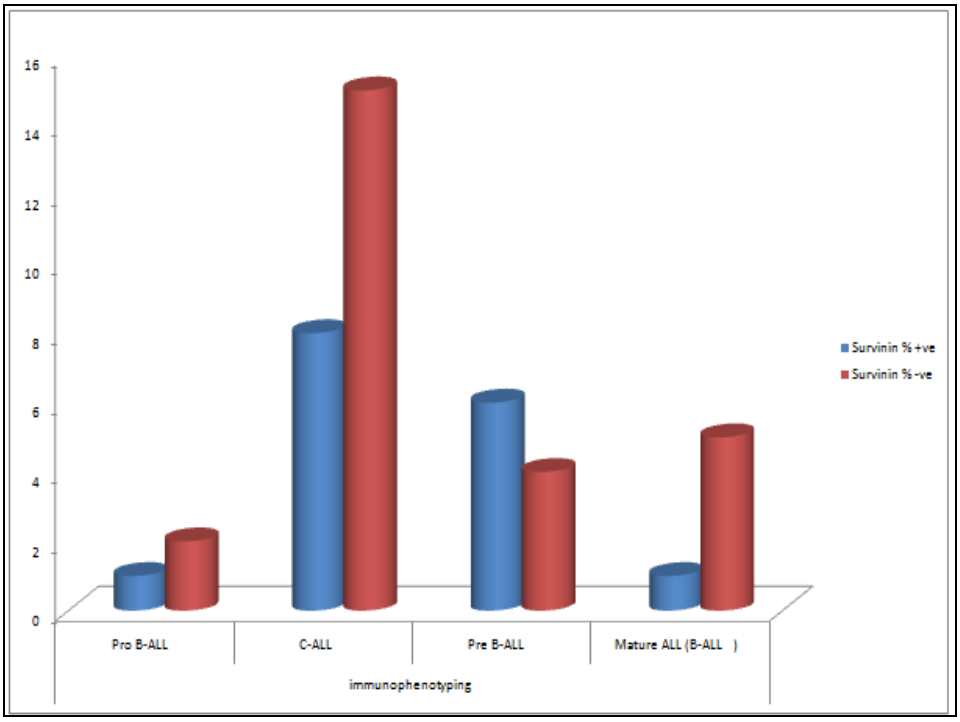


Figure (25): Illustrates the difference between positive and negative survivin expression and Immunophenotyping subtypes in both denovo and relapsed cases.

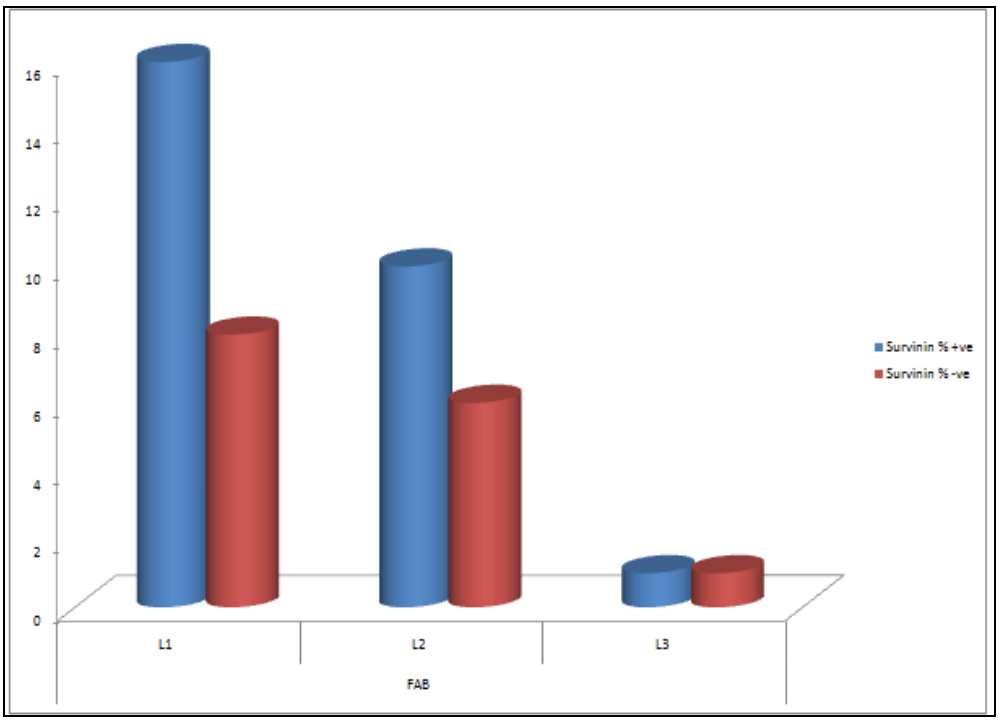


Figure (26): Illustrates the difference between positive and negative survivin expression and FAB subtypes in both denovo and relapsed cases.

Table (15): Comparison of the survivin MFI and laboratory data in Denovo cases.

Parameters		Survivin MFI Median	Range	Test of significance (U)	p -value
TLC ($\times 10^9$/l)	**>50**	42.1	(22.4-1071)	121.5	>0.05
	<50	51.8	(12.5-434.5)		
Hb (g/dl)	**<10**	46.8	(12.5-279.5)	95.5	>0.05
	>10	231	(25.7-1071)		
Platelets ($\times 10^9$/l)	**<30**	50.1	(22.4-1071)	142.5	>0.05
	>30	45.2	(12.5-434.5)		
FAB		**Median**	**Range**	**Test of significance (K)**	**p -value**
L1		62.2	(12.5-208.9)	1.14	>0.05
L2		42.05	(22.7-1071)		
L3		25.5	(48.3-279.5)		
Immunophenotyping		**Median**	**Range**	**Test of significance (K)**	**p -value**
Pro-B ALL		67.4	(34.-100.6)	0.65	>0.05
C-ALL		51.8	(12.5-208.9)		
Pre-B ALL		100.8	(25.7-1071)		
Mature ALL		38.9	(23.2-279.5)		

P>0.05= not significant

This table shows that there is no significant relation between survivin MFI and laboratory data (TLC, Hb, Platelets, FAB and immunophenotyping) in de-novo ALL groups.

Table (16): Correlation between survivin MFI, expression and some prognostic criteria in denovo cases.

Prognostic criteria	Survivin MFI		Survivin expression	
	Test of significance		Test of significance	
	(r)test	P value	(r)test	P value
Age(years)	0.21	>0.05	-0.1	>0.05
TLC($\times 10^9$/l)	0.18	>0.05	-0.24	>0.05
Hb(g/dl)	-0.2	>0.05	-0.2	>0.05
FAB	0.1	>0.05	0.2	>0.05
Immunophenotyping	0.17	>0.05	-0.22	>0.05
CNS manifestations	0.06	>0.05	0.33	>0.05

This table shows that there is no significant correlation between survivin (MFI & %) and some prognostic criteria as (Age, TLC, Hemoglobin, FAB, Immunophenotyping and CNS manifestations) in de-novo group.

Table (17): Association between Survivin expression (percentage and MFI) and response to therapy in denovo patients:

Clinical outcome **Survivin expression**	**Remission (n=26)**	**Resistance (n=7)**	**Dead (n=2)**	**Test of significance (Z) test**	**P value**
+ve **Survivin**	6(23%)	2(28.6%)	2(100%)	Z1=1.383 Z2=0.597 Z3=1.721	>0.05
-ve	20(77%)	5(71.4%)	0(0%)		
Clinical outcome **Survivin MFI**	**Remission (n=26)**	**Resistance (n=7)**	**Dead (n=2)**	**Test of significance (F)**	**p-value**
Mean ±SD	96.58 ±100.2[a]	221.3±385.4 [b]	55.3±9.8 [c]	0.3	<0.05*

*= Significant

Z1= difference between Remission and Resistance.
Z2= difference between Resistance and Dead.
Z3= difference between Remission and Dead.

a = difference between Remission and Resistance ($p<0.05$).
b = difference between Resistance and Dead ($p<0.05$).
c = difference between Remission and Dead ($p<0.05$).

- This table shows that there is no significant difference in Survivin expression between ALL patients according to their clinical outcome.
- But it shows that there is a significant difference in the MFI of survivin between ALL patients according to their clinical outcome.