Assessment of Livin Expression in Childhood B-lineage Acute Lymphoblastic Leukemia Patients

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ABSTRACT
Background: When it comes to childhood acute lymphoblastic leukemia (ALL) patients, Livin is related with good prognostic characteristics.
Objective: The aim of the work was to assess livin expression in childhood B-lineage acute lymphoblastic leukemia to determine its significance.
Patients and Methods: From September 2018 and September 2019, at Zagazig University Hospitals Clinical Pathology and Pediatric departments, our case control trial was conducted on 50 subjects, patients' group (25 cases of newly diagnosed ALL) and control group (25 healthy children). Assessment of livin expression was done using real time polymerase chain reaction (PCR).
Results: There was statistically significant difference between the age of livin positive and livin negative cases (the mean age of livin positive cases was 6.2±3 years while the mean age of livin negative cases was 12.3±1.4 years). Mean livin expression level was significantly higher in age group < 10 than in age group group ≥ 10 years (294.53± 154.03 in age group <10 years versus 31 ±12.37 in age group ≥ 10). In livin negative group there were 3 patients with leucocytic count< 50.000/mm3 at diagnosis while 5 patients with leucocytic count ≥ 50.000/mm3 or more at diagnosis compared to 14 and 3 patients among livin positive group respectively (p=0.025). Regarding age, sex, WBCs, and risk stratification, there were statistically significant differences between the livin negative and positive groups.
Conclusion: It could be concluded that livin was associated with favorable prognostic factors among ALL patients: (age<10 years, female patients, WBCs <50,000/mm3).
Keywords: Livin expression, Acute Lymphoblastic Leukemia.

INTRODUCTION

Around 80% of all pediatric leukemias are acute lymphoblastic leukemia (ALL), making it the most frequent form of childhood cancer [1].

Apoptosis is a self-sustaining process in which a large number of genes are activated, expressed, and regulated to cause programmed cell death in order to eliminate undesirable or defective cells [2].

There is a class of apoptotic regulators, known as Inhibitor apoptotic Proteins (IAPs), that promote cancer cells' resistance to apoptosis, notably in cancer treatment and chemotherapy. In order to reestablish the apoptotic response to proapoptotic stimuli, disrupting the binding of IAPs to their functional partners is a viable method [3].

Cell cycle and proliferation are affected as well as apoptosis when Livin/ML-IAP/BIRC7 is present, making it an important player in these processes. Livin, which is over-expressed in a number of cancers, has an anti-apoptotic effect mediated by the direct suppression of caspase 3, but also caspases 7 and 9 as well as DIABLO [4].

Different isoforms of livin may play different functions in apoptosis. Truncated proteins are formed when Livin isoforms are cleaved. To be effective, the shortened form of the protein, known as livin (t- livin), must also have the ability to cause cell death. Thus, livin has both the ability to shield csll cells from cell death and the ability to promote it once it is cleaved [5].

More specifically, livin was found to be related with positive prognostic characteristics in children with acute lymphoblastic leukemia (ALL) [6].

It was the goal of this study to assess the significance of livin expression in childhood B -lineage acute lymphoblastic leukemia.

PATIENTS AND METHODS

This case-control trial study included a total of 25 cases of newly diagnosed All and 25 healthy children served as control, attending at Departments of Clinical Pathology and Pediatrics, Zagazig University Hospitals, Egypt. This study was conducted between July 2020 to July 2021.

The included 50 subjects were divided into two groups; **Control Group:** Included 25 apparently healthy children with mean age 9.1±3.6 years; 60% of them were males (15 males) and 40% of them were females (10 females), and **Case Group:** Included 25 newly diagnosed ALL patients who attended the Pediatric Hematology and Oncology unit with mean age 8.1±3.9 years; (56%) of them were males (14 males), and (44%) of them were females (11females).

*Ethical Consideration***:**
This study was ethically approved by Zagazig University's research ethics committee. Written informed consent of all the participants' parents was

obtained and submitted them to Zagazig University (ZU-IRB#3598). We adhered to the Helsinki Declaration, the ethical norm of the World Medical Association for human testing.

Inclusion Criteria: De novo cases of ALL (didn't receive any chemotherapy before), and age: < 18 years, of both sexes.

Exclusion Criteria: Cases having malignant disorder other than acute lymphoblastic leukemia, and patients exhibiting an immunophenotype of T- cell acute lymphoblastic leukemia.

Patients were classified according to their risk into standard risk and high risk based on age and WBC count at diagnosis: Standard risk: age from 1 to less than 10 years, WBC less than 50×10^9/L. High risk: age less than 1 year or more than 10 years, WBC more than 50×10^9/L.

All participants were subjected to history taking, clinical examination and laboratory assessment of livin expression using real time polymerase chain reaction (PCR).

Statistical analysis

The collected data were coded, processed and analyzed using the SPSS (Statistical Package for Social Sciences) version 22 for Windows® (IBM SPSS Inc, Chicago, IL, USA). Numbers and percentages are used to represent data (percent) or mean ± SD. Different qualitative factors were examined using the Chi square (X2) test. If the significant probability was less than 0.05, the threshold for statistical significance, the results were considered statistically significant and highly significant. P value < 0.05 was considered significant.

RESULTS

(Table 1) shows demographic and clinical characteristics of control and case groups, with significant statistically differences between them regarding lymphadenopathy, splenomegaly and hepatomegaly.

(Table 2) shows mean ± SD of CBC characteristics of control and case groups with statistically highly significant differences as regard Hemoglobin (HB), white blood cells (WBC) and platelets (PLT).

(Table 3) shows mean ± SD of liver, kidney functions and livin in control and cases. No statistically significant differences between cases and controls as regard any of the tests except LDH and livin which means show highly significant increase in case than control (P<0.001).

(Table 4) shows there was statistically significant increase in livin negative than positive cases regarding mean age, statistically significant differences between both groups regarding age and FAB distribution and risk stratification, while no significant differences regarding other items.

(Table 5) shows relation between livin expression and laboratory findings: As regard WBC there was statistically significant difference between livin +ve cases and livin –ve cases: in livin –ve cases 3 patients presented with WBCs <50.000/mm^3 while 5 patients presented with WBCs ≥50.000/mm^3 compared to 14 and 3 among livin +ve cases respectively (p=0.025). A significant difference existed between the two groupings in terms of BM blast (P=0.05). Livin +ve and -ve patients had no statistically significant differences with regard to the following: Hb, PLT; PT; PTT; SGOT; SGPT; SGOT; SGPT; Total bilirubin; TP; Albumin; Urea; LDH; creatinine; and peripheral blast.

(Table 6) shows that mean livin expression was significantly higher in age group < 10 years than those who are > 10 years (p <0.001). Mean livin expression was higher in patients with Pre-B ALL than those of mature progenitor B-ALL< and also significantly higher in standard risk group than high risk group (P=0.01).

Table (1): Demographic and clinical characteristics of control and cases:

Variables	Control		Case		Test***	P value
	N=25		N=25			
Age (years) (mean±SD)	9.10±3.60		8.10±3.90		-0.862**	0.393
Sex						
▪ Male	14	56.0%	15	60.0%	0.082	1.00
▪ Female	11	44.0%	10	40.0%		
Lymphadenopathy						
▪ Negative	2	8.0%	25	100.0%	42.593	<0.001*
▪ Positive	23	92.0%	0	0.0%		
Splenomegaly						
▪ Negative	3	12.0%	25	100.0%	39.28	<0.001*
▪ Positive	22	88.0%	0	0%		
Hepatomegaly						
▪ Negative	4	16.0%	25	100.0%	36.21	<0.001*
▪ Positive	21	84.0%	0	0.0%		

**independent sample t test ***Chi square test

Table (2): Complete Blood Count of control and cases:

Variables(mean±SD)	Control		Case		Test	P value
	N=25		N=25			
▪ HB (g/dl)	12.2±0.5		8.5±1.7		-10.641**	<0.001*
▪ WBC (x10³ /mm³)	8.2±1.6		37.6±28.1		-4.134***	<0.001*
WBC						
▪ <50.000/mm³	25	59.5%	17	40.5%	9.524	0.004*
▪ ≥50.000/mm³	0	0.0%	8	100.0%		
	Mean ± SD		Mean ± SD			
▪ PLT: (x10³/mm³)	300.0±69		61.0±69.0		-5.620***	<0.001*

Table (3): Comparison between controls and cases regarding laboratory findings

Variable (mean±SD)	Control N=25	Case N=25	Test	P value
Total bilirubin (mg/dl)	0.8±0.2	0.8±0.2	-0.091***	0.928
Direct bilirubin(mg/dl)	0.30±0.08	0.30±0.09	-0.093***	0.926
Total Protein(g/dl)	7.0±0.7	6.6±0.7	-1.747***	0.087
Albumin(g/dl)	4.0±0.6	3.9±0.6	-0.817**	0.418
SGOT (U/L)	26.2±4.3	28.6±4.5	1.029**	0.309
SGPT(U/L)	28.5±5.5	29.9±4.2	1.029**	0.309
PT (second)	12.0±0.6	12.6±0.8	-1.174***	0.241
PTT (second)	30±3.0	29.0±3	-0.980**	0.332
LDH (U/L)	158±17	310±37	-6.071***	<0.001*
Creatinine (mg/dl)	0.39±0.08	0.40±0.07	-0.675***	0.500
Urea(mg/dl)	14.5±3.3	13.6±3.4	-0.963**	0.335
Livin	179.31±15.66	0.054±0.01	-6.065***	<0.001*

Table (4): Relation between livin expression and Demographic & clinicopathological characteristics among livin negative and positive subgroups

Variable	Livin Expression				Test**	P value
	Negative		Positive			
	N=8	%	N=17	%		
Age						
▪ <10	0	0.0%	15	100.0%	17.64	<0.001*
▪ ≥10	8	80.0%	2	20.0%		
Sex						
▪ Male	7	50.0%	7	50.0%	4.723	0.042*
▪ Female	1	9.1%	10	90.9%		
Lymphadenopathy						
▪ Negative	0	0.0%	2	100.0%	1.023	1.00
▪ Positive	8	34.8%	15	65.2%		
Splenomegaly						
▪ Negative	1	33.3%	2	66.7%	0.003	1.00
▪ Positive	7	31.8%	15	68.2%		
Hepatomegaly						
▪ Negative	1	25.0%	3	75.0%	0.107	1.00
▪ Positive	7	33.3%	14	66.7%		
CNS Infiltration						
▪ Negative	7	29.2%	17	70.8%	2.214	0.320
▪ Positive	1	100.0%	0	0.0%		
Immunophenotyping						
▪ Pre-B –ALL	4	21.1%	15	78.9%	4.360	0.059
▪ mature &progenetor B-ALL	4	66.7%	2	33.3%		
FAB						
▪ L1	3	60.0%	2	40.0%	7.17	0.017*
▪ L2	3	16.7%	15	83.3%		
▪ L3	2	100.0%	0	0.0%		
Risk						
▪ High	8	61.5%	5	38.5%	10.860	0.002*
▪ Standard	0	0.0%	12	100.0%		

Table (5): Relation between livin expression and laboratory findings:

Variable	Livin Expression				Test **	P value
	Negative N=8		Positive N=17			
	No.	%	No.	%		
WBC						
<50.000/mm^3	3	37.5	14	82.4	5.029	0.025*
≥50.000/mm^3	5	62.5	3	17.6		
	Mean ± SD		Mean ± SD			
HB:(g/dl)	9.0±1.4		8.2±1.7		-0.993**	0.321
PLT:(x10^3/mm^3)	35.50±3.53		72.53±8.14		-1.962	0.091
PT	14.1±2.7		15.9±2.3		-0.147	0.883
PTT	29.13±3.23		28.59±3.12		-0.444**	0.657
Total bilirubin:(mg/dl)	0.8±0.2		0.8±0.2		-0.213	0.831
Direct bilirubin: (mg/dl)	0.30±0.08		0.30±0.04		-0.126	0.900
Total Protein:(g/dl)	6.7±0.6		6.6±0.7		-0.530**	0.596
Albumin(g/dl)	3.9±0.7		3.9±0.6		-0.264**	0.792
SGOT	32.3±.1		26.8±4.9		-1.547**	0.122
SGPT	31.1±4.4		29.4±5.7		-0.234**	0.815
Urea(mg/dl)	12.12±1.31		13.44±2.19		-1.321**	0.131
LDH(U/L)	312.0±49.0		310.0±32.0		-0.176	0.860
Creatinine (mg/dl)	0.40±0.07		0.40±0.08		-0.119	0.905
BM blast	79.75±9.81		68.65±12.94		-1.960	0.050*
Peripheral Blast	18.83±1.65		14.85±2.79		-0.796	0.426

Table (6): Relation between livin expression and some prognostic factors:

Variable		Positive Livin expression			
		N=17	Mean± SD	Test**	P value
Age	<10	15	294.53±153.03	-2.367	<0.001*
	≥10	2	31.0±12.73		
Sex	Male	7	284.29±184.90	-0.732	0.464
	Female	10	249.0±163.41		
LN	Negative	2	178.50±228.40	-0.746	0.456
	Positive	15	274.87±165.19		
Splenomegaly	Negative	2	313.50±4.95	-0.075	0.941
	Positive	15	256.87±178.27		
Hepatomegaly	Negative	3	363.00±158.75	-1.009	0.313
	Positive	14	242.21±167.36		
WBC	<50.000/mm^3	14	249.85±155.15	-1.708	0.089
	≥50.000/mm^3	3	117.33±72.91		
Immunophenotyping	Pre-B –ALL	15	296.33±149.62	-2.237	0.025*
	Mature & Progenetor B-ALL	2	17.50±0.71	-0.298	0.765
FAB	L1	2	291.00±28.28		
	L2	15	259.87±178.90		
Risk	High	5	82.80±131.25	-2.584	0.01*
	Standard	12	338.83±116.90		

DISCUSSION

The most frequent childhood cancer is acute lymphoblastic leukemia (ALL) [7]. Understanding of the disease's biology, customized treatment, and better supportive care have all led to an increase of rates of surviving [8].

Cells use apoptosis, one of several types of programmed cell death (PCD), to get rid of unneeded or damaged cells. Cancer cells are known for their ability to resist apoptosis [3].

Inhibitor of apoptosis proteins (IAPs) family belongs to the large and heterogenous group of antiapoptotic factors that are frequently over expressed in cancer cells including leukemia. As IAPs proteins also regulate several key points that control the cell's decision to live or die, they are considered to be promising targets for prognostic and therapeutic modalities [9].

Livin is one of the IAPs family which generally interacts with caspase 3, caspase 7, caspase 9, and also can inhibit SMAC/DIABLO which promotes caspases activation by binding to IAP-family members [5].

Livin plays a dual role in tumorigenesis (antiapoptotic in full length and proapoptotic in the cleaved or truncated form [10].

It was found that there was no statistically significant difference between controls and cases as regard any of the tests except LDH which means show highly significant increase in cases than controls (158± 17 in control group versus 310± 37) (P<0.001).

Also mean livin expression level was significantly higher in case group in comparison to control group (179.31±185.66 in control group versus 0 .054±0.021 in case group) (P<0.001).

In this study it was found that livin expression was more in age group less than 10 years (15 cases) than those in age group more than 10 years (2 cases).

This is in agreement with **Ibrahim et al.** [6] who found that livin expression was more in age < 10 years than age group ≥10 years.

Livin expression might be related to the age of patients. Children, not fully matured, have a variety of different biologic features distinguishing them from adults.

Children aged 1 to < 10 years have a more favorable outcome than infants and children aged >10 years this may be due to high frequency of more favorable underlying biologic features of lymphoblasts of patients of this age group [11].

As regard sex distribution in this study it was found significant difference between negative and positive cases (livin –ve cases were 7 males and 1 female while livin +Ve cases were 7 males and 10 females.

Choi et al. [12], found livin expression was higher in females 33% 0f females expressed livin while 21% of males expressed livin. While **Ibrahim et al.** [6] found no significant difference as regard sex between negative and positive cases (12 males and 15 females versus 27 males and 26 females).

Like many other diseases and cancers, childhood ALL risk shows consistent sex differences. Relapse and secondary malignancies are more common in males this may be due to the occurrence of testicular relapse [13].

As regard WBCs it was found that in livin negative subgroup (8 cases): there were 3 patients (37.5%) with leucocytic count < 50.000/mm³ at diagnosis and 5 patients(62.5%) with leucocytic count ≥ 50.000/mm³ or more at diagnosis. Compared to 14 (82.4%) and 3 patients (17.6%) among livin positive group (17 cases) respectively (p=0.025).

As WBCs count at diagnosis is an adverse prognostic factor [14] so, it could be concluded that livin may be associated with good prognosis of childhood acute lymphoblastic leukemia.

This was in agreement with **Ibrahim et al.** [6] who found in livin negative group (27 cases) 10 patients with leucocytic count < 50.000 /mm³ at diagnosis and 17 patients with leucocytic count ≥ 50.000/mm³ or more at diagnosis. Compared to 45 and 8 patients among livin positive group (53 cases) respectively (p<0.001).

In this study it was found that livin expression was significantly higher in standard risk group patients than high risk group patients. Mean livin expression in standard risk group was 338.83±116.90 while in high-risk group was 82.8±131.25.

This was in agreement with **Ibrahim et al.** [6] who found mean livin expression level in standard risk group was 128.13 (0.84-519.1) versus 8.6 (4.6-110.6) in high-risk group.

As regard B.M blast it was found that there was significant difference in B.M blast between livin negative cases and livin positive case. B.M blast was significantly higher in livin negative subgroup. (Mean±SD of B.M blast in livin negative and livin positive cases was79.75±9.81 and 68.65±12.94 respectively).

This was in agreement with **Zareifar et al.** [15] who found that in livin positive group (35 patients) there were 6 patients with blast <50% and 29 patients with blasts > 50% , while in livin negative group (8 patients) there were 2 patients with blasts < 50% and 6 patients with blasts > 50%.

Choi et al. [12], demonstrated that the existence of positive prognostic variables at diagnosis was, although not always, related with Livin expression in childhood ALL. Furthermore, a positive early response to chemotherapy was linked to high levels of Livin expression. In addition, it was found that Livin expression rates and livin expression levels were higher in patients with favorable clinical aspects compared to those in patients with negative clinical features.

Positive correlation between livin and good prognostic factors of childhood ALL may be explained by evidence that leukemic cells contain the cleaved form of livin and expressed Livin. In other words, this cleaved version hasn't been tested in other types of

cancer [16]. Perhaps the cleaved form possessed proapoptotic properties [15].

The results of the current study are also in contrast with **El-Mesallamy et al.** [17] as they revealed the existence of poor prognostic variables at diagnosis was linked to elevated livin expression in ALL patients. High livin expression was also linked to a decreased EFS and OS in both groups of participants. Leukemia cells that produce high levels of livin are less responsive to chemotherapeutic agent-induced apoptosis.

Also, **Zareifar et al.** [15], found that Livin showed significant associations with some poor prognostic factors of childhood leukemia.

The controversy between the previous results and our results may be explained by **Abd-Elrahman et al.** [10] **and Lazar et al.** [18] who observed the proapoptotic activity of truncated form of livin (t-livin) which is more predominant in children [6].

CONCLUSION

It could be concluded that livin was associated with favorable prognostic factors among ALL patients: (age<10 years, female patients, WBCs <50,000/mm^3).

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