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ORIGINAL ARTICILE

Assessment of B-cell Activating Factor (BAFF) in children with Acute Lymphoblastic Leukemia

Ola Aly Hussein[(1)], Mervat Atfy mohamed[(2)], Ghada Mohamed Alakkad[(1)], Nahla Zidan[(1)], Asmaa Hassan Samy[(1)]

1: Clinical Pathology Department, Faculty of Medicine, Zagazig University, Zagazig, Egypt
2:Pediatrics Department, Faculty of Medicine, Zagazig University, Zagazig, Egypt.

Corresponding author:
Asmaa Hassan Samy Ali Behairy
Email:
asmaa.h.behairy@gmail.com

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ABSTRACT

Background:Acute lymphoblastic leukemia (ALL) is the most common childhood malignancy. BAFF is member of TNFSF13B whose level is elevated in cases of ALL. **Aim**: To assess serum BAFF level in children with ALL and correlate its level with the clinical, laboratory findings and known prognostic factors as well as its relation to response to treatment.**Subjects and Methods**: This study was conducted at Zagazig University Hospitals in the period from April 2018 to June 2019 on 24 Egyptian children with newly diagnosed ALL and 24 apparently healthy children of matched age and sex as a control group. Patient history, clinical and laboratory examination results were taken, including complete blood count, bone marrow aspiration with cytochemistry, immunophenotyping, and estimation of serum BAFF level using ELISA kits.**Results:** We found statistically significant higher BAFF levels among de novo childhood acute lymphoblastic leukemia patients compared with the apparently healthy control group (P value < 0.0001).High serum BAFF was significantly correlated to patients' poor treatment response(P value < 0.05) but not patients' outcome (P value > 0.05).**Conclusion**: We found a significant relation between serum BAFF level and response to treatment so assessment of serum BAFF level at the time of diagnosis may be a predictor for response to treatment. This finding recommends that patients with high BAFF level at the time of diagnosis be subjected to intensified course of therapy.

Key Words: Acute lymphoblastic leukemia (ALL), B-cell activating factor (BAFF), enzyme-linked immunosorbent assay (ELISA).

INTRODUCTION

Acute lymphoblastic leukemia (ALL) is a malignant disorder of lymphoid pro-genitor cells and the most common type of malignant neoplasms in children and adolescents**[1]**. Is a serious hematic disease characterized by the overproduction and continuous multiplication of malignant and immature WBCs (referred to as lymphoblasts or blasts) **[2]**.

Increasing evidence indicate that microenvironmental causes play critical role in

cancer biology and that malignant cells are responsive to multiple extrinsic factors from their microenvironment**[3].**

These stimuli involve both soluble factors and receptor/ligand interactions, which mediates or influence processes such as tumor development, maintenance, drug-resistance, and immune evasion. Studies indicate that the leukemia microenvironment supports ALL cells developing in the bone marrow by providing survival/proliferation signals and by functioning as potential niches for chemotherapy-resistant tumor cells **[3].**

There is emerging evidence that the tumor necrosis factor super-family member B-cell activating factor (BAFF) (along with its receptors) is a critical factor for the growth and survival of both normal and malignant clone of B-cell **[4].**

It has been reported that BAFF can augment tumor cell growth of B-cell by either stimulating proliferation, inhibiting apoptosis or protecting malignant cells against drug induced apoptosis [5].

In accordance with this, there is emerging evidence that B lineage neoplasm have aberrant expression of BAFF **[6]**.

So, we here aimed to assess serum BAFF level in children with ALL and correlate its level with the clinical, laboratory findings and known prognostic factors as well as its relation to response to treatment.

SUBJECTS AND METHODS

The work has been carried out in accordance with The Code of Ethics of the World Medical Association (Declaration of Helsinki) for studies involving humans.

Technical design:

Site of the study :

The study was conducted on admitted de novo acute lymphoblastic leukemia patients at Clinical Pathology and Pediatric Departments, Faculty of Medicine, Zagazig University Hospitals.

Time of the study :

From April 2018 to June 2019.

Sample size :

This study was done on 48 individuals who are categorized into two groups:

Control group: It included 24 apparently healthy children. They were 15 males (62.5%) and 9 females (37.5%) with male to female ratio 1.7 : 1. Their ages ranged from (2-14) years and the median value was 7 years.

They matched well with the patients as regard age and sex.

Patients group: It included 24 children newly diagnosed with ALL before receiving induction therapy. They were 14 males (58.3%) and 10 females (41.7%) with male to female ratio 1.4 : 1. Their ages ranged from (3-15) years and the median value was 7.5 years.

Each group was subjected to the following:

Patients group:

Complete history taking.

Clinical examination was done particularly for bone aches, fever, pallor, purpura, hepatomegaly, splenomegaly, lymphadenopathy and manifestations of CNS involvement.

Routine laboratory investgations for control group and before first induction therapy for patients group:

Complete blood picture and blood smear examination.

ESR.

Liver, kidney function tests , lactate dehydrogenase and serum minerals &electrolytes (Na^{+}, K^{+}, Mg^{++}, Ph^{++} and Ca^{++}).

Bone marrow aspiration and examination followed by cytochemistry and immunophenotyping for patients group only.

Cytogenetic examination for patients group only.

Specific investigation:

Estimation of serum BAFF level using enzyme-linked immunosorbent assay technique (ELISA) **(El Fatah et al., 2015)** [6] for both control and patients groups at the onset of disease before receiving first induction of therapy . Thereafter, the patients group were assessed on follow-up by:

- Response to treatment was assessed for patients group at day 28 from induction chemotherapy

by peripheral blood picture and bone marrow aspirate.

- Patients' clinical outcome after 12 months duration was assessed.

Operational design:

Type of the study: case control study.

Administrative design:

Informed consents were taken from parents of all subjects participating in this study. Approval was taken from Zagazig Institutional Review Board (IRB).

Evaluation of BAFF levels:

BAFF level was measured for all enrolled patients at diagnosis and compared with serum BAFF levels of the controls. BAFF was quantitatively measured in serum using Human BAFF ELISA Kit-1 × 96 provided by SUNRED company (No.18,Keyuan Road, DaXing Industry Zone, Beijing, China).

The optical density (OD) was measured spectrophotometrically at a wavelength of 450 nm. The OD value is proportional to the concentration of B-Cell Activating Factor (BAFF). The concentration of (BAFF) in the samples can be calculated by comparing the OD of the samples to the standard curve.

Statistical methods

All data were collected, tabulated using Microsoft Excel 2010, and then statistically analyzed using SPSS 18.0 for windows (SPSS Inc., Chicago, IL, USA). Continuous Quantitative variables e.g. age, disease duration, lab results, etc... were expressed as the mean ± SD & median and range, while Categorical Qualitative variables were expressed as absolute & relative frequencies (percentage).

Unpaired Student's t-test, Chi-Square test (X^2), Spearman's correlation, Kruskal Wallis test and Mann Whitney U (MW) test were used for statistical analysis. ROC curve for determination of BAFF cut off and Kaplan-Meier curve for estimation of overall survival were used.

RESULTS

Hematological data and cytogenetic risk of B - ALL patients group presented into table (1) and showing that Hb mean ± SD was 7.7 ± 1.7 & the range is (4.8 - 12), platelet mediane is 39 & the range is (7 - 216), PB Blast cells (%) mean ± SD was 39.2 ± 15.9 & the range was (15 - 65), regarding BM cellularity 92% of cases were hypercellular, while 8% of cases were normcellular, regarding BM blast cells (%) the mean ± SD was 84 ± 7.6 and the range was (65 – 97), regarding FAB classification 92% of cases were L1, while 8% of cases were L2, regarding immunophenotyping 8.3% of cases were Pro-B-ALLm while 91.7% were C-ALL and as regard cytogenetic risk of 16 patients who underwent cytogenetic studies 69% of them were favorablem while 31% wereunfavorable.

Comparison between control group and B - ALL patients group regarding serum BAFF level presented into table (2) which shows that there was a highly statistically significant difference between the two groups regarding serum BAFF level ($P < 0.0001$).

Relation of serum BAFF levels to some demographic data and clinical finding of B - ALL patients group was presented into table (S1) and shows that there was a highly statistically significant difference between serum BAFF level and positive & negative findings of purpura ($P < 0.01$), while there was no statistically significant difference between serum BAFF levels and sex, positive & negative findings of other clinical data (P-values > 0.05).

Correlation of serum BAFF level with age and laboratory findings of B – ALL patients group presented into table (3) which shows that there was a statistically significant correlations between serum BAFF level and both PB & BM blast cells, while there were no statistically significant correlations between serum BAFF and other laboratory findings.

Relation between serum BAFF level and response to treatment among of the B – ALL patients group presented into table (4) which shows that there was a statistically significant relation between serum BAFF level and response to treatement ($P < 0.05$).

Relation between serum BAFF level and patients' outcome presented into table (5) which shows that there was no significant relation between serum BAFF level and patients' outcome.

Table (1): Hematological data and cytogenetic risk of B - ALL patients group.

Parameter	Value
WBCs (4-11x10^9/L)	
Median	26
(Range)	(10 – 82)
Hb (11-15gm/dl)	
Mean ± SD	7.7 ± 1.7
(Range)	(4.8 - 12)
Platelets (150-450x10^9/L)	
Median	39
(Range)	(7 – 216)
PB Blast cells(%)	
Mean ± SD	39.2 ± 15.9
(Range)	(15 – 65)
BM cellularity	
Hypercellular	22 (92%)
Normcellular	2 (8%)
BM Blast cells(%)	
Mean ± SD	84 ± 7.6
(Range)	(65 – 97)
FAB classification	
L1	22 (92%)
L2	2 (8%)
Immunophenotyping	
Pro-B-ALL	2 (8.3%)
C-ALL	22 (91.7%)
Cytogenetic risk of 16 patients who underwent cytogenetic studies	
Favorable	11 (69%)
Unfavorable	5 (31%)

Table (2): Comparison between control group and B - ALL patients group regarding serum BAFF level.

Subjects BAFF (pg/ml)	Control group (no.=24)	Patients group (no.=24)	MW	P-value
Median (Range)	**270 (200 – 320)**	**1250 (650 – 5000)**	**-5.96**	**< 0.0001* (HS)**

Table (4): Correlation of serum BAFF level with age and laboratory findings of B – ALL patients group.

	BAFF pg/ml		
	R	**P- Value**	**S**
Age	-0.9	0.66	(NS)
TLC	-0.08	0.7	NS
Hb	-0.07	0.75	NS
PLT	0.24	0.25	NS
PB blasts	0.47	0.02*	(S)
BM blasts	0.58	0.003*	(S)
CD19	-0.03	0.90	NS
CD20	-0.14	0.51	NS
HLA DR	-0.06	0.79	NS
CD10	0.14	0.53	NS
CD34	-0.28	0.18	NS
Different cytogentics done for 16 patients only	KW 3.8	0.58	NS

Table (4):): Relation between serum BAFF level and response to treatment among of the B – ALL patients group.

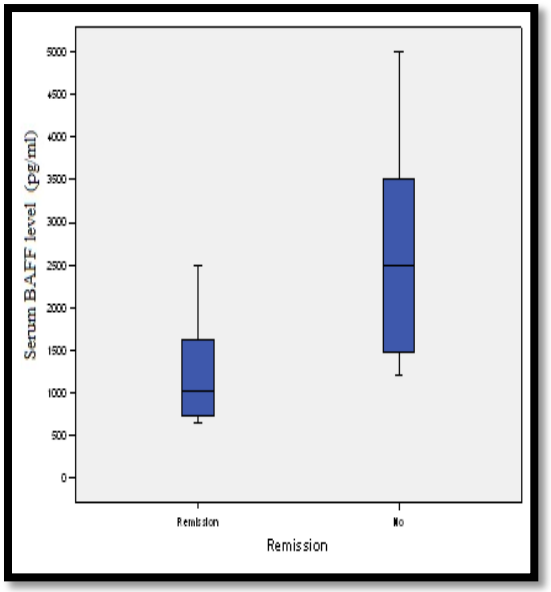
sponse to Treatment	BAFF pg/ml		P	
	Median	Range	MW	P-Value
Remission (no.=16)	1050	650 -2500	-2.52	0.011* (S)
No remission (no.=8)	2500	1225 -5000		

Table (5): Relation between serum BAFF level and patients' outcome.

Outcome	BAFF pg/ml		P	
	Median	Range	MW	P-Value
Survived (no.=21)	1250	(650 – 5000)	-0.57	0.62 (NS)
Died (no.=3)	2500	(2000 - 4000)		

Figure (1): Box-plots:

(A) Box-plot diagram represents serum BAFF level in relation to response to treatment among of the B – ALL patients group.



(B) Box-plot diagram represents the serum BAFF level in relation to patients 'outcome.

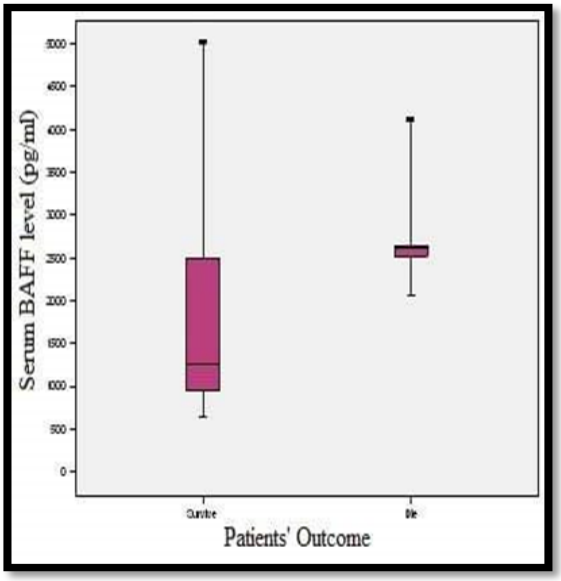
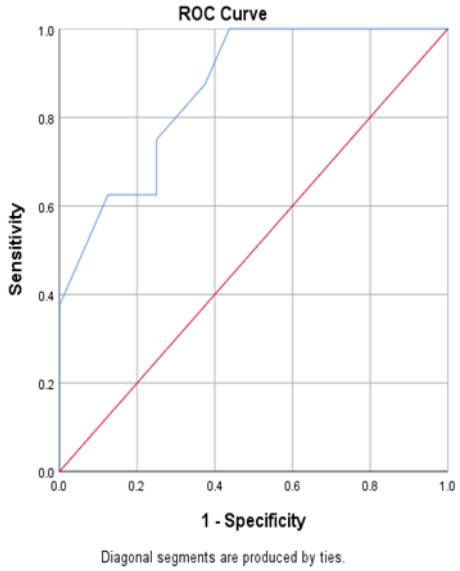
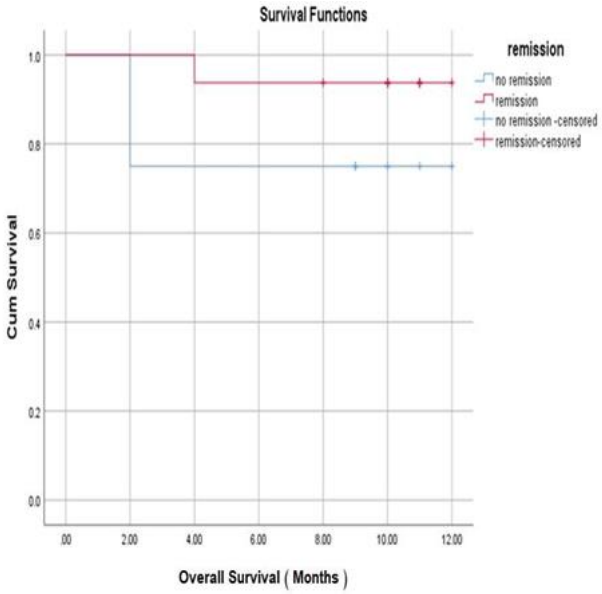


Figure (2): ROC and Kaplan-Meier curves

(A) Receiver operating characteristic (ROC) curve of serum BAFF in predicting treatment outcome of B - ALL patients group.



(B) Kaplan Meier curve for overall survival in the B - ALL patients group according to remission state.



DISCUSSION

Acute lymphoblastic leukemia (ALL) is the most common cancer in children. Current treatment strategies for childhood ALL result in long term remission for approximately (90%) of patients. However, the therapeutic response is worse among those who relapse [7].

A frequent site of relapse is the BM [8], which may be a particularly natural protective site for such cells. Therefore, many studies are focusing on the BM microenvironment as a possible target for treatment by investigating the interactions between cell surface receptors on the ALL cells that are engaged by factors produced in the BM [9].

Tumor necrosis factor (TNF) superfamily ligands such as B cell-activating factor (BAFF), a proliferation inducing ligand (APRIL) and TNF-related apoptosis inducing ligand (TRAIL), can play an important role in the pathogenesis and development of various B cell malignancies involving B-ALL[10].

Researches on the BM microenvironment stated that there is emerging evidence BAFF, along with its receptors, is a critical factor for growth and survival of both normal and malignant clones of B-cells **[4].** It has been reported that BAFF can augment tumor cell growth of B-cells by either stimulating proliferation, inhibiting apoptosis or protecting malignant cells against drug-induced apoptosis **[11**]. In accordance with this, there is emerging evidence that B-lineage neoplasms have aberrant expression of BAFF **[6]**.

The present study aimed at assessment of serum BAFF level in children patients with B-ALL and correlates its level with the clinical, laboratory findings and known prognostic factors as well as its relation to response to treatment.

In the current study, 92% of the patients had hypercellular B M, while 8% only had normocellular BM. This is consistent with **Nguyen et al. [11]** who confirmed hypercellular bone marrow in cases of ALL due to leukemic infiltration.

In our study, the peripheral blood blast cells ranged from 15-65% with a mean ± SD of 39.2 ± 15.9 , while the B M blast cells ranged from 65 – 97% with a mean ± SD of 84 ± 7.6, similar results reported by **El Fatah et al.[6]** with mean ± SD of peripheral blood &

peripheral blood blast were 38.50 ± 13.31 80.9 ± 10.98; respectively.

According to FAB classification which was based on morphological finding , the more common encountered FAB subtype was L1 (92%) , followed by L2 (8%) and these findings not consistent with the studies done by **Shingade et al. [12]** who showed that L2 was the most encountered FAB subtype in their patients(48%) and this may be due to that we selected B – ALL patients only for enrollment in this study not all ALL patients as done by **Shingade et al. [12].**

As regard immunological classification in our study, C-ALL comprised 92%, while pro-B-ALL occurred only in 8% of patients, which was close to that of **Armitage [13]** who stated that C-ALL is the most common encountered subtype in children (89%).

Regarding cytogenetic risk, our patients were classified into favorable (67%) and unfavorable patients (33%). In this study, the favorable group was the dominant which was comparable to those reported by **Moorman, et al. [14]** with percentage of 75%.

In our study, the median of serum BAFF was 270 pg/ml and the range was (200-320) for the control group but for the B - ALL patients group, the median of serum BAFF was 1250 pg/ml and the range was (650-5000). There was a highly statistically significant increase in the serum BAFF level in the patients group compared with the control group ($P < 0.0001$). These findings are in agreement with **El Fatah et al. [6]** and **Bienertova-Vasku et al. [4]** who found that serum BAFF level was significantly increased in the ALL patients compared with their control subjects ($P < 0.001$).

This may be explained by the fact that BAFF can augment tumor cell growth of B-cells by either stimulating proliferation, inhibiting apoptosis or protecting malignant cells against drug-induced apoptosis. Also B-cells can reach survival advantage during positive selection by becoming responsive to BAFF **[11]**.

Regarding laboratory findings in our study, statistically significant correlations were detected between serum BAFF level, both peripheral blood and bone marrow blast cells % at initial diagnosis (P-values : 0.02 & 0.003); respectively. This can be explained by BAFF role in prolonging the survival of cells and protecting it from apoptosis **[5]**. However no correlations were found regarding other laboratory parameters and this is in agreement with the results obtained by **Bienertova-Vaskua et al. [4]** and **El Fatah et al. [6]** who found no correlations between serum BAFF level and laboratory findings.

In our study, no statistically significant difference was reported in relation between serum BAFF level and different cytogenetic abnormalities ($P > 0.05$).

In our study, assessment of serum BAFF level at a time of diagnosis in relation to response to treatment showed a statistically significant relation between them ($P = 0.011$). As patients with higher BAFF levels with median of 2500 pg/ml and range (1225 – 5000) pg/ml showed no remission, while patients with lower BAFF levels with median of 1050 pg/ml and range (650 – 2500) pg/ml entered in complete remission. This is in agreement with **El Fatah et al. [6]** who reported that patients that showed no remission have higher serum BAFF levels, while patients with lower BAFF levels entered in complete remission.

This finding suggest the role of serum BAFF levels as early predictor for response to therapy and recommend that patients with high serum BAFF level at time of diagnosis should be subjected to intensified course of therapy. If this level more than 10000 pg/ml , these patients considered as high-risk ALL patients as reported by **Bienertova-Vaskua et al. [4]**.

In this study we found that BAFF had no relation with disease outcome. This may be caused by the limited number of patients included in the study and the same was reported by **El Fatah et al. [6].**

The majority of ALL cases are of B-cell lineage. **Parameswaran et al. [9]** had previously discovered that these leukemia cells have a protein on their surface called BAFF-R. It was known that this BAFF-R was present on mature B-cells but finding it on pre-B cells was surprising and also presented a therapeutic target for selectively killing the pre-B ALL cells and by using of the fact that BAFF protein specifically binds to the BAFF-R protein and then is allowed entry into the cell. They tested a toxin-BAFF fusion protein. Using a "Trojan horse" approach, the investigators showed that when ALL cells were exposed to the BAFF-toxin, the ALL cells bound the BAFF-toxin, transported it inside the cell, and were then killed. The BAFF-R is only present on certain blood-forming cells, so the BAFF-toxin is not expected to harm any other cells, making it much less toxic than standard chemotherapy **[9].**

So, this therapy was discovered killing acute lymphoblastic leukemia (ALL) cells. Using a fusion toxin that specifically targets cancer cells, this novel therapeutic may offer a future alternative to patients who become resistant to chemotherapy.

Limitations of this study were limited number of patients included in the study, short duration of the study, short period of follow up and limited financial sources.

CONCLUSION

There was a positive correlation between serum BAFF level and both peripheral blood & bone marrow blast cells % at initial diagnosis which may be related to its role in ALL pathogenesis.

Significant relation was observed between serum BAFF level and response to treatement, so the higher BAFF level, the greater the need for more intensive therapy.

RECOMMENDATIONS

More studies on large numbers of patients are needed to confirm these results.

Follow-up for long duration of patients who exhibit very high levels of BAFF.

Further research studies for BAFF are strongly needed to support its potential therapeutic target in pediatric ALL.

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