Prognostic Significance of Plasma APRIL Level in Patients with Chronic Lymphocytic Leukemia

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ABSTRACT:

BACKGROUND:

A proliferation-inducing ligand (APRIL) is a member of tumor necrosis factor family which plays an important role in B-cell development. It activates chronic lymphocytic leukemia (CLL) cells by reacting with its receptors, enhancing immune recognition, proliferation, and survival of the leukemia cells.

OBJECTIVE:

To assess the level of APRIL in newly diagnosed CLL patients in comparison with the control group and to correlate between the plasma APRIL level and hematological parameters and clinical Binet stages.

PATIENTS AND METHODS:

This case-control study was conducted on 40 adult newly diagnosed CLL patients with 40 healthy individuals served as controls. Plasma APRIL levels were tested by ELISA.

RESULTS:

plasma APRIL level in CLL patients was significantly higher than those of healthy control group (*P*-value < 0.001). There were statistically insignificant correlations between APRIL level and hematological parameters. There was significant association between absence of splenomegaly and higher APRIL level (*P*=0.011), while no significant statistical differences were found with lymphadenopathy and hepatomegaly. There was no significant association between plasma APRIL level and Binet stages (*P*=0.180).

CONCLUSION:

There were insignificant correlations between APRIL level and hematological parameters and Binet stage. APRIL level was not found to be a useful marker to predict prognosis in patients with CLL.

KEY WORDS: APRIL, CLL, Binet staging

INTRODUCTION:

Chronic lymphocytic leukemia (CLL) is a chronic B-cell lymphoproliferative disorder characterized by the accumulation of monoclonal B cells with the appearance of small mature lymphocytes.[1] The tumor necrosis factor superfamily member a proliferation-inducing ligand (APRIL) has been shown to play an important role in B-cell biology. APRIL expression has been reported, under physiological conditions, in several hematopoietic cells (neutrophils, monocytes, macrophages, dendritic cells, B and T lymphocytes and osteoclasts). It has also been detected in solid tumors and in B-cell-derived malignancies.[2] It interacts with two canonical receptors, the B-cell maturation antigen (BCMA) and the transmembrane activator, calcium modulator, and cyclophilin ligand interactor (TACI).[2] Furthermore, it has been shown to react also with the heparan sulfate side chain of proteoglycans, which is supposed to facilitate TACI and/or BCMA signaling.[3] APRIL plays an important role in B-cell development; it activates CLL cells by reacting with its receptors, enhancing immune recognition, proliferation, and survival of the leukemia cells. APRIL induces activation of the canonical NF-κB pathway and protects CLL cells from apoptosis.[4,5] The study aimed to correlate between the plasma APRIL level and hematological parameters and clinical Binet stages.

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PATIENTS, MATERIALS AND METHODS:

This case-control study was conducted on forty adult, newly diagnosed, CLL patients attending the Hematology outpatient clinic at Oncology Teaching Hospital of the Medical City, Baghdad, Iraq. Their median age was 62 years, 21 of them were males and 19 were females.

Data were collected for each patient using questionnaires form including: name, age, gender, main symptoms and physical signs mainly the presence of lymphadenopathy, splenomegaly and hepatomegaly. Clinical Binet staging system was applied.[6] Diagnosis was based on peripheral blood samples morphology and immunophenotyping using a four-color flow cytometer (BD FACS Canto™II Flow Cytometer, USA), all patients had absolute lymphocyte count > 5×10^9/L with CLL score of 4 or 5.[(7)] Plasma APRIL level assay was done by sandwich enzyme-linked immunosorbent assay (ELISA) using the Human APRIL, Abcam, (USA). All controls were negative for C- reactive protein testing.

Statistical analyses: Parametric variables are presented as median, range and inter-quartile range (IQR), while qualitative var

iables are presented as number and percentage. Mann-Whitney U tests were used for the difference of quantitative variables between the study groups. Pearson correlation was used to test the correlations between APRIL and other variables. *P*-value of <0.05 was considered statistically significant.

RESULT:

The median age of CLL patients was 62 years (range, 43-76 years), and the median age of the control group was 62 years (range, 40-72 years). CLL was observed more in males 21/40 (52.5%) than in females 19/40 (47.5%) with an M: F ratio of 1.1:1. The most frequent presenting signs of patients were lymphadenopathy (LAP) and splenomegaly (75% and 60%, respectively) followed by hepatomegaly (20%). According to Binet staging system, of the 40 cases studied, 18 patients were in stage A (45%), 16 patients were in stage B (40%) and 6 were in stage C (15%). The median WBC count was 37.8 $\times10^9$/L, while the median absolute lymphocyte count (ALC) was 28.05 $\times10^9$/L, the median of Hemoglobin (Hb) concentration was 12.2 g/dL and that of platelet count (PLT) was 174 $\times10^9$/L.

In patients with CLL the median plasma APRIL level was 0.852 ng/mL which is significantly higher than that in controls with a *P*-value of <0.001 (Table 1).

Table 1: Comparison of APRIL in control and patients groups.

APRIL	Control n=40	Patients n=40	*P*-value*
Median (ng/mL)	0.305	0.852	<0.001
Range	0.24 - 0.42	0.40 - 5.77	
IQR	0.06	1.28	

* Mann Whitney test

There was insignificant correlation between APRIL level and age, WBC, ALC, Hb, and PLT of the patients (Table 2).

Table 2: Pearson correlations of some hematological parameters with APRIL level in patients group.

Variables	APRIL	
	R	*P*
Age (years)	-0.196	0.225
WBC ($\times10^9$/L)	0.076	0.641
ALC ($\times10^9$/L)	0.062	0.705
Hb (g/dL)	0.124	0.447
PLT ($\times10^9$/L)	<0.001	0.999

Interestingly, there was a significant association between splenomegaly and a lower APRIL level (*P*=0.011), while no significant statistical differences were found with LAP and hepatomegaly (Table 3).

Table 3: Association of APRIL with the most common presenting signs in patients group.

	APRIL	Median	Range	IQR*	P-value*
LAP	Absent n=10	0.8	0.42 - 1.91	0.78	0.794
	Present n=30	0.92	0.40 - 5.77	1.3	
Splenomegaly	Absent n=16	1.64	0.44 - 5.77	1.27	0.011
	Present n=24	0.54	0.40 - 5.60	1.13	
Hepatomegaly	Absent n=32	0.92	0.40 - 5.77	1.26	0.102
	Present n=8	0.48	0.40 - 1.74	1.15	

*Mann Whitney test
*IQR: inter-quartile range

There was insignificant relationship between plasma APRIL level and the Binet clinical stages (Table 4).

Table 4 : Comparison of APRIL according to Binet stage of CLL.

APRIL	Binet stages		P-value*
	A N=18	B+C N=22	
Median (ng/mL)	0.98	0.55	0.180
Range	0.42-5.6	0.40 - 5.77	
IQR	1.33	1.29	

*Mann Whitney test

The median level of APRIL in patients who need treatment at presentation was (1.09 ng/mL) and in those who did not need treatment was (0.59 ng/mL). There was no significant relation between APRIL and both groups (Table 5).

Table 5: Comparison of APRIL level at presentation between patients groups who did not need treatment and those who need treatment in 40 CLL patients.

APRIL	Do not need treatment N=17	Need treatment N=23	*P*-value*
Median (ng/mL)	0.59	1.09	0.481
Range	0.4-1.91	0.4-5.77	
IQR	1.15	1.35	

*Mann Whitney test

DISCUSSION:

The plasma APRIL levels in CLL patients were significantly higher as compared with the normal control group indicating that APRIL protein is overexpressed in a large fraction of B-CLL tumors. These were comparable to the results obtained by western studies done by Planelles et al (2004).[8], Ferrer et al. (Spain) (2011),[9] and Tecchio et al. (Italy) (2011) [10] who reported that serum soluble APRIL level was higher in CLL patients than in healthy individuals.

There was no significant association detected between APRIL levels and gender which was in agreement with that reported by Junak et al (2009).[11] and an Egyptian study(2016).[12]

In concordance with the study of Junak et al(2009).[11] and Esatoglu et al. (Turkey) (2014),[13] there was statistically insignificant relation between APRIL and age. In contrast, an Egyptian study in 2016[12] showed a positive relation between

age and APRIL; this difference may be attributed to the difference in population structure.

Furthermore, there was no correlation between APRIL level and Hb concentration and PLT which was comparable to that reported by Tecchio et al. (Italy)(2011) [10] and a study in Egypt 2017.[14] But it was in contrast to other studies by El Shora O et al (2016).[12] and Junak et al(2009).[11] in which there was a negative correlation between Hb level, PLT and APRIL level; this may be due to difference in sample size.

Correlation of APRIL level with WBC and ALC was statistically insignificant, this result was consistent with that reported by Esatoglu et al. (Turkey)(2014),[13] but in contrast, El Shora O et al(2016).[12] reported a positive correlation between APRIL level and that of WBC and ALC.

Association of APRIL with lymphadenopathy was statistically insignificant in contrast to an Egyptian study(2017),[14] which reported that higher serum APRIL levels were found among CLL patients with lymphadenopathy.

Significant association was found between higher level of APRIL and the absence of splenomegaly and no significant association was found with hepatomegaly but unfortunately no previous studies was found to compare with.

Relationship between APRIL and clinical Binet staging system was statistically insignificant which is comparable to the study reported by Tecchio et al. (Italy)(2011),[10] and Esatoglu et al. (Turkey)(2014),[13] however, the Egyptian study in 2016[12] reported significantly higher levels of APRIL with high-risk modified Rai stage of CLL; this could be due to the inclusion in the Egyptian study of only 10% of patients in the low-risk group and 52.5% of patients in the high risk group, whereas in our study 45% of patients were in group A.

Though the median level of APRIL at presentation in those who needed treatment was higher than those who did not need treatment but the difference was statistically insignificant, unfortunately no other studies were found to compare with.

The involvement of APRIL in the pathogenesis of lymphoproliferative diseases prompted us to explore its prognostic role in patients with B-CLL; Planelles et al(2004).[8] had demonstrated that among 95 patients affected by CLL, those with $APRIL_{high}$ group had significantly poorer overall survival (OS). Junak et al(2009).[11] have analyzed APRIL plasma levels with regard to OS in a cohort of 183 patients with B-CLL, demonstrating that $APRIL_{high}$ group correlated with more advanced (Rai stages III and IV) and predicted worse OS. However, Tecchio et al(2011) [10] did not observe a predictive role of APRIL in OS, and they also did not found any association between APRIL levels and number of clinical–biological characteristics of malignant B-lymphocytes such as age, gender, clinical stage, Hb level, ALC or platelet count, GFR-b2M, CD38 or ZAP70. Unfortunately, the current study lacks information about Progression–free survival and OS because of the limited time of the study and lacked the opportunity to follow-up the studied patients.

CONCLUSION:

APRIL may play an important role in the pathogenesis of CLL where its level is significantly higher than that in control group. However, no significant correlations between plasma APRIL level and hematological parameters and Binet stage were found to predict prognosis in patients with CLL.

REFERENCES:

1. Del Principe MI, Del Poeta G, Buccisano F, Maurillo L, Venditti A, Zucchetto A, et al. Clinical significance of ZAP-70 protein expression in B-cell chronic lymphocytic leukemia. Blood. 2006; 108:853-61.
2. Dillon S, Gross J, Ansell SM, Novak AJ. An APRIL to remember: novel TNF ligands as therapeutic targets. Nat Rev 2006; 5:235-46.
3. Ingold K, Zumsteg A, Tardivel A, Huard B, Steiner QG, Cachero TG, et al. Identification of proteoglycans as the APRIL-specific binding partners. J Exp Med 2005; 201:1375-83. doi: 10.1084/jem.20042309
4. Endo T, Nishio M, Enzler T, Cottam HB, Fukuda T, Jameset DF, et al. BAFF and APRIL support chronic lymphocytic leukemia B-cell survival through activation of the canonical NF-kappaB pathway. Blood 2007; 109:703-10.
5. Nishio M, Endo T, Tsukada N, Ohata J, Kitada S, Reed JC, et al. Nurse-like cells express BAFF and APRIL which can promote survival of chronic lymphocytic leukemia cells via a paracrine pathway distinct from that of SDF-1alpha. Blood 2005; 106:1012-20.

6. Kay NE, Hamblin TJ, Jelinek DF, Dewald GW, Byrd JC, Farag S, et al. Chronic Lymphocytic Leukemia. Hematology Am Soc Hematol Educ Program (2002) 2002 (1):193-213. https://doi.org/10.1182/asheducation-2002.1.193
7. Montserrat E and Hillmen P. Chronic lymphocytic leukaemia and other chronic B-cell disorders. In: Hoffbrand V, Higgs DR, Keeling DM, Mehta AB (eds). Postgraduate Haematology. 7th edition. UK: Wiley Blackwell; 2016. p. 500-523.
8. Planelles L, Carvalho-Pinto CE, Hardenberg G, Smaniotto S, Savino W, Gómez-Caro R, et al. APRIL promotes B-1 cell associated neoplasm. Cancer Cell 2004;6:399-408. doi.org/10.1016/j.ccr.2004.08.033
9. Ferrer G, Hodgson K, Pereira A, Otero MJ, Elena M, Colomer D, et al. Combined analysis of levels of serum B-cell activating factor and a proliferation-inducing ligand as predictor of disease progression in patients with chronic lymphocytic leukemia. Leukemia & Lymphoma 2011;52:2064-68. DOI: 10.3109/10428194.2011.591008
10. Tecchio C, Nichele I, Mosna F, Zampieri F, Leso A, Al-Khaffaf A, et al. A proliferation-inducing ligand (APRIL) serum levels predict time to first treatment in patients affected by B-cell chronic lymphocytic leukemia. Eur J Haematol 2011; 87:228-34.
11. Junak A, Husb I, Chocholskab S, Szczepanekb E, Siekluckaa M, Dmoszyn A, et al. BAFF and APRIL expression in B-cell chronic lymphocytic leukemia: correlation with biological and clinical features. Leuk Res 2009;33:1319-27.
12. El Shora O, Shahbah A. Serum-soluble APRIL influences survival in patients with chronic lymphocytic leukemia. Egypt J Haematol 2016; 41:194-99.
13. Esatoglu SN, Keskin D, Kutnu M, Elverdi T, Salihoglu A, Eskazan AE. The prognostic value of serum APRIL levels in patients with chronic lymphocytic leukemia. Blood 2014;124:5648. https://doi.org/10.1182/blood.V124.21.5648.5648.
14. Shalaby NA, Eissa DSM, El-Said NMA. Evaluation of a proliferation-inducing ligand (APRIL) level in patients with b-cell chronic lymphocytic leukemia. Thesis submitted for partial fulfillment of Master Degree in Clinical Pathology, Faculty of Medicine, Ain Shams University; 2017. Available from: http://srv4.eulc.edu.eg/eulc_v5/Libraries/Thesis/BrowseThesisPages.aspx?fn=ThesisPicBody&BibID=12454066&TotalNoOfRecord=162&PageNo=1&PageDirection=First.