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Original article

Study of Philadelphia Like Acute Lymphoblastic Leukemia in Adult Patients using CRLF2 expression and JAK2 r683 mutation

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ABSTRACT:

Background: A Philadelphia-like acute lymphoblastic leukemia (Ph-like ALL) is a high-risk B-cell ALL subtype that is identified by a gene expression profile similar to Philadelphia chromosome-positive ALL, and lacking the BCR-ABL1 fusion. In this study, we analyzed molecular and clinical data to examine the coexistence and prognostic implications of CRLF2 overexpression and JAK2 r683 mutations in adult Ph-like ALL; we evaluate the potential utility of these biomarkers for risk stratification. **Methods:** In this study, we enrolled newly diagnosed fifty adult Ph-like ALL patients. Fluorescence In Situ Hybridization (FISH) was employed to evaluate CRLF2 expression, and Sanger sequencing was employed to identify JAK2 mutations. **Results:** The mean age of the 50 patients was 38.86 years, with a range of 18 to 64 years. Typically, 70% of the patients were male. Concerning CRLF2, only nine patients (18%) had expressed CRLF2 and all showed wild type JAK2 r6839 expression. CRLF2 demonstrated a significant connection with cytogenetic risk ($p < 0.003$). Moreover, CRLF2 manifested significant connection with relapse-free survival (RFS) and overall survival (OS), with positive CRLF2 correlating with reduced RFS and diminished OS, Positive CRLF2 significantly increase hazard of mortality by 7.54 folds while it significantly increases hazard of relapse by 6 folds.
Conclusion: CRLF2 has a prognostic role in adult Ph-like ALL, it may be used as a marker of bad prognosis together with other parameters to classify patients who need more aggressive therapy.
Keywords: cytokine receptor-like factor 2 (CRLF2), Ph-like ALL, JAK2 mutations.

INTRODUCTION

A prevailing childhood cancer, acute lymphoblastic leukemia (ALL), exhibited an elevated cure rate and favorable prognosis. Adults can also get ALL, albeit it happens less frequently and has a much worse prognosis due to numerous relapses. The poor prognosis of adult ALL is caused by a number of variables, including comorbidities, diminished performance level, worse compliance, and an elevated prevalence of high-risk genetic subgroups [1]
Philadelphia (Ph)-like, called BCR-ABL1-like ALL, experiencing prevalence about 15% of B-ALL, is a novel genetic subtype that shares clinical characteristics with Ph+ ALL, according to a 2009 study by Den Boer et al. The reported prevalence of this genetic subgroup of ALL differed significantly between the studies, hence its exact prevalence is still unknown. Similar to Ph+ chromosomal, studies have also indicated that Ph-like B-ALL serves as a prognostic indicator for elevated treatment failure rates, worse overall survival (OS) outcomes, and heightened minimum residual disease at the conclusion of induction therapy. Patients may be susceptible to tyrosine kinase inhibitors due to gene fusions or rearrangements that involve erythropoietin receptor, EPOR, ABL1/2, Janus kinase 2,JAK2, and platelet-derived growth factor receptor beta,PDGFRβ. Early detection of these genetic abnormalities

is crucial for treatment choices and prognosis [2]

The receptor protein encoded by the thymic stromal lymphopoietin receptor (TSLPR), also known as cytokine receptor-like factor 2 (CRLF2), is involved in activating STAT, potentially through JAK pathways. The control of the immune system depends on these pathways. A subpopulation of individuals with high-risk ALL who have an extraordinarily poor prognosis have been shown to have CRLF2 rearrangements and one recurrent mutation that causes CRLF2 overexpression. The CRLF2 gene encodes a subunit of the TSLPR, which is mostly expressed in early B- and T-cell progenitors, dendritic, and mast cells, located in the pseudoautosomal regions Xp22.3 and Yp11.3 [3]

CRLF2 alteration is a common anomaly (50–60%) and typically manifests in older individuals with greater white blood cell counts, unlike non-CRLF2-rearranged Ph-like ALL. Furthermore, compared to other ethnic groups, CRLF2 seemed to cluster in Hispanic patients (hispanic patients comprised 78% of those with CRLF2 overexpression) Immunoglobulin heavy locus, IGH-CRLF2 accounted for the bulk of rearrangements (57.6%-76%), then P2RY8-CRLF2 (17%-21%). Thymic stromal lymphopoietin (TSLP) heterodimeric receptors consist of the IL7R-alpha subunit and monomers of cytokine receptor-like factor 2, encoded by CRLF2 [4]. Interestingly, CRLF2 or IL7R mutations, supporting constitutive receptor dimerization and downstream JAK/STAT activation, are frequently present in CRLF2 rearrangement cases without JAK2 mutations. [5]

The majority of the JAK2 mutations in BCR-ABL are missense variants, grouped together in exon 16 of the pseudo kinase domain [6]. In contrast to the JAK2 V617F mutations associated with myeloproliferative neoplasms, the CRLF2 expression, in conjunction with JAK2 mutations observed in ALL, enables factor-independent transformation of cell lines in vitro [7].

Although the exact processes underlying CRLF2 overexpression in leukemia remain unclear, multiple studies indicate that it affects prognosis. Preclinical models have investigated JAK2 and mTOR inhibitors as possible treatments for CRLF2-rearranged ALL [8].

Severe outcomes in the Ph-like subtype of ALL are linked to genetic alterations affecting Janus kinase 2 (JAK2). The identification of activating JAK2 point mutations and JAK2 fusion genes in ALL signifies a notable progress for prospective targeted therapeutics, especially considering the effectiveness of kinase inhibitors in chronic myeloid leukemia treatment. However, the molecular mechanisms by which these alterations activate JAK2 and facilitate downstream signaling are mostly unexamined [9].

The high-risk Ph-like ALL subtype has been found to contain gain-of-function mutations in JAK2, which only occur in conjunction with CRLF2 rearrangements (CRLF2r), resulting in CRLF2 overexpression. Moreover, roughly half of Ph-like ALL individuals have activating point mutations in JAK1 or JAK2, and about 50% of these patients have CRLF2 overexpression [10].

Mutants such as JAK2 R683G disrupt the autoinhibitory connection between the kinase domain and JH2, as evidenced by the mutant-JAK2 activation. The transition of JAK2 from a suppressed, quiescent state to a partially active one has the potential to facilitate malignant transformation. The molecular activation mechanisms of ALL-linked JAK2 R683 mutations remain incompletely elucidated; nevertheless, further structural investigations may enhance future pharmacological strategies aimed at targeting JAK2 R683-mutant JAK2 [8].

So, in this study we hypothesize that CRLF2 overexpression and JAK2 R683G mutations are ated with poorer prognosis in adult Ph-like ALL.

METHODS

Patients:

A cohort study includes fifty newly Ph Negative B- cell ALL adult patients from Oncology Unit Zagazig University Hospitals and Clinical Pathology Department during the period from May 2021 to January 2024, with a minimum follow-up duration of 24 months. ..The rate from 4 to 5 cases per month the sample size was 50patients in the study period.

Ethical consideration:

Patients were informed of the procedure and any risks, and their written informed consent was acquired. The Ethical Committee of Zagazig University Faculty of Medicine approved this work (IRB approval number 4907-6 11 2018). The study followed the ethical guidelines of the Declaration of Helsinki.

Inclusion criteria: Newly diagnosed Ph Negative B-cell type ALL patients aged from 18 to 65 years were included in the study **while** Pediatrics and pregnant female**,** Relapsed or refractory disease, T-Cell type of ALL and Ph positive B-cell type ALL patients were excluded.

Initial assessment for all patients

Sampling: Under complete aseptic conditions 2ml of venous blood was collected on EDTA tubes with good mixing for CBC and reticulocyte counting and JAK2 analysis by sequencing, 10 ml of venous blood collected on heparin tubes for conventional karyotyping for Ph chromosome, 1.6 ml venous blood collected on sodium citrate tubes for ESR. Bone marrow aspirate and 300 ul of BM samples are collected for Immunophenotyping and stained blood smears using leishman stain for counting and assessment of morphology. One ml of BM sample was collected on heparin tubes for analysis of CRLF2 by FISH.

All patients will be subjected during Initial assessment to: **Full history**, **Complete physical examination** and **Laboratory investigation including: Hematological investigation:** Complete blood picture and peripheral blood film CBC were performed on an automated cell counter, model XN 2000 (Sysmex, Japan) and reticulocyte count. ESR was done by Westergren method. Bone marrow examination and Immunophenotyping (BD, FACSCalibur; San Jose, California, USA) (Cell Quest software (BD Biosciences). Chromosomal Study was by various techniques (Conventional Karyotyping, FISH for t 9,22 to exclude Ph positive cases).

- **CRLF2 rearrangement detection by FISH:**

Principle: In fixed cytogenetic samples, Fluorescence In Situ Hybridization (FISH) is employed to detect DNA sequences on metaphase chromosomes or within interphase nuclei. This technique is a potent adjunct to traditional cytogenetics, as it employs DNA probes that hybridize to entire chromosomes or single, distinctive sequences. The target DNA is prepared for annealing with a fluorescently labeled DNA probe that has a complementary sequence and is also denatured after fixation and denaturation. The DNA is counterstained for visualization after unbound and non-specifically bound DNA probes are removed following hybridization. The hybridized probe can be observed on the target substrate using fluorescence microscopy.

Probe Specification: CRLF2., Breakapart probe (Cytocell®, Cambridge, UK) CRLF2, Xp22.33/Yp11.32, Green**.,** CRLF2, Xp22.33/Yp11.32, Red

The probes are provided premixed in hybridization solution (Formamide; Dextran Sulphate; saline sodium citrate, SSC) and are ready to use. For optimal visualisation of the probe, A 100-watt mercury lamp and plan apochromat objectives of 63x or 100x magnification were used. The triple bandpass filter DAPI/FITC/TRITC was utilized for simultaneous observation of all fluorophores and DAPI on the BX63F image microscope.

Expected Results: Two red/green signals (which may be represented as yellow, Y) are anticipated in a normal cell (2Y). A translocation will generate one red, green, and yellow signal. The translocated chromosomes will provide one green and one red signal, but the normal chromosome will yield a fused red/green signal that may manifest as yellow. Cells displaying a solitary green signal and a combined red/green signal (perhaps seeming yellow) may experience intrachromosomal loss, resulting in producing the P2RY8-CRLF2 fusion gene.

- **JAK2r683g mutation detection by sequencing:**

Genomic DNA was obtained from anticoagulated whole blood samples utilizing (QIAamp DNA Blood Mini Kits). PCR amplicons were generated using the following primer pairs for JAK2 E16: Forward Sequence 5' -> 3' CTCAATGCATGCCTCCAA Reverse Sequence 5' -> 3' ACAACATGCCCTTTACACC **[11].** PCR

amplified products were first purified with QIAquick gel extraction kit (Qiagen Inc. Valencia CA) and then DNA cycle sequencing of the purified PCR product was done with Bigdye Terminator V3.1 cycle sequencing kit (Perkin-Elmer, Foster city, CA) cat-number 4336817 then secondary purificating of the sequencing reaction was done by Centrisep (spin column) purification Kit CS-901 for one hundred reactions. The DNA sequencing was performed with the Applied Biosystems 3130 automated DNA sequencer (ABI, 3130, USA). Based on sequence analysis for JAK2 on Gene Bank site: JAK2r683g mutation was negative in all positive cases of CRLF2 (all samples of JAK2 at position 683 were (A) base pair).

Statistical analysis

Data analysis was conducted with SPSS (Statistical Package for the Social Sciences) version 28. Categorical variables were defined by their absolute frequencies and compared utilizing the chi-square and Fisher's exact tests when appropriate. The Kolmogorov–Smirnov test was employed to validate the assumptions necessary for parametric tests. Independent sample t-tests were used for normally distributed data, while Mann-Whitney tests were utilized for non-normally distributed data to compare quantitative data between two groups. Survival analysis and the Kaplan-Meier plot were utilized to measure the proportion of individuals surviving for a certain length post-treatment and to analyze the anticipated time until the occurrence of a singular event, either death or recurrence. The threshold for significance was deemed at 5% (P<0.05).

RESULTS

Subjects are classified into two subgroups based on the results of CRLF2: 41 patients (82%) are negative and 9 patients (18%) are positive. All of the nine patients showed wild JAK2 r6839 expression **(Table 1)**.

As regard **(Table 2),** CRFL2 was more with older age, 38.86 ± 14.18 (P value 0.04). CRLF2 did not reveal significant correlation with gender of patients (P value: 0.7). The patients with positive CRLF2 manifested significantly elevated lymphadenopathy (P value: < 0.001). CRLF2 did not demonstrate significant correlation with either fever or organomegaly (P value > 0.05). There is significant elevation of hemoglobin, WBCs and ESR, (P value: 0.04, P value: <0.001 and Pvalue:0.038 respectively) among positive CRLF2 while is statistically non-significant relation between CRLF2 and all BM blast cells, nor platelet count (P vale: 0.77 and 0.94 respectively).

The CRLF2 revealed a significant relation with cytogenetic risk, (P value: 0.003) (poor risk significantly associated with positive expression). The correlation between CRLF2 and immunophenotyping by FCM is non-significant (P value: 0.477) **(Table 3).**

There is significant relation connection positive CRLF2 and death (P value: 0.021). Positive CRLF2 significantly increases risk of mortality by 7.54 folds. However, positive CRLF2 did not reveal significant correlation with remission or relapse. The positive CRLF2 status non-significantly elevates the risk of relapse by sixfold (P value: 0.095) **(Table 4).**

A significant correlation exists between CRLF2 and RFS, with positive CRLF2 correlating with mitigated RFS and significant connection between CRLF2 and OS (positive CRLF2 is significantly associated with lower OS) **(Table 5) (Figure 1).**

Table (1) Distribution of studied patients based on CRLF2 expression:

	N=50	**%**
CRLF2		
Negative	41	82%
Positive	9	18%
JAK2 r6839	N=9	
Wild	9	100%
Mutant	0	0

Table (2) Relation between CRLF2 expression and demographic, clinical and laboratory data:

	Negative N=41 (%)	**Positive N=9 (%)**	χ^2	**P**
Gender				
Female	13 (31.7%)	2 (22.2%)	Fisher	0.705
Male	28 (68.3%)	7 (77.8%)		
	Mean ± SD	**Mean ± SD**	T	P
Age (year)	36.95 ± 13.7	47.56 ± 13.76	-2.01	0.041*
Organomegaly				
Absent	27 (65.9%)	4 (44.4%)		
Spleen	8 (19.5%)	4 (44.4%)	3.813	0.282
Liver	4 (9.8%)	0 (0%)		
Liver and spleen	2 (4.9%)	1 (11.1%)		
Lymphadenopathy				
Absent	38 (92.7%)	3 (33.3%)	Fisher	<0.001**
Present	3 (7.3%)	6 (66.7%)		
Fever				
Absent	17 (41.5%)	1 (11.1%)	Fisher	0.13
Present	24 (58.5%)	8 (88.9%)		
Hemoglobin (g/dl)	9.02 ± 2.09	8.36 ± 2.15	-2.101	0.041*
	Median (IQR)	**Median (IQR)**	Z	P
WBCs ($10^3/mm^3$)	11(2.85 – 29)	65(51 – 147.5)	-4.155	<0.001**
BM blast cell	72(52 – 88.5)	67(45.5 – 85)	-0.291	0.771
Platelet count ($10^3/mm^3$)	35(14 – 67.5)	34(22 – 51)	-0.076	0.94
ESR at 1st hour (mm/hr)	80(64 – 95.5)	102(77.5 – 120)	-2.072	0.038*

*p < 0.05: significant; p ≤ 0.001: highly significant. Statistical tests: independent sample t-test, χ^2 (Chi-square), and Z (Mann–Whitney) test.

Table (3) Relation between CRLF2 expression and result of flowcytometry and cytogenetic risk:

	Negative N=41 (%)	**Positive N=9 (%)**	χ^2	**p**
Flowcytometry				
C-ALL	27 (65.9%)	6 (66.7%)	1.61	0.477
Pre B	13 (31.7%)	2 (22.2%)		
Pro-B	1 (2.4%)	1 (11.1%)		
Cytogenetic risk				
Poor	5 (12.2%)	8 (88.9%)		
Intermediate	11 (26.8%)	1 (11.1%)	14.303	0.003*
Standard	25 (60.9%)	0 (0%)		

*p<0.05 is statistically significant t independent sample t test χ^2Chi square test

Table (4) Relation between CRLF2 expression and outcome

	Negative N=41 (%)	**Positive N=9 (%)**	χ^2	**P**
Remission				
Absent (resistant)	11 (26.8%)	4 (44.4%)	Fisher	0.423
Present	30 (73.2%)	5 (55.6%)		
COR (95% CI)	2.18(0.49 – 9.64)			
Relapse				
Absent	24 (80%)	2 (40%)	Fisher	0.095
Present	6 (20%)	3 (60%)		
COR (95% CI)	6 (0.81 – 44.35)			
Death				
Absent	28 (68.3%)	2 (22.2%)	Fisher	0.021*
Present	13 (31.7%)	7 (77.8%)		
COR (95% CI)	7.54(1.37 – 41.41)			

χ^2Chi square test COR odds ratio CI Confidence interval *p<0.05 is statistically significant

Table (5): Kaplan– Meier survival curves illustrating relapse free survival and overall survival time crude differences in patients as regard CRLF2expressions

CRLF2	**Total N**	**N of relapsers**	**Censored**		**Survival time, Months**		**P**
			N	**%**	**Mean**		
					Estimate ±SD	**95% CI**	
Negative	30	6	24	80%	84.18 ± 7.16	70.14– 98.21	0.035*
Positive	5	3	2	40%	28.25 ± 9.37	9.89 – 46.62	
Overall	35	9	26	74.3%	78.14± 7.28	63.88 – 92.4	
CRLF2	**Total N**	**N of deaths**	**Censored**		**Survival time, Months**		**P**
			N	**%**	**Mean**		
					Estimate ±SD	**95% CI**	
Negative	41	13	28	68.3%	79.93 ± 6.01	68.15 – 91.71	<0.001**
Positive	9	7	2	22.2%	26.06 ± 8.92	8.58 – 43.53	
Overall	50	20	30	60%	70.52 ± 6.09	58.58 – 82.54	

*p<0.05 is statistically significant p for Mantel cox

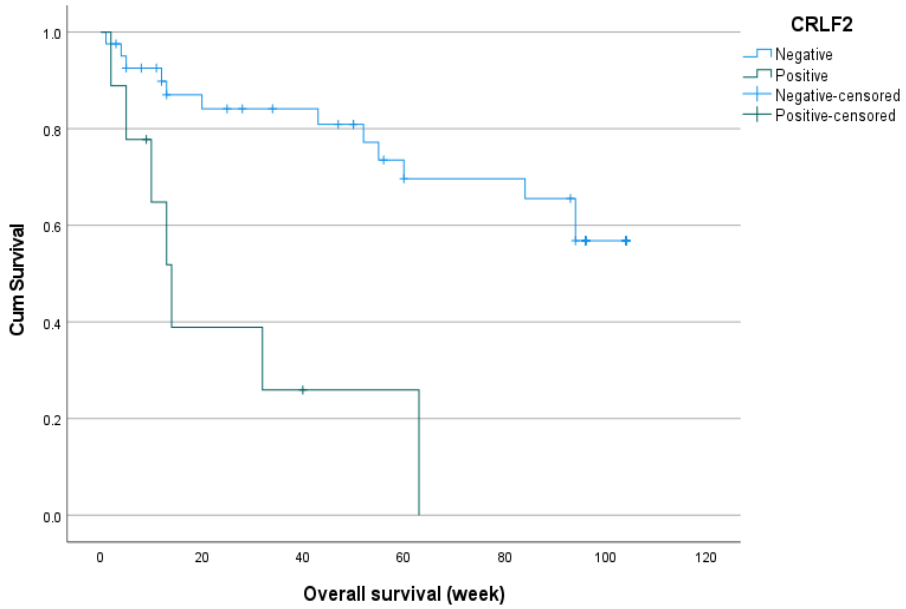

Figure (1) Kaplan Meier plot showing relation between CRLF2 and overall survival

DISCUSSION

The aggressive type of B-ALL cancer, Ph-like ALL, exhibited a poor prognosis and a high relapse rate. Although resistant B-ALL has responded well to targeted therapies versus the cell surface proteins CD22 or 19, patients may relapse as a result of antigen loss, thus alternative antigens must be targeted. Fifty percent of Ph-like ALL patients had overexpression of the cytokine receptor-like CRFL2 [12].

Ph-like ALL is classified into seven molecular subtypes, the most prevalent of which are CRLF2 and ABL-class rearrangements. These subtypes differ in specific downstream signaling cascades. Utilizing FISH, whole transcriptome sequencing, and PCR enables a comprehensive understanding of ALL cytogenetics and molecular biology to investigate the function of targeted medicines in such uncommon clinical situations, more research is required. [10].

A precise prognostic evaluation is essential to ALL treatment. Among clinical variables, poor performance status and advanced age are linked to reduced OS and complete remission (CR) rates. The single best predictor of CR and OS in ALL is cytogenetic alterations and genetic traits, even though clinical variables play a significant role in directing treatment. [13].

The assessment of newly diagnosed B-cell ALL patients should include an effort to identify the Ph-like phenotype. A conclusive diagnosis should depend on the detection of a genetic anomaly in the cell signaling-related gene rather than on the gene expression phenotype. RNA sequencing enables the identification of a Ph-like phenotype and a comprehensive analysis of atypical translocations; nevertheless, this method is technically intricate and unavailable in most centers. One screening method involves identifying a certain phenotype using a restricted selection of genes. A panel of FISH probes or PCR assays targeting the prevalent ABL, JAK/EPOR, and CRLF2 translocations may serve as a screening method [14].

Here, we assessed the prognostic importance of CRLF2 rearrangement, 50 Ph negative adult ALL patients from Zagazig university hospitals were tested for CRLF2 by FISH and positive cases for CRLF2 were tested by sequencing to search for JAK2 r683g mutation. This study from May 2021 to January 2024, including a follow-up duration of two years.

In the present study, 18% of the patients had CRLF2 rearrangement and 82 % without CRLF2 rearrangement. This percentage is similar to the study by Hasssan, et al in Egyptian National Cancer Institute in Egypt, which reported 18.3% positive CRLF2 in

pediatric patients [15]. Also, Chen, et al., [16] and Yano, et al., [17] revealed 17-18% of B-ALL patients experienced CRLF2 overexpression. Kamal, et al at Alexandria university in Egypt reported positive CRLF2 in 56.7% of pediatric ALL instances [18]. In adults with BCP ALL, Herold et al. [19] manifested an incidence of 13% of Ph-like ALL within it CRLF2 rearrangement was (6 out of 16; *P*=0.002). Cario et al., [20] and Palmi et al., [21] reported lower prevalence rates of CRLF2 over expression 5% and 9% respectively.

Herein, the patient age spanned from 18 to 65 years, with a mean age of 26.5 years. Patients experiencing CRLF2 rearrangement were significantly older (mean: 47.5 years), compared to patients without CRLF2 rearrangement (mean: 36.9 years, P = 0.041), aligning with Vesely et al. [22].

Herein, no significant difference in CRLF2 gene expression between males and females was found. This result is in concordance with Asai. et al., [23] and Pastorczak. et al., [24] who studied its expression on 386 patients.

Higher WBC counts were in positive CRLF2 at presentation than non-CRLF2 ALL, statistically significant (median: 65 vs. 11 x 10^3/uL, P = <0.001), aligned with outcomes of Jain et al, revealing a significant connection between CRLF2 mutation and WBC count [25]. However, Pastorczak. et al., [24] and Asai. et al., [23] manifested no significant difference in WBCs count between high and low CRLF2 gene expression (p=0.91, p=0.87respectevly).

In this study, hemoglobin levels were mitigated in CRLF2 positive patients, unlike negative CRLF2 mutation (P = 0.041)

Our outcomes are different from the findings of Jain et al., [25], reporting an insignificant correlation of CRLF2 mutation with baseline hemoglobin level., this may be due to their study in sample size and patients populations.

Here, there is significant connection between CRLF2 and RFS and OS (positive CRLF2 significantly linked to mitigated RFS and OS). Positive CRLF2 significantly increase hazard of mortality by 7.54 folds while it significantly increases hazard of relapse by 6 folds. Aligning with the outcomes of Harvey et al [26], who indicated that CRLF2 rearrangement and overexpression were linked to unfavorable prognoses, with a 4-year RFS rate of 35% for CRLF2 changes compared to 71% for cases without such changes. Furthermore, a research conducted by Boer et al. [27] evaluated patients from multiple Dutch-Belgian HOVON trials and found that EFS and OS were worse in the Ph-like ALL subgroup compared to the other B-ALL subgroup; nevertheless, the observed difference was not significant, aligning with Harvey et al. [28], who reported that patients positive for CRLF2 had much worse 5-year event-free survival (EFS; 63.3% ±3.1 vs 82.1% ±0.7, p<0.0001) and OS (79.6% ±2.6 vs 90.5% ±0.6, p<0.0001), unlike those negative for CRLF2. In contrast to the current investigation, Palmi et al. [21] exhibited that CRLF2 overexpression did not impact OS in pediatric ALL (p = 0.35)., this may be due to different age group in this study where pediatric patients were included.

Based on sequence analysis for JAK2, JAK2r683g was negative in all positive cases of CRLF2, this outcome may be attributed to the limited number of patients in the current study. Likewise, Dou et al. [29] could not identify JAK2 mutations in the whole group of 271 Chinese children. In contrary to the present study Hasssan, et al [15] reported (6.1%) JAK2 R683G mutation in their study in the Egyptian National Cancer Institute. In 16 out of 187 (8.6%) of BCR-ABL1 negative, high-risk pediatric ALL patients, Mullighan et al. [30] identified JAK2 mutations. The amino acid residue R683 is essential for the negative autoregulation of JAK2 activity within the JH2 domain, and it was present in 13 of the 16 mutant instances. Steeghs et al. [31] reported that JAK2 mutations were present in 3.5% (16/461) of the BCP ALL patients. Cario et al. [20] found the JAK2 R683G mutation in one of 49 (2%) pediatric patients who exhibited elevated CRLF2 expression. The differences in frequency may be attributed to the existence of other mutation sites within JAK2, apart from the JAK2 R683G site, as well as the limited sample size in the current study.

The limitation of this study was a small number of individuals involved, so we recommend involving large number of

patients and using more long base pair fragment primers to increase the chance for detection of JAK2 r683g mutation or other mutations in JAK2.

CONCLUSIONS

CRLF2 expression upon diagnosis significantly influenced the clinical outcome in Adult Ph-like ALL. CRLF2 gene expression level was found to be correlated with risk stratification, high WBCs, OS and RFS. Hence it may have a prognostic role in adult Ph-like ALL, it may be used as a marker of bad prognosis together with other parameters to classify patients who need more aggressive therapy.

Author Contributions: E.I.A selected the study idea, designed the experimental procedures, and revised the findings; H.S.G, N.G.A, A.M.A, and E.M.A. gathered, analyzed, and evaluated the patient data, encompassing practical investigations; A.A.E. recruited and prepared the patients for the study; all authors have reviewed, revised, and agreed to the published version of the manuscript.

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