ISSN NO. 2320-5407

Journal homepage: http://www.journalijar.com

INTERNATIONAL JOURNAL OF ADVANCED RESEARCH

RESEARCH ARTICLE

Expression and prognostic impact of caspase 8 associated protein 2 (CASP8AP2) in childhood acute lymphoblastic leukemia.

Dr. Adel Marzouk Ali Agha[1], Dr. Safia Mohammed Diab[2], Dr. Doaa Mahmoud El-Ghannam[3], Dr. Mona Mohamed El- Behisy[4], *Amira Ahmed Shawky Abd El- Ghany[5].

1. Prof. of Clinical & Chemical Pathology, Benha Faculty of Medicine, Egypt.
2. Prof. of Clinical &Chemical Pathology, Benha Faculty of Medicine,Egypt.
3. Prof. of Clinical & Chemical Pathology, Mansoura Faculty of Medicine, Egypt.
4. Lecturer of Clinical & Chemical Pathology, Benha Faculty of Medicine, Egypt.
5. MSC of Clinical & Chemical Pathology, Mansoura student hospital, Egypt.

Manuscript Info

Manuscript History:

Received: 17 January 2016
Final Accepted: 29 February 2016
Published Online: March 2016

Key words:
Childhood leukemia, Acute lymphoblastic leukemia, CASP8AP2, Prognostic factor, Bone marrow relapse.

Corresponding Author

Amira Ahmed Shawky Abd El- Ghany.

Abstract

Objective: To measure the expression level of caspase 8 associated protein 2 (CASP8AP2) in newly diagnosed bone marrow childhood acute lymphoblastic leukemia (ALL) samples and analyze its associations with bone marrow relapse, disease free survival (DFS) as well as overall survival (OS).

Materials and Methods: Fifty newly diagnosed ALL cases were evaluated using clinical and laboratory measures. CASP8AP2 expression was measured in bone marrow samples using real-time quantitative PCR (qRT-PCR). Another 25 blood samples from healthy reference control subjects with matched age and sex were also measured for CASP8AP2 expression.

Results: Patients with high CASP8AP2 expression have significantly better OS (p value = 0.017) and DFS (p value = 0.018) than those with low CASP8AP2 expression. There was a significantly high bone marrow relapse rate in CASP8AP2 low-expression group with (p value = 0.018). From receiver operating characteristic (ROC) curve, the best cut-off values were established for CASP8AP2 for the prediction of development of relapse in the present study group was 0.319, with a sensitivity of 100% and a specificity of 91.3%. The area under the curve was 0.916.

Conclusion: Relapse showed statistically low CASP8AP2 expression versus those who continued complete remission (CCR). So, CASP8AP2 expression was reported as an independent prognostic factor in childhood ALL.

.

Introduction:-

Leukemia is the most common cause of childhood malignancy under the age of 15 years with an annual incidence rate of 43 cases per million, leukemia represents 31% of all cancer cases occurring among children younger than 15 years of age : more than 75% of these cases are acute lymphoblastic leukemia (ALL). Boys are slightly more frequently affected than girls (sex ratio 1.3:1.0) for ALL **[1]**.

The outcome of childhood acute leukemia shows dramatic improvement over the last decades. ALL achieves an overall survival of over 80%. This success was achieved, in part, through the implementation of risk-stratified therapy. Patients presenting with features that are associated with a higher risk of relapse receive more intensive treatment, while those with features linked to a more favorable outcome are treated with more modest, less toxic therapy **[2].**

As a result of the accumulation of knowledge on the molecular biology of malignancies, new diagnostic modalities are beginning to be incorporated into diagnostic and therapeutic strategies**.** One of these modalities is the real time quantitative reverse transcriptase polymerase chain reaction (qRT-PCR) that allows the determination of messenger RNA expression levels and, therefore, allows researchers to examine the expression patterns of a large number of genes at the RNA level **[3,4].**

Caspase-8 associated protein 2 (*CASP8AP2)* is located at 6q15, also known as Fas-associated death domain containing protein (FADD)-like IL-1-converting enzyme (FLICE) associated huge protein (FLASH), CED-4, KIAA1315 or RIP25 **[5]**, encodes a protein with multiple functions; although it has been traditionally recognized as a key mediator of apoptosis, several studies have demonstrated that also participates in cell division, nuclear factor kappaB (NF-Kb) signaling, c-Myb activation, S phase progression, histone transcription and 3´-end maturation of histone mRNAs. CASP8AP2 interacts with the death-effector domain (DED) of caspase 8 and hence it plays an important regulatory role in Fas-mediated apoptosis **[6].**

In this study, we measured the expression of CASP8AP2 in 50 newly diagnosed bone marrow samples using qRT-PCR, and analyzed its associations with bone marrow relapse, disease free survival as well as overall survival in pediatric ALL.

Materials and methods:-

Patients:-

This prospective study was carried out on 50 childhood ALL cases; 31 males and 19 females; their ages ranged from 1year to 14 years (mean 6.52±3.808) admitted to Children Hospital, Faculty of Medicine, Mansoura University with ≥ 70% leukemic cells in diagnostic bone marrow samples . They were subclassified according to FAB subtype; 46 B ALL and 4 T-ALL and had been followed up until remission or death for periods up to 24 month during 2013 to 2015. Patients with history of exposure to chemotherapy/ radiotherapy and secondary ALL patients were excluded from this study. Another 25 healthy reference control subjects with matched sex (15 males and 10 females) and age (1-13 years, mean 5.24±3.745 years) enrolled in this study.

An informed consent was obtained from the legal guardian of each patient or control before enrollment. This study was approved by the ethical committee of Benha University.

All patients were subjected to a thorough assessment of history, complete clinical examination, routine chemical investigations **[7]**, complete blood count, bone marrow examination, immunophenotyping (cMPO, CD13, CD33, CD34, CD10, CD19, CD22, CD3, CD7, CD20 and HLA-DR)**[8]**.

Relative CASP8AP2 gene expression evaluation using quantitative real- time polymerase chain reaction (qRT-PCR):-

- **Purification of cellular mRNA from patient's blood.**

For extraction of RNA from blood, QIAamp RNA Blood Mini kit (QIAGEN GmbH, Hilden, Germany) is used. Erythrocytes are selectively lysed and leukocytes are recovered by centrifugation. The leukocytes are then lysed using highly denaturing conditions that immediately inactivate RNases, allowing the isolation of intact RNA. After homogenization of the lysate by a brief centrifugation through a QIAshredder spin column, ethanol is added to adjust binding conditions and the sample is applied to the QIAamp spin column. RNA is bound to the silica membrane during a brief centrifugation step. Contaminants are washed away and total RNA is eluted in 30 µl or more of RNase-free water for direct use in any downstream application. Since the procedure relies on intact leukocytes, frozen blood cannot be used. RNA extract can be stored under -25 ºC to be used later **[9, 10].**

- **RNA reverse transcription (RT).**

To synthesize single complementary strand of DNA (cDNA) from total RNA we used High Capacity cDNA Reverse Transcription Kit (Applied Biosystem, Foster City, CA, USA). After thermal cycle end, the cDNA of the extracted RNA is then produced **[11].**

- **Primers and TaqMan probe.**

Sequence Detection Primers (forward and reverse) for target CASP8AP2 gene (900 nM) each and TaqMan probe for CASP8AP2 (250 nM) were designed using TaqMan® Gene Expression Master Mix kit (Applied Biosystem, Foster City, CA, USA) **[11].**

- ✓ Forward primer: 5´CACTTGCCACTTCTACAAGTC-3´.
- ✓ Reverse primer: 5´TGGCGGCTAAATATGCAAATG-3´.
- ✓ TaqMan® Probe for the target CASP8AP2 gene: 5´FAM-TGTCAGAAAAGAGGGCCATCATTTAAA-TAMRA3´.

- **Quantitative RT-PCR (qRT-PCR) of CASP8AP2.**

Quantitative RT-PCR reactions and fluorescence measurements were performed on an ABI PRISM® 7000 real-time PCR system (PE Applied Biosystems, USA). A relative quantification of CASP8AP2 gene expression was normalized to the endogenous housekeeping gene glyceraldehyde-3-phosphate dehydrogenase (GAPDH). The *GAPDH* gene was used as the internal control and the quantification of relative expression was performed by real-time PCR using real-time cycler, 1.125 pmol of each primer, 1.0 pmol probe and 5 µL cDNAs. The reaction was carried out at 95 ◦C for 15 min, followed by 50 cycles of 15 s at 95 ◦C and 1 min at 60 ◦C. GAPDH was amplified as an internal control to check the quantity and quality of the cDNAs in the assay. The ratio of CASP8AP2/ GAPDH was calculated as the expression index of CASP8AP2 **[12].**

The comparative cycle threshold (*C*t) method was used to determine the relative expression levels of CASP8AP2. The mean of the cycle number difference was calculated. Data were presented as $2^{-\Delta\Delta Ct}$, where *C*t is the threshold cycle and Δ*C*t is the *C*t value of target amplification minus that of reference amplification (Δ*C*t = GAPDH− CASP8AP2) **[12].** Using the $-\Delta\Delta Ct$, a relative quantification in the means of fold change [fold change $2(-\Delta\Delta Ct)$ $2(-\Delta\Delta$ Ct)] is measured using a reference of 1 for the control **[10].**

Statistical analysis:-

The statistical analysis of data was done by using ***excel*** program and ***SPSS*** (statistical package for social science) program (SPSS, Inc, Chicago, IL) version 16. Kolmogorov-Smirnov test was done to test the normality of data distribution. Significant data was considered to be nonparametric. Qualitative data were presented as frequency and percentage. Chi square test was used to compare groups. Quantitative data were presented as mean and standard deviation. For comparison between two groups; student t-test, and Mann-whitney test (for non parametric data) were used. For comparison between more than two groups; ANOVA and Kruskal wallis (for non parametric data) were used. Kaplan–Meier test was used for survival analysis and the statistical significance of differences among curves was determined by Log-Rank test. Prediction of survival was done using multivariate analysis applying age, TLC and CASP8AP2 expression state as covariates. Receiver operating characteristic (ROC) curve used to assess the prognostic effect of CASP8AP2 for bone marrow relapse. *P* value less than 0.05 indicated statistical significance.

Results:-

Delta cycle threshold (△CT) values of CASP8AP2 gene and CASP8AP2 gene expression ratio values between studied groups:-

There was significant differences between cases and control regarding △CT and expression of CASP8AP2. Median CASP8AP2 expression in ALL group was 1.080 which was low as regard control 34.3 with (p value < 0.001). ALL group was classified according to median expression into high and low groups **(table 1).**

Table (1). Statistical comparison according to △CT values of CASP8AP2 gene and CASP8AP2 gene expression ratio values between studied groups.

Caspase gene expression values	ALL (n=50)				Control (n=25)				*p
	Mean	SD	Median	Range	Mean	SD	Median	Range	
△CT of CASP8AP2 gene	27.639	3.358	26.875	24.4-45	4.0012	1.89170	5.1	0.58-5.5	<0.001
CASP8AP2 gene expression	5.614	13.508	1.080	$1X10^{-6}$ - 64.89	26.793	17.632	34.3	1.49-45.25	<0.001

*P < 0.05 is significant .

Receiver Operating Characteristic curve (ROC) analysis was conducted to identify the optimal CASP8AP2 expression level for potential prediction of development of relapse within ALL patients. From this curve, the best cut-off values were established for CASP8AP2, for the prediction of development of relapse in the present study group was 0.319, with a sensitivity of 100% and a specificity of 91.3%. The area under the curve was 0.916.

There was a significantly high bone marrow relapse rate in CASP8AP2 low-expression group with (p value = 0.018). A poor OS was found in the CASP8AP2 low-expression group (p = 0.017; Fig.1), as well as a poor DFS (p = 0.018; Fig.2). The findings indicated that the expression of CASP8AP2 detected in diagnosed bone marrow samples could be a useful indicator for bone marrow relapse.

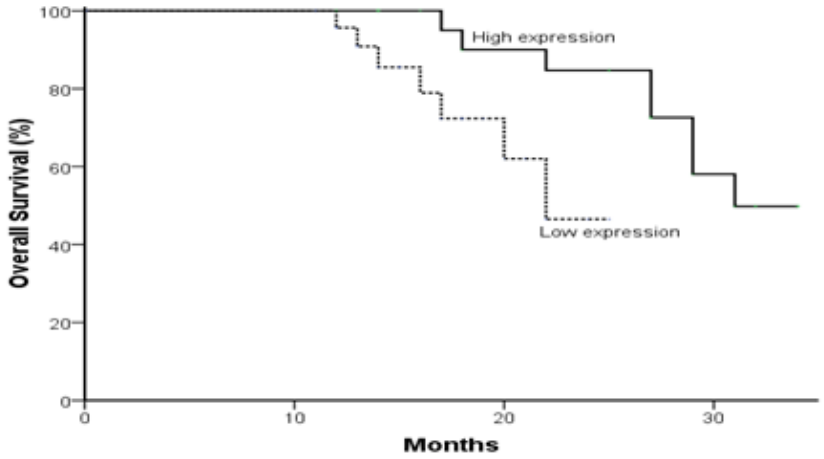

Figure (1). Overall survival according to CASP8AP2 expression in ALL patients.

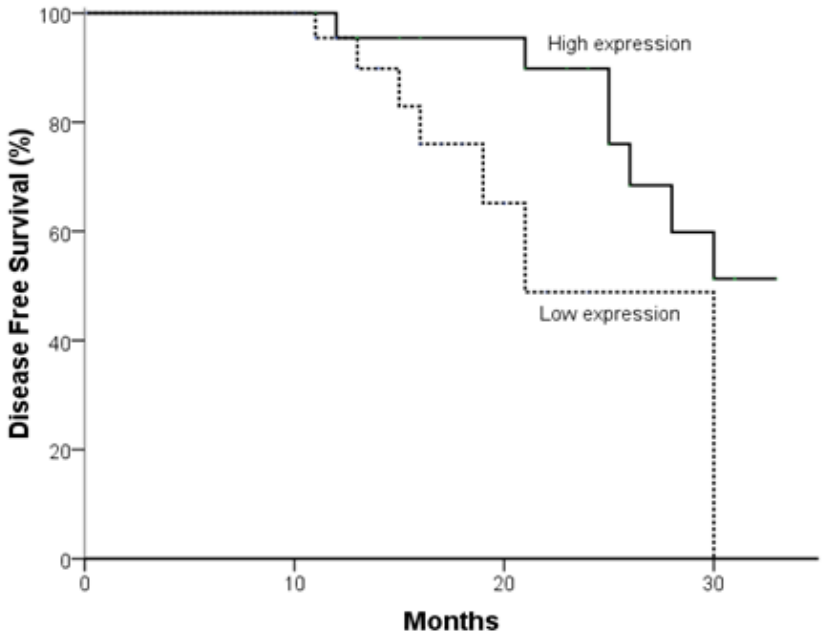

Figure (2). Disease free survival according to CASP8AP2 expression in ALL patients.

Statistical comparison of mean CASP8AP2 expression between cases and control regarding clinical data:-

No statistical differences were found between mean CASP8AP2 expression regarding age, sex, clinical presentation, laboratory data, immunophenotyping and BCR- ABL with (p value > 0.05 for each). Moreover, no statistical differences were found between number of cases in low and high caspase expression with (p value > 0.05 for each) **(table 2)**.

(Table2)The relationships of CASP8AP2 to common clinicobiological features.

Features	Total number	High CASP8AP2 expression	Low CASP8AP2 expression	* P value
*** Age**				
< 1 y	2	0	2	
1-9y	37	18	19	0.241
>= 10y	11	7	4	
*** Gender**				
Male	31	13	18	0.244
Female	19	12	7	
***WBC**				
<50x109/L	25	12	12	0.889
>=50x109/L	25	13	13	
***Immunophenotype**				
B-ALL	46	23	23	1.000
T-ALL	4	2	2	
***CNS involvement**				
No	48	24	24	1.000
Yes	2	1	1	
***Testicular involvement**				
No	48	25	23	
Yes	2	0	2	1.000
***Treatment response**				
Good	33	17	16	0.941
Poor	17	8	9	
***BCR-ABL**				
Positive	2	0	2	0.490
Negative	48	25	23	

*P < 0.05 is significant

Multivariate analyses for DFS and OS:-

Prediction of survival was done using multivariate analysis applying age, sex, TLC, BM blasts, CNS involvement, Testicular involvement, BCR-ABL, Treatment response and CASP8AP2 expression as covariates. Multivariate analysis showed that high Caspase expression is afavorable prognostic factor for DFS and OS (p=0.032, HR (hazard ratio) =0.265, 95% CI (Confidence interval) = 0.078-0.894; p=0.022, HR=0.191, 95% CI=0.046-0.785 respectively), otherwise, multivariate analysis did not show any significant differences in DFS and OS regarding any other covariate with (p value > 0.05) **(table 3).**

Table (3). Multivariate analysis for DFS and OS as dependent parameters studied with other covariates

Covariates	DFS				OS			
	*P	**HR	‖95% CI		*P	**HR	‖95% CI	
Age (years)	0.920	1.008	0.870	1.167	0.646	0.968	0.840	1.114
Sex (Males versus females)	0.867	0.907	0.288	2.853	0.191	0.489	0.167	1.428
TLC ($x10^9$/L)	0.307	1.005	0.996	1.014	0.511	1.003	0.994	1.012
BM blasts (%)	0.282	1.036	0.972	1.104	0.286	1.029	0.976	1.085
CNS involvement	0.720	1.106	0.770	2.877	0.654	0.534	0.187	1.534
Testicular involvement	0.756	0.132	0.654	1.645	0.224	0.654	0.345	2.765
BCR-ABL	0.534	1.756	0.245	2.109	0.234	1.876	0.354	1.987
Treatment response	0.654	1.987	0.985	2.765	0.654	1.264	0.954	1.165
Caspase expression (High versus low)	0.032	0.265	0.078	0.894	0.022	0.191	0.046	0.785

*P < 0.05 is significant, **HR(hazard ratio),‖ Confidence interval at 95%.

Discussion:-

Acute Lymphoblastic Leukemia is a malignant disorder of lymphoid progenitor cells that proliferate and replace the normal hematopoietic cells of the bone marrow resulting in a marked decrease in normal blood cell production **[13].**

The expression of CASP8AP2 was reported as an independent prognostic factor by **Flotho et al [9]; Remke et al.[14]; Jiao et al.[12]; Juárez-Velázquez et al.[15]; Li et al.[16] and Cui et al.[17]** and this was confirmed by our study. While, **Kang et al.[18]; Yang et. al.[19]and Yang et. al.[20]** failed to show prognostic significance for this gene expression.

Jiao et al. noted that in Yang's report the percentage of leukemic cells in diagnostic bone marrow samples was not mentioned, which could imply that a proportion of CASP8AP2 expression was from normal cells rather than leukemic cells.

Kang et al. aimed to find a multi-gene pattern to identify those patients with high risk of relapse; therefore only patients with high risk B-precursor ALL were included in their study. CASP8AP2 was found to have relatively weak signals and had no discriminating ability in their cohort. Nevertheless, it implied the correlation of CASP8AP2 expression and clinical stratification, as well as its potential prognostic significance. **Jiao et al.** study revealed that the prediction significance of CASP8AP2 alone was no better than the current clinical risk stratification. Both CASP8AP2 expression and clinical risk stratification showed a better prediction significance than that of clinical risk stratification or CASP8AP2 alone.

Flotho et al., Remke et al. and Jiao et al. reported that the patients with low expression of CASP8AP2 were more subject to high minimal residual disease (MRD) as well as poor RFS and OS.

Biologic basis of the variation of CASP8AP2 expression could be deletions at band 6q15-16.1, which are often detected in patients with T cell ALL. **Remke et al.** identify 6q15-16.1 deletions as a risk factor predicting an unfavorable MRD response therefore, may contribute to the definition of a molecularly defined high risk group.

Furthermore, other possibilities of CASP8AP2 silencing should be considered: aberrant methylation in the promoter of CASP8AP2 has been reported in cancer cells, contributing to prolonged cell survival and resulting in drug resistance **[16,21].**

Li et al. concluded that the abnormal hypermethylation of the two CpG sites (at -1189 and -1176) of the promoter of the CASP8AP2 gene is possibly associated with leukemogenesis in childhood ALL. The treatment outcome is more poor in patients with hypermethylation than that in patients with low methylation. The combination of the methylation level of the two CpG sites and MRD level at the end induction remission is able to predict relapse more effectively.

Milovic-Holm et al.[22] made the intriguing observation that activation of the Fas receptor triggers the translocation of CASP8AP2 from the promyelocytic leukemia (PML) nuclear bodies to the cytoplasm, where it associates with caspase-8 at the mitochondrial surface, thereby activating the mitochondrial apoptotic pathway. This process was facilitated through the Crm1- dependent nuclear export pathway. Therefore low expression of CASP8AP2 could result in an apoptotic aberration and drug resistance of leukemic cells.

Bongiorno-Borbone et al.[23] and Yang et al.[24] has been reported that CASP8AP2 is as a component of Cajal bodies and histone locus bodies, involved in histone transcription and S-phase progression.

Kiriyama et al.[25]demonstrate that not only the expression of CASP8AP2 but also the expression of Arsenite Resistance Protein 2 (ARS2), a protein involved in the formation of microRNA and the interaction between CASP8AP2 and ARS2 play important roles in S phase progression**.**

It has been recognized that the leukemic cells proliferating slowly were resistant to the common chemotherapeutic agents. Possibly, the mechanism of resistance to chemotherapy of leukemic cell with low ARS2 and CASP8AP2 expression was correlated to the decreased proliferation capacity. To a greater extent, low expression of both ARS2 and CASP8AP2 would induce growth arrest and hindrance to proliferation of leukemic cells, which might in turn result more refractory to multi-agent chemotherapy of leukemic cells than single low expression of either of the two genes**[17]**.

De Cola et al.[26] show that the transcription factor p73 regulates histone gene transcription in the S-phase. p73 binds to CASP8AP2 at histone gene promoters and contributes to their transcription.

Moreover, Caspase-8 forms a complex with CASP8AP2 and tumour necrosis factor receptor-associated factor2 (TRAF2) to initiate the nuclear factor kappaB (NF-κB) activation pathway during tumour necrosis factor (TNF) signaling in fibroblast and epithelial cell lines**[27].** However, the fact that CASP8AP2 activates NF-kB may explain the observation that in many cell types, TNF treatment did not induce apoptosis in the absence of gene expression **[28].**

NF-κB activation can prevent the induction of apoptosis by up-regulating anti-apoptotic mechanisms such as anti-apoptotic Bcl-2 family members **[29],** inhibitor of apoptosis proteins (IAPs) **[30].**

CASP8AP2 seems to be an upstream component of various receptor mediated signals including TNF-alpha and most likely has a dual function in apoptosis and NF-kB signaling **[31].**

The c-Myb oncoprotein is a DNA-binding transcription factor with a key role in early stages of hematopoiesis. CASP8AP2 interacts with the DNA-binding domain of c-Myb and enhances c-Myb-dependent reporter activity and expression of endogenous c-Myb target genes. Chromatin immunoprecipitation assays revealed that CASP8AP2 and c-Myb both associate with the MYC promoter region as well as with the intronic enhancer of the c-Myb target gene adenosine deaminase (ADA)**[32,33]**.

Tumor Necrosis Factor alpha Receptor- and Fas-associated CASP8AP2 inhibit Transcriptional Activity of the Glucocorticoid Receptor by Binding to and Interfering with Its Interaction with p160 Type Nuclear Receptor Coactivators **[34]**.

H2A histone family member Z (*H2AFZ*) (also known as *H2AZ*, *H2A/Z* or *H2A.Z*, located at 4q24) is a highly conserved basal histone that is expressed independently of DNA replication and is involved in diverse biological processes, including the transcriptional activation of genes**[35,36],** chromosomal segregation and stability **[37,38],** heterochromatic gene silencing **[36]** and cell cycle progression **[35].**

Flotho et al.[39] and Juárez-Velázquez et al.[15] found that Patients who showed low expression of both *H2AFZ* and *CASP8AP2* genes had a suboptimal treatment response and eventually relapsed.

Very little is known regarding the regulation of CASP8AP2 function. CASP8AP2 has been shown to interact with the Small ubiquitin-related modifier (SUMO)-conjugation enzyme, Ubc9. Sumoylation attenuates the transcriptional activity of CASP8AP2 as measured by the Gal4 tethering assay **[40].**

Vennemann and Hofmann [41] show that arsenic trioxide, a drug known to potentiate SUMO modification and degradation of promyelocytic leukemia (PML), triggers recruitment of CASP8AP2 to PML bodies and concomitant loss of CASP8AP2 protein. SUMO targets CASP8AP2 for proteasome-dependent degradation, which is associated with recruitment of CASP8AP2 to PML bodies.

Conclusion:-

From the present study, we could conclude that:

Relapse showed statistically low CASP8AP2 expression versus those who continued complete remission. Interestingly, CR had higher CASP8AP2 expression than refractory disease and induction death, although non significant differences. Also, Patients with high CASP8AP2 expression have significantly better OS and DFS than those with low CASP8AP2 expression. The expression of CASP8AP2 was reported as an independent prognostic factor.

Further studies of caspases and other regulators of apoptotic cascade are crucial in the study of leukemias and are likely to yield important insights into the behaviour and responsiveness to treatment of leukemic cells.

Financial support and sponsorship:-
Nil.

Conflicts of interest:- There are no conflicts of interest.

References:-

1. Borowitz MJ, Devidas M, Hunger SP, et al.: Clinical significance of minimal residual disease in childhood acute lymphoblastic leukemia and its relationship to other prognostic factors: a Children's Oncology Group study. Blood 2008; 111 (12): 5477-5485.
2. Silverman LB, Stevenson K^{B}E, O'Brien JE, et al.: Long-term results of DanaFarber Cancer Institute ALL Consortium protocols for children with newly diagnosed acute lymphoblastic leukemia (1985-2000). Leukemia 2010; 24 (2): 320-334.
3. Gregers J, Christensen IJ, Dalhoff K, et al.: The association of reduced folate carrier 80G>A polymorphism to outcome in childhood acute lymphoblastic leukemia interacts with chromosome 21 copy number. Blood 2010; 115 (23): 4671-4677.
4. Choi YH, Kim KB, Kim HH, Hong GS, Kwon YK, et al.: FLASH coordinates NF-kappa B activity via TRAF2. J Biol Chem 2007; 276: 25073–25077.
5. Imai Y, Kimura T, Murakami A, Yajima N, Sakamaki K, et al.: The CED-4-homologous protein FLASH is involved in Fas-mediated activation of caspase-8 during apoptosis. Nature 1999; 398: 777–785.
6. Juárez-Velázquez M.R., Salas-Labadía C., A. Reyes-León, M.P. Navarrete-Meneses, E.M. Fuentes-Pananá and P. Pérez-Vera: Clinical Epidemiology of Acute Lymphoblastic Leukemia - From the Molecules to the Clinic; Genetic Markers in the Prognosis of Childhood Acute Lymphoblastic Leukemia 2013; http://dx.doi.org/10.5772/54288.
7. Arneson W, Brickell J. *Clinical chemistry a laboratory perspective*.
8. Philadelphia, PA: F. A. Davis Company; 2007.
9. Swansbury J. Cancer cytogenetics: methods and protocols; cytogenetic techniques for myeloid disorders. *Methods Mol Biol* 2003; 220:43–57.
10. Flotho C, Coustan-Smith E, Pei D, Iwamoto S, Song G, Cheng C, et al. Genes contributing to minimal residual disease in childhood acute lymphoblastic leukemia: prognostic significance of CASP8AP2. Blood 2006;108:1050–7.
11. Q*iagen sample and assay technology* for total RNA purification from human whole blood. Volume 2. Qiagen, QIAamp® RNA Blood Mini Handbook 2010:18–21.
12. Beillard E, Pallisgaard N, van der Velden VHJ, Bi W, Dee R, van der Schoot E, et al. Evaluation of candidate control genes for diagnosis and residual disease detection in leukemic patients using 'real-time' quantitative reversetranscriptase polymerase chain reaction (RQ-PCR)—a Europe against cancer program. Leukemia 2003;17:2474–86.
13. Jiao Y, Cui L, Gao C, Li W, Zhao X, Liu S, et al.: CASP8AP2 is a promising prognostic indicator in pediatric acute lymphoblastic leukemia. Leuk. Res. 2012; Jan;36(1):67–71.
14. Whitlock JA, Maloney KW, Carroll WL, et al. : Down syndrome childhood acute lymphoblastic leukemia has a unique spectrum of sentinel cytogenetic lesions that influences treatment outcome: a report from the Children's Oncology Group. Blood 2012, 116(7):1045-1050.
15. Remke M, Pfister S, Kox C, Toedt G, Becker N, Benner A, et al.: High-resolution genomic profiling of childhood T-ALL reveals frequent copy-number alterations affecting the TGF-beta and PI3K-AKT pathways and deletions at 6q15-16.1 as a genomic marker for unfavorable early treatment response. Blood 2009, Jul 30;114(5):1053–62.
16. Juarez-Velazquez Rocio n1, Consuelo Salas-Labada1, Roberto Rivera-Luna3, Liliana Velasco-Hidalgo4, Gerardo López-Hernndez5, Norma López-Santiago6, Rogelio Paredes-Aguilera6, Aarón Dominguez-López7, Roberto Bernldez8 & Patricia Pérez-Vera1: Significance of *CASP8AP2* and *H2AFZ* expression in survival and risk of relapse in children with acute lymphoblastic leukemia *Leukemia & Lymphoma* 2014, October,55(10): 2305–2311.
17. Li Z.G, Ying Jiao a, Wei-Jing Li a, Guo-Ren Dengb, Lei Cuia, Chao Gaoa, Xiao-Xi Zhaoa, Min-Yuan Wua, Hong-Ti Jia c,: Hypermethylation of two CpG sites upstream of CASP8AP2 promoter influences gene expression and treatment outcome in childhood acute lymphoblastic leukemia; Leukemia Research 2013, 37 1287– 1293.
18. Cui L., Chao Gaob, Rui-Dong Zhangb, Ying Jiaob, Wei-Jing Lib, Xiao-Xi Zhaob,Shu-Guang Liub, Zhi-Xia Yueb, Hu-Yong Zhengb, Guo-Ren Dengc, Min-Yuan Wub,Zhi-Gang Lib,1, Hong-Ti Jiaa,∗,1: Low expressions of ARS2 and CASP8AP2 predict relapse and poorprognosis in pediatric acute lymphoblastic leukemia patients treatedon China CCLG-ALL 2008 protocolLeukemia Research 2015, 39,115–123.
19. Kang H, Ming Chen I, Wilson CS, Bedrick EJ, Harvey RC, Atlas SR, et al.: Gene expression classifiers for relapse-free survival and minimal residual disease improve risk classification and outcome prediction in pediatric B-precursor acute lymphoblastic leukemia. Blood 2010;115:1394–405.

20. Yang Y.Li, Shu-Rung Linc, Jiann-Shiuh Chend, et al., (2010): Expression and prognostic significance of the apoptotic genes *BCL2L13*, *Livin*, and *CASP8AP2* in childhood acute lymphoblastic leukemia Leukemia Research 2010, 34 ,18–23.
21. Yang X, Xu B, Sabath I, et al.: FLASH is required for the endonucleolytic cleavage of histone pre-mRNAs but is dispensable for the 5' exonucleolytic degradation of the downstream cleavage product. Mol Cell Biol;2011,31:1492–1502.
22. Lee K-D, Pai M-Y, Hsu C-C, et al.: Targeted Casp8AP2 methylation increases drug resistance in mesenchymal stem cells and cancer cells. Biochem Biophys Res Commun;2012,422:578–585.
23. Milovic-Holm K, Krieghoff E, Jensen K, Will H, Hofmann TG: FLASH links the CD95 signaling pathway to the cell nucleus and nuclear bodies. Embo J,2007, 26: 391–401.
24. Bongiorno-Borbone L, De Cola A, Vernole P, Finos L, Barcaroli D, et al.: FLASH and NPAT positive but not Coilin positive Cajal Bodies correlate with cell ploidy. Cell Cycle 7,2008,: 2357–2367.
25. Yang XC, Burch BD, Yan Y, Marzluff WF, Dominski Z (2009): FLASH, a proapoptotic protein involved in activation of caspase-8, is essential for 3- end processing of histone pre-mRNAs. Mol Cell 2009, 36: 267–278.
26. Kiriyama M, Kobayashi Y, Saito M, Ishikawa F, Yonehara S : Interaction of FLASH with arsenite resistance protein 2 is involved in cell cycle progression at S phase. Mol Cell Biol 2009, 29: 4729–4741.
27. De Cola A, Bongiorno-Borbone L, Bianchi E, Barcaroli D, Carletti E, et al.: FLASH is essential during early embryogenesis and cooperates with p73 to regulate histone gene transcription. Oncogene. DeltaNp73 isoform, allowing apoptosis to occur. Cell Death Differ 2012,11,685–687.
28. Jun JI, Chung CW, Lee HJ, Pyo JO, Lee KN, et al. : Role of FLASH in caspase-8-mediated activation of NF-kappaB: dominant-negative function of FLASH mutant in NF-kappaB signaling pathway. Oncogene 2005, 24: 688–696.
29. Lamkanfi M. , Wim Declercq, Tom Vanden Berghe, and Peter Vandenabeele: Caspases leave the beaten track: caspase-mediated activation of NF-κB The Journal of Cell Biology 2006, Vol. 173, No. 2, April 24, 165–171.
30. Wang, C.Y., D.C. Guttridge, M.W. Mayo, and A.S. Baldwin Jr.: NF-kappaB induces expression of the Bcl-2 homologue A1/Bfl -1 to preferentially suppress chemotherapy-induced apoptosis. *Mol. Cell. Biol.1999*,19:5923–5929.
31. Weber, G.F., and A.S. Menko.: The canonical intrinsic mitochondrial death pathway has a non-apoptotic role in signaling lens cell differentiation. *J. Biol. Chem.2005*, 280:22135–22145.
32. Chen S, Evans HG, Evans DR: FLASH Knockdown Sensitizes Cells To Fas-Mediated Apoptosis via Down-Regulation of the Anti-Apoptotic Proteins, MCL-1 and Cflip Short.2012, PLoS ONE 7(3): e32971. doi:10.1371/journal.pone.0032971.
33. Alm-Kristiansen AH, Saether T, Matre V, Gilfillan S, Dahle O, et al.: FLASH acts as a co-activator of the transcription factor c-Myb and localizes to active RNA polymerase II foci. Oncogene 2008, 27: 4644–4656.
34. Alm-Kristiansen AH, Lorenzo PI, Molvaersmyr AK, Matre V, Ledsaak M, et al.: PIAS1 interacts with FLASH and enhances its co-activation of c-Myb. Mol Cancer 2011, 10: 21.
35. Kino T, Ichijo T, Chrousos G: "FLASH interacts with p160 coactivator subtypes and differentially suppresses transcriptional activity of steroid hormone receptors". *J. Steroid Biochem. Mol. Biol.* 2004,92 (5):357;63. doi:10.1016/j.jsbmb.2004.09.003. PMID 15698540.
36. Nekrasov M, Amrichova J, Parker BJ, et al.: Histone H2A.Z inheritance during the cell cycle and its impact on promoter organization and dynamics. Nat Struct Mol Biol; 2012,19:1076–1083.
37. Soboleva TA, Nekrasov M, Pahwa A, et al.: A unique H2A histone variant occupies the transcriptional start site of active genes. Nat Struct Mol Biol; 2012,19:25–30.
38. Greaves IK, Rangasamy D, Ridgway P, et al.: H2A.Z contributes to the unique 3D structure of the centromere. Proc Natl Acad Sci USA 2007,104:525–530.
39. Huh YH, Sherley JL.: Molecular cloaking of H2A.Z on mortal DNA chromosomes during nonrandom segregation. Stem Cells 2011,29:1620–1627.
40. Flotho C, Coustan-Smith E, Pei D, Cheng C, Song G, Pui C-H, et al: A set of genes that regulate cell proliferation predicts treatment outcome in childhood acute lymphoblastic leukemia. Blood. 2007, Aug 15;110(4):1271–7.
41. Alm-Kristiansen AH, Norman IL, Matre V, Gabrielsen OS: SUMO modification regulates the transcriptional activity of FLASH. Biochem Biophys Res Commun 2009, 387: 494–499.
42. Vennemann A. and Hofmann T.G. :*SUMO regulates proteasome-dependent degradation of FLASH/Casp8AP2Cell Cycle 2013, 12:12, 1914–1921; June 15,; © Landes Bioscience.