Prognostic value of ABCA2 and ABCA3 Genes expression in pediatric Acute Lymphoblastic Leukemia

Amira Al-Ramlawy*, Hanaa Abdel-masseih, Raida S. Yahya , Camelia Abdel-Malak

Abstract— Acute lymphoblastic leukemia (ALL) is a highly aggressive hematological-malignancy resulting from the proliferation and expansion of lymphoid blasts in the blood, bone marrow and other organs. Multidrug resistance (MDR) is an important cause of treatment failure in ALL. The role of ABCA2 and ABCA3 in drug transport is not entirely clear, but much of the evidence suggests that these carrier proteins have a role in MDR by causing an accumulation of drugs in the lysosomes and possibly their efflux from the cell. **Aims:** The aim of this work was to study and investigate the mRNA expression profile of ABCA2, ABCA3 in newly diagnosed children with ALL and healthy children, and evaluate their prognostic value to disease outcome. **Subjects and Methods:** This study was carried out on 50 newly diagnosed children with ALL, with age ranged from 2-18 years and 20 healthy children with matching in age and sex. Mononuclear cells were isolated from the bone marrow and peripheral blood for patients. Evaluation of gene expression for ABCA2 and ABCA3 genes using quantitative real- time polymerase chain reaction (qRT-PCR), for all groups. Complete Blood Picture, liver, kidney function tests, and serum LDH were measured using investigated measurements. **Results:** There was a significant difference of the ABCA2 and ABCA3 levels among different groups of ALL (patients and control group) at ($P < 0.05$). The response to treatment assessed after (16 months), our results indicated that there was a strong and significant relationship between the expression of ABCA2 &ABCA3 genes, and response to treatment &some known prognostic factors. **Conclusion**: Our findings suggested that, the expression of ABCA2 & ABCA3 genes, are very important in determination the severity of Acute Lymphoblastic Leukemia, which helps in future predictability of the disease and appropriate treatment methods and the susceptibility for drug resistance.

Index Terms— ABCA2 transporter, ABCA3 transporter, multidrug resistance, childhood acute lymphoblastic leukemia
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1 INTRODUCTION

Leukemia is one of, the commonly reported cancer worldwide [21]. Acute lymphoblastic leukemia (ALL) is a highly aggressive hematological-malignancy resulting from the proliferation and expansion of lymphoid blasts in the blood, bone marrow and other organs [6]**.** Approximately 80% of children cancer, of acute lymphoblastic leukemia [38]. Where ALL is one of the four types of haematological malignancies records highest frequency among children, [13] .ALL is a common form of leukemia in children below 5 years of age, occurs with a bimodal distribution with an early peak in children 4 – 5 years old followed by a second peak at ~ 50 years of age [19], and including 34% of all cases among children under 15 years old [23]**.**

A major factor responsible for the failure of chemotherapy in the treatment of cancer and relapse, is the development of multidrug resistance (MDR)**,** [5]**,** [27],.MDR is a multifactorial phenomenon [37], generated by mechanisms such as the increased efflux of a wide range of chemotherapeutics from the cells [31] **.** During the recent decades, various mechanisms responsible for drug resistance have been extensively studied both in vivo and in vitro [18].

A well-established cause of cancer cell MDR is through the increased expression of the ATP binding cassette (ABC) transporter superfamily, which can export a variety of chemotherapeutics out of the cell [7]**,** ABC family form one of the largest protein families, members of this family found in all living organisms, this wild-spread of these proteins, suggests a fundamental role, [20]**.** In humans, 48 members of ABC transporters have been identified so far, that have been subdivided into seven families (called ABC A–G) according to their structural features. ABC proteins are mainly involved in molecular trafficking processes, such as the transport of (vitamins, lipid, cholesterol, phospholipids, glycolipids, bile salts, steroids, toxins, drugs, and metabolites) across biological membranes [32] .

ABCA2 transports drugs from the cytoplasm into the lysosomal compartment, where they may become degraded and exported from the cell. The aforementioned mechanism may contribute to MDR. It has been reported that ABCA2 may induce resistance to mitoxantrone, estrogen derivatives and estramustine. It is resistant to the aforementioned compounds [1],[3], [8] ,[16] [12].

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ABCA3 is located at intracellular membranes [42], highly expressed in alveolar epithelial type II cells. It is localized to the limiting membrane of lamellar bodies, acidic and lipid rich organelles for production, storage and regulated secretion of pulmonary surfactant [17] ,it does not confer a classical drug efflux but seems to be involved in intracellular sequestration and the vesicular transport of its physiological substrates as chemotherapeutic agents as daunorubicin [41], ABCA3 also expressed in ALL, [5].

2 Material and Methods

2.1. Subjects:

This study was carried out on 50 newly diagnosed children with ALL, with age ranged from 2-18 years, (33 Male & 17 Female), selected from Hematology Oncology Unit – Mansoura University Children Hospital and Oncology Center in Mansoura University and, 20 healthy children (9 Male &11 Female) with matching in age and sex. Any patient had a history of any treatment such as (chemotherapy/ radiotherapy and secondary ALL patients and any other diseases may interfere with our study such as (uncontrolled diabetes, hypertension, infections, marked hepatic or renal dysfunctions) were excluded.

Our studied groups divided into 2 groups, 1- (50) patients at newly diagnosed, 2- (20) control (healthy children).All cases undergo hematological, biochemical examination and gene expression of ABCA2 and ABCA3 genes by qRT-PCR

Our patient's group undergo a follow up for 16 months & the prognosis for disease outcome was assessed by some known prognostic factors.

2.2. Methods:

Blood sampling: blood from vein puncture were collected and divided into three tubes; first that contained dipotassium ethylene diamine tetra-acetic acid (K2EDTA) for complete blood cell counts (CBC). CBC was determined in each sample within 2 hours of collection. Second part was taken in plain tube to be used for biochemistry tests, and the third part used for qRT-PCR of ABCA2 & ABCA3 genes.

2.2.1. Hematological & Biochemical tests:

CBC was determined using the electronic counter (CELL-DYN 3700, Abbott, Canda) to determine hemoglobin (HB), total leukocyte count (WBCs), platelets count. Liver and kidney functions measured on an automated biochemistry analyzer (Hitachi 917; Roche Diagnostics, Mannheim, Germany). Serum Aspartate Aminotransferase (AST), Alanine Aminotransferase (ALT), enzymes activity were determined according to the method of [36].Determination of serum creatinine, serum Albumin, serum Uric Acid, and lactate dehydrogenase (LDH) were determined according to [11].

Bone marrow sampling: Bone marrow aspiration was done under complete aseptic conditions at time of diagnosis: small part of aspirate was used for spreading smears to be examined by Leishman stains, 1 ml was dispended into sterile tube containing K-EDTA to be used for the flow cytometry for immunophenotyping detection and 1ml of the aspirate was used for qRT-PCR of ABCA&ABCA3 genes.

2.2.2. Immunophenotyping:

Bone marrow aspiration smears: for morphological diagnosis of ALL. Cytochemical analysis (Sigma Diagnostics, St. Lowis, Missouri, USA) of air-dried peripheral blood and/or bone marrow smears. Immunophenotyping of Leukemia blasts, had an important role in distinguished between AML and ALL and ALL sub-classifying using, (COULTER EPICS XL™ Flow cytometer coulter electronics, Florida, USA). The panel of monoclonal antibody included, (CD7), (CD10),(CD19),(CD22),(CD34), Positivity with flow cytometry was defined as an expression in at least 20% of cells in the gated populations of interest, compared to internal negative control cells [4].

2.2.3. Quantitative real- time polymerase chain reaction (qRT-PCR).

The total RNA was extracted using Qiagen (QIAamp RNA blood mini kit), the amount of total RNA extracted assessed using (Eppendorf), then 2 µg of total RNA were used for synthesis of cDNA, using Applied Biosystem™ High Capacity cDNA reverse transcription kit, using random primer scheme, according to specific temperature protocol provided (25ºC for 10 minutes, 37ºC for 120 minutes, 85ºC for 5 minutes, and 4ºC for ∞). cDNA resulting from the previous steps was kept at -20ºC for the next steps.

For the gene expression method, quantitative real time polymerase chain reaction(qRT-PCR) was performed, by assessed mRNA levels using, Applied Biosystems. TaqMan® Universal Master Mix II, with specific protocol and melting curve plan analysis, qRT-PCR performed in 40 cycles, (95ºC for 10 minutes, 95ºC for 15 sec, and 60ºC for 1 minute). We used Beta-Actin (ACTB) as a housekeeping gene for internal control.

Primer sequence for the endogenous control Beta-Actin (ACTB) available at (Thermo Fisher catalogue No 4326315E).

The primer sequence for our studied genes was summarized in (Table 1).

Table 1: Primer sequences of *ABCA2, ABCA3*

Gene	Primer sequence (5′ to 3′)	Primer Length (base pairs)	Amplicon Length (base pairs)	Tm	GC%
ABCA2	F:CCGCACCATCCTTCTGTCCACCCACC	26	263	70.7	65.3
	R:TGCGGATGAACTGGGACACCTGGAGC	26		70.3	61.5
ABCA3	F:GGCCATCATCATCACCTCCCACAGCA	26	177	66.7	57.7
	R:AGCGCCTCCTGTTGCCCTTCACTCTG	26		68.7	61.5

Abbreviations: F, forward; R, reverse; *T*m, melting temperature.

Statistical analysis

Statistical analyses of data done by using excel program and SPSS 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. Comparison between more than two groups; ANOVA and Kruskal wallis (for non- parametric data) were used.

3. RESULTS

3.1 At diagnosed.

By comparing our studied genes expression in patients group at diagnosed with control group, we found that, there was a significant increase in the expression of ABCA2 gene in patients compared with the control group with (2.101 ± 1.277) vs (0.47 ± 0.19), (mean± S.D, P= 0.001) respectively, Fig 1, also, there was a significant increase in the expression of ABCA3 gene in patients at diagnosed compared with the control group with (2.267± 1.006) vs (0.2 ± 0.19) (mean± S.D, P= 0.001), respectively. Fig 1 .There was a significant difference in some hematological & biochemical measurements & non -significant difference in other parameters as mentioned in table (2).

Table (2) Hematological& biochemical parameters in studied groups at diagnosed.

Parameter	Control Mean± S.D(N=20)	patients Mean± S.D(N=50)	P value
Age(years)	13.05± 2.28	11.87± 4.238	0.244
Gender	M 9 45%	M 33 66%	
	F 11 55%	F 17 34%	
Hb (g/dl)	12.495±1.1650	8.964 ±1.607	0.001*
Platelets(x109/L)	218.00±54.42	59.1600±25.173	0.001*
WBCs (x109/L)	7.600±2.31	34.128±6.728	0.001*
ALT (IU/L)	22.10±4.77	21.07±4.375	.365
AST(IU/L)	22.01±3.83	22.374±5.44	.783
Creatinine(mg/dl)	0.68±0.13	1.257±0.654	.0001*
Uric Acid(mg/dl)	4.32±1.12	7.067±1.813	.0001*
LDH(U/L)	325.58±48.21	591.242±222.20	.0001*
Blasts (PB)		67.300±15.508	
		Minimum 29% / Maximum 94%	
Blasts (BM)		30% / 95%	
		Mean ± S.D (N=50) 73.900 ±15.527	
FAB		L1 7 14%	
		L2 39 78%	
		L3 4 8%	

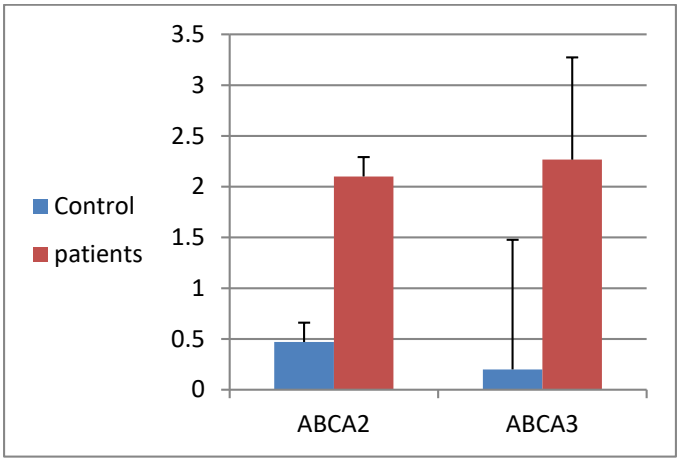

Fig 1: the expression of ABCA2 & ABCA3 among studied groups

Follow up assessment.

1- Prognostic value of studied genes.

The assessment to therapy response, carried out after the period of 16 months, and the following results have been recorded. **1-ABCA2 gene expression** (Median = 1.75), as regarding to (low<1.75:high>1.75) gene expression, we found that the complete remission cases recorded with percentage (84%:44%), and for relapse cases the percentage among low to high expression were (16%:56%) and that is significant P=0.007*, total death observed with percentage (4%:32%) regarding to (low: high) gene expression, and this is also significant with P=0.01*. **2 -ABCA3 gene expression** (Median = 2.21), regarding to the median of the gene expression and ratio (low <2.21: high >2.21), our results found that, complete remission cases with percentage (80%:48%), relapse cases were (20%:52%), and this is a significant P= 0.038*, while total death recorded with percentage (4%: 32%), and this is also a significant p= 0.01*. table 3. These date may refers to the role of drug resistance genes in the increasing percentage of relapse cases.

Table 3: Outcome findings according to the expression of our studied genes.

ABCA2	Low expression M<1.75	High expression M>1.75	P
Complete remission	21 (84%)	14 (44%)	0.007*
relapse	4 (16%)	11 (56%)	
Total death	1 (4%)	8 (32%)	0.01*
ABCA3	Low expression M<2.21	High expression M>2.21	
Complete remission	20 (80%)	12 (48%)	0.038*
relapse	5 (20%)	13 (52%)	
Total death	1 (4%)	8 (32%)	0.01*

M: Median

2- Relationships between genes expression and some prognostic factors.

Our follow up investigations were assessed on some prognostic factors, and they revealed that: there was an inverse relationship between the expression of ABCA2 gene and the level of hemoglobin, (r= - 0.653 , P= 0.001*) Fig 2.a
Also, there was an inverse relationship between the expression of ABCA3 gene and the level of Hemoglobin, (r= - 0.628, P= 0.001*). Fig 2.b
While there was a direct relationship between the increased expression of ABCA2 and blasts in peripheral blood & bone marrow (r= 0.591, r= 0.567, P= 0.001) respectively, fig 2.c,& fig 2.e, and, there was a direct relationship between the increased expression of ABCA3 gene and blasts in peripheral blood & bone marrow (r= 0.483, r=0.532, P= 0.001*) respectively, Fig 2.d& fig 2.f . (r: person correlation coefficient, P: probability)

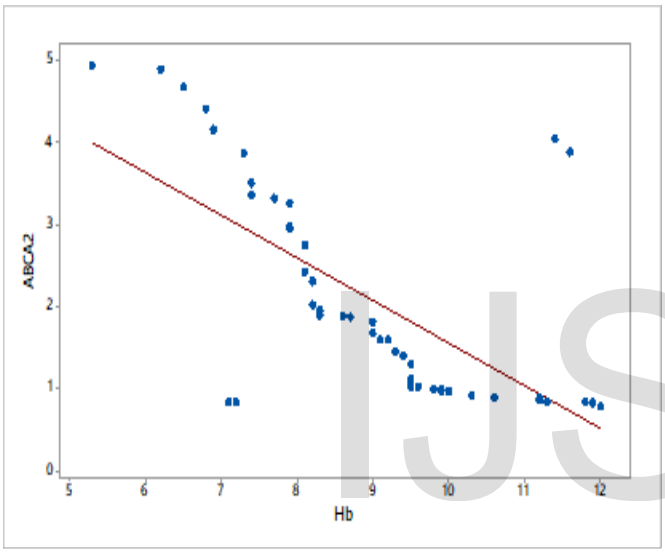

Figure 2.a: correlation between Hb level & ABCA2 expression

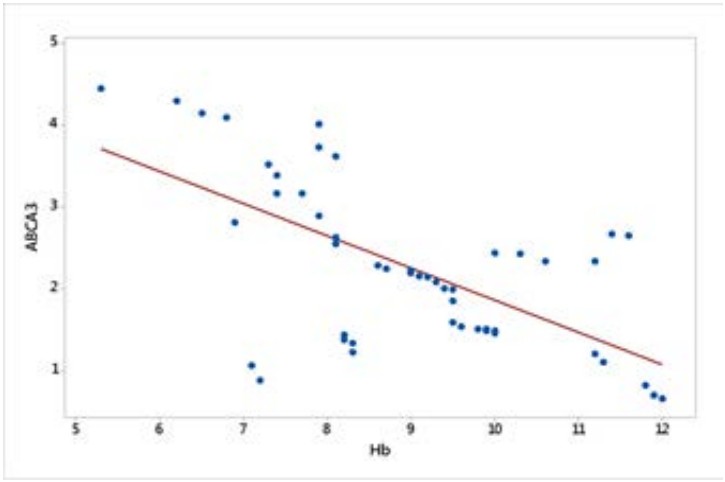

Figure 2.b: correlation between Hb level & ABCA3 level.

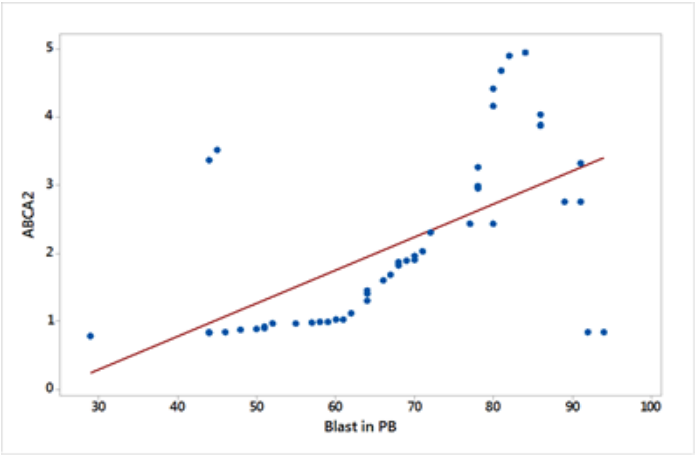

Figure 2.c: correlation between PB blasts & ABCA2 level.

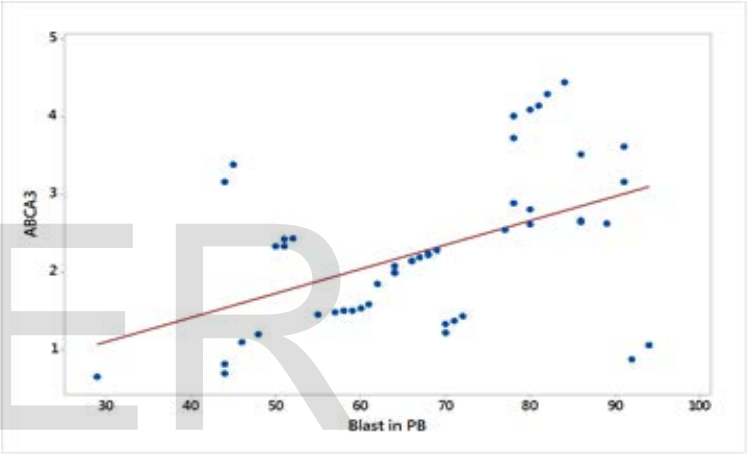

Figure: 2.d: correlation between PB blasts& ABCA3 expression.

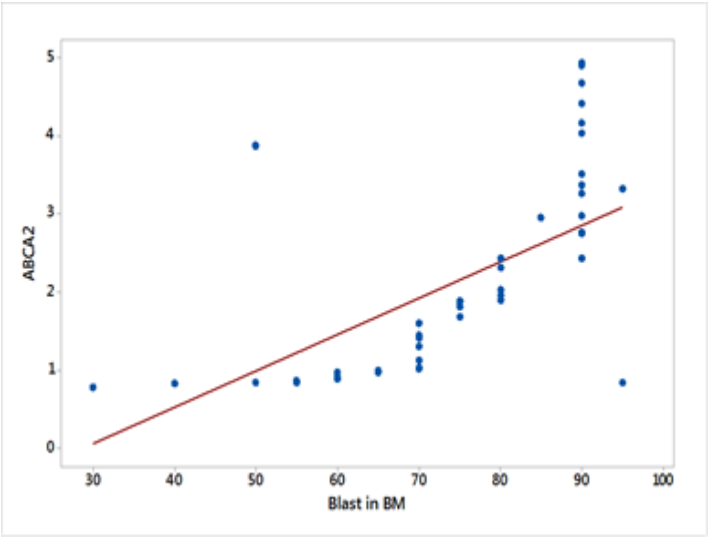

Figure: 2.e: correlation between BM blasts &ABCA2 expression.

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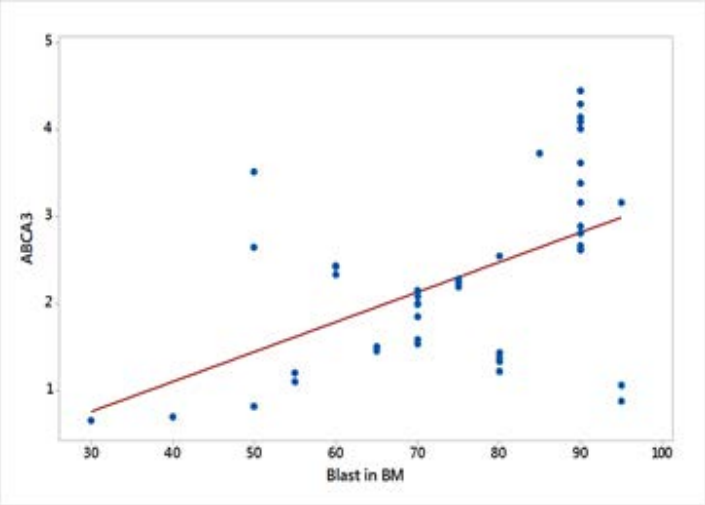

Figure2.f: correlation between BM blasts& ABCA3 expression.

3 - Phenotyping expression of studied genes.

Our results recorded that, the increased level of ABCA2 gene expression was correlated with the high expression of CD7 & CD10, with P= (0.002, 0,003)* respectively. And the high expression of ABCA3 gene was correlated to the increased expression of CD34 with P=0.004*. Table 4 & fig 3 a& b.

Table 4: Phenotyping of studied genes.

		ABCA2		P
		Median	Range	
CD7	negative	1.60	.78-4.68	0.002*
	Positive	3.51	1.12-4.94	
CD10	negative	0.89	.78-0.99	0.003*
	Positive	1.90	.84-4.94	
		ABCA3		
CD34	negative	1.99	.65-4.45	0.004*
	Positive	2.24	.70-4.30	

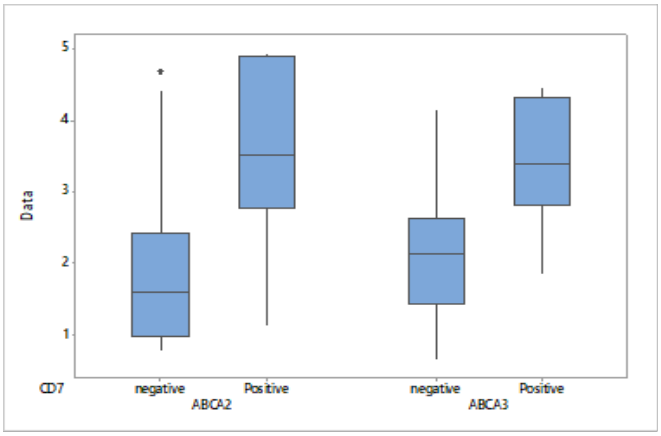

Figure 3.a: CD7 expression in ABCA2 gene.

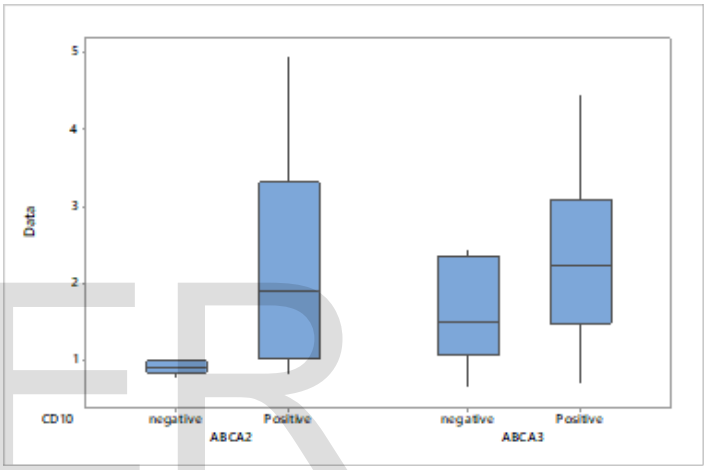

Figure 3.b: CD10 expression in ABCA2 gene.

Data expressed as Median (Range)
P: Probability *: significance <0.05
Test used: Mann-Whitney test

Discussion

Acute lymphoblastic leukemia (ALL) is the most common type of cancer in children [28], [34], although the complete remission of pediatric ALL around 70-80%, in some cases, 20-30% of patients still show drug resistance and relapse [26], [24] ,resistance to chemotherapy reduced the rate of treatment success and increase the risk of relapse [33], [39].The main cause of MDR is the increased expression of ATP-binding cassette transporters, or ABC transporters, [35].This present study carried out on 50 patients and 20 control (healthy) children, gene expression for both genes (ABCA2 &ABCA3) assessed on all groups at diagnosed, and revealed that, there was a significant increase in studied genes expression between patients and control group at diagnosed with (P<0.005)*. Some studies found that there was a higher expression of genes in patients than healthy individuals but not a significant at diagnosed, and the difference observed significantly after therapy assessment only [35],our studied

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cases were examined for Hb level, WBCs count, and platelets count, and there was a high significant decrease between patients and healthy children in the level of HB, elevated WBCs count and reduced number of platelets with P<0.005, ALT &AST did not show any significant while there were an observed increase in creatinine, uric acid, and LDH in patients compared to healthy group with p<0.005, Blasts % in patients peripheral blood ranged from (29-94%) with mean of (67.300±15.50) ,While blasts in patient's bone marrow, ranged from (30-95%) with mean of (73.90±15.52). According to FAB classification, L1: L2:L3 in patients were, 14%: 78%: 8%, respectively, there was no-significant difference in age, between patients and control groups (11.87± 4.238), (13.05 ± 2.28) (mean± S.D, P= 0.244) respectively, and no-significant difference in sex also,as they are matched in age & sex. Our results in hematological& biochemical parameters came in agreement with these studies,[29], [9], [21], [2], [40], [10], [22].

All patients included in this study were followed up for 16 months for prognosis outcome, and the following data obtained, the response for chemotherapy treatment differ among cases, **For ABCA2 gene**, patients show complete remission, with low expression level(<1.75) were 21 cases with percentage 84% ,while in high expression level (>1.75), 14 cases with percentage of 44% , while in relapse cases, patients with low gene expression were 4 with percentage 16%, and 11 cases with percentage 56% in high expression, and this is significant p=0.007* and death recorded in 1 case at low expression with percentage 4%, and 8 cases with percentage 32% of high expression with P=0.01*.**table 3**

For ABCA3 gene, patients show complete remission, with low expression level(<2.21) were 20 cases with percentage 80% while in high expression level (>2.21), 12 cases with percentage of 48% , while in relapse cases, patients with low gene expression were 5 with percentage 20%, and 13 cases with percentage 52% in high expression, and this is significant p=0.038* and death recorded in 1 case of low expression with percentage 4%, and in 8cases with percentage 32% of high expression with P=0.01*. from the previous date we observed that the number of relapse cases increase with the high gene expression for both studied genes, and number of died cases also increase with high gene expression while number of remission cases decrease with the high gene expression level, and that may prove the role of drug resistance genes in treatment failure.

These findings are a good predictor for the relation between the gene expression and drug resistance [35], [1].The role of ABCA2 protein in drug transport and its role in MDR are still under investigation, where the role of this gene by saving drugs in the lysosome and probably their efflux from the cell, [3], [30], [14]. Some studied mentioned the role of ABCA3 in ALL & their role in drug resistance, which still under investigation, [41] [42], [15], [35].

Until now, the pathophysiological role of ABCA2 and ABCA3 is under investigation and their precise function in leukemia has remained undefined.

Our study found also a relationship between the expression of our studied genes (ABCA2& ABCA3) and some prognostic factor, there was an inverse relation between the overexpression of these genes and decreased in hemoglobin level with P=0.001*, reduced in hemoglobin level is a strong prognostic factor specially when it correlate with the expression of studied genes, also there was a direct relationship with the increased gene expression and blasts in peripheral blood and bone marrow with p= 0.001*,our phenotype findings referred to, that the over expression of ABCA2 gene is correlated with the increased expression of CD7 &CD10 with significant value P=(0.002*, 0.003*) respectively, where the increased expression of ABCA3 gene correlated with high expression of CD34, P= 0.004*, these results come compatible with Rahgozar et al., 2014,[35]. All previous data may be a good risk for treatment failure & multidrug resistance in ALL, the exact function of ABC transporters in increasing the risk of resistance to therapy remain to be established.

Conclusion

The expression of ABCA2& ABCA3 genes was high in patients compared to control group, and this may be a good risk of relapse.

By followed up for patients during 16 months, we found that the both genes related to increase the percentage of relapse cases specially in patients who have a high gene expression more than those who have low gene expression .and this may be related to their role in drug resistance, thus the treatment failure.

REFERENCES

[1] Aberuyi, N., Rahgozar, S., and Moafi, A. (2014). The role of ATP-binding cassette transporter A2 in childhood acute lymphoblastic leukemia multidrug resistance, Vol 4).

[2] aime-Perez, J. C., Garcia-Arellano, G., Herrera-Garza, J. L., Marfil-Rivera, L. J., and Gomez-Almaguer, D. (2019). Revisiting the complete blood count and clinical findings at diagnosis of childhood acute lymphoblastic leukemia: 10-year experience at a single center. Hematology, transfusion and cell therapy 41, 57-61.

[3] Albrecht, C. and E. Viturro, The ABCA subfamily-gene and protein structures, functions and associated hereditary diseases. Pflugers Archiv-European Journal of Physiology, 2007. 453(5): p.581-589.

[4] Bain, B.J., Barnett, D., Linch, D., Matutes, E. and Reilly, J.T (2002):Resived guideline on immunophenotyping in acute leukemias and chronic lymphoproliferative disorders. Clin Lab Haematol; 24:1-13.

[5] Bakos E, Homolya L. Portrait of multifaceted transporter, the multidrug resistance-associated protein 1 (MRP1/ABCC1). Pflugers Arch 2007; 453:621-41; PMID:17187268; http://dx.doi. org/10.1007/s00424-006-0160-8.

[6] Bassan R, Hoelzer D. Modern therapy of acute lymphoblastic leukemia. J Clin Oncol 2011; 29:532-43; PMID:21220592; http://dx.doi.org/10.1200/JCO.2010.30.1382.

[7] Begicevic, R.-R., and Falasca, M. (2017). ABC Transporters in Cancer Stem Cells: Beyond Chemoresistance. International journal of molecular sciences 18, 2362.

[8] Beljanski, V., et al., Characterization of the ATPase activity of human ATP-binding cassette transporter-2 (ABCA2). In Vivo, 2005. 19(4): P.657-660.

[9] Belson, M., Kingsley, B., and Holmes, A. (2007). Risk factors for acute leukemia in children: a review. Environmental health perspectives 115, 138-145.

[10] Bhatia, N. G., Sneha, L. M., Selvan, S. M., and Scott, J. J. X. (2013). Acute renal failure as an initial manifestation of acute lymphoblastic leukemia. Indian journal of nephrology 23, 292-293.

[11] Bonsens, K. and Taussky D.H. (1984). "Determination of serum creatinine." J Ch Inv 27: 648-660.

[12] Boonstra, R., et al., Mitoxantrone resistance in a small cell lung cancer cell line is associated with ABCA2 upregulation. British journal of cancer, 2004.

[13] Campos-Sanchez, E., et al., Acute lymphoblastic leukemia and developmental biology: A crucial interrelationship. Cell Cycle, 2011. 10(20): p. 3473-3486.

[14] Coelho, A.C. and P.C. Cotrim, The Role of ABC Transporters in Drug-Resistant Leishmania, in drug Resistance in Leishmania Parasites. 2013,springer. p. 237-258.

[15] Efferth T. Gillet JP. Sauerbrey A, Zintl F, Bertholet V, de Longueville F, Remade J, Steinbach D. Expression profiling of ATP-binding cassette transporters in childhood T-cell acute lymphoblastic leukemia. Mol Cancer Ther 2006; 5:1986-94; PMlD:l6928819.

[16] Efferth, T., Inhibition of ATP-Binding Cassette Transporters by Chinese Herbs and Phytochemicals, in Evidence and Rational Based Research on Chinese Drug. 2013, Springer. Vienna, P. 283-331.

[17] Engelbrecht, S., Kaltenborn, E., Griese, M., and Kern, S. (2010). The surfactant lipid transporter ABCA3 is N-terminally cleaved inside LAMP3-positive vesicles. FEBS Letters 584, 4306-4312.

[18] Filipits M. Mechanisms of cancer: multidrug resistance. Drug Discov Today Dis Mech 2004; 1:22934; http://dx.doi.org/10.1016/j.ddmec.2004.10.001.

[19] Fullmer, A., O'Brien, S., Kantarjian, H. & Jabbour, E. (2010) Emerging therapy for the treatment of acute lymphoblastic leukemia. Expert Opin Emerg Drugs, 15, 1-11.

[20] Gottesman MM, Fojo T, Bates SE (2002). Multidrug resistance in cancer, role of ATP-dependant transporters. Nat Rev Cancer, 2, 48-58.

[21] Jemal, A., et al., Global cancer statistics. a cancer journal for clinicians, 2011. 61(2): P. 69-90.

[22] JKornberg, A., and Polliack, A. (1980). Serum lactic dehydrogenase (LDH) levels in acute leukemia: Marked elevations in lymphoblastic leukemia, Vol 56).

[23] Kaatsch, P., Epidemiology of childhood cancer. Cancer treatment reviews, 2010.36(4): P. 277-285.

[24] Kennedy JA, Barabé F, Poeppl AG, Wang JC and Dick JE: Comment on ' Tumor growth need not be driven by rare cancer stem cells ' . Science 318: 1722, 2007.

[25] Klein I, Sarkadi B, Varadi A (1999). Biochim Biophys Acta, 1461. 237-62

[26] Le Viseur C , Hotfilder M, Bomken S , Wilson K , RÖttgers S , Schrauder A, Rosemann A, Irving J, Stam RW, Shultz LD, et al: In childhood acute lymphoblastic leukemia, blasts at different stages of immunophenotypic maturation have stem cell properties. Cancer Cell 14: 47-58, 2008.

[27] Leonard GD, Fojo T, Bates SE. The role of ABC transporters in clinical practice. Oncologist 2003; 8:411-24;PMID:14530494; http://dx.doi.org/10.1634/theoncologist.8-5-411.

[28] Linet MS, Ries LA, Smith MA, Tarone RE and Devesa SS: Cancer surveillance series: Recent trends in childhood cancer incidence and mortality in the United States. J Nat1 Cancer Inst 91: 1051-1051-1058, 1999.

[29] Lustosa de Sousa, D. W., de Almeida Ferreira, F. V., Cavalcante Félix, F. H., and de Oliveira Lopes, M. V. (2015). Acute lymphoblastic leukemia in children and adolescents: prognostic factors and analysis of survival. Revista brasileira de hematologia e hemoterapia 37, 223-229.

[30] Mack, J.T., C.B. Brown, and K.D. Tew, ABCA2 as a therapeutic target in cancer and nervous system disorders. Expert Opin Ther Targets, 2008. 12(4): p. 491-504.

[31] Mansoori, B., Mohammadi, A., Davudian, S., Shirjang, S., and Baradaran, B. (2017). The Different Mechanisms of Cancer Drug Resistance: A Brief Review. Advanced pharmaceutical bulletin 7, 339-348.

[32] Molday, R. S., Zhong, M., and Quazi, F. (2009). The role of the photoreceptor ABC transporter ABCA4 in lipid transport and Stargardt macular degeneration. Biochim Biophys Acta 1791, 573-583.

[33] Pui C-H, Relling MV and Downing JR: Acute lymphoblastic leukemia. N Eng1 J Med 350: 1535-1548, 2004.

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[34] Pui C-H: Acute lymphoblastic leukemia in children. Curr Opin Oncol 12: 3-12, 2000.

[35] Rahgozar, S., Moafi, A., Abedi, M., Entezar-E-Ghaem, M., Moshtaghian, J., Ghaedi, K., Esmaeili, A., and Montazeri, F. (2014a). mRNA expression profile of multidrug-resistant genes in acute lymphoblastic leukemia of children, a prognostic value for ABCA3 and ABCA2. Cancer biology & therapy 15, 35-41.

[36] Reichling, J. J. and Kaplan M. M. (1988). "Clinical use of serum enzymes in liver disease." Dig Dis Sci 33(12): 1601-1614.

[37] Shukla, S., Z.-S. Chen, and S.V. Ambudkar, Tyrosine kinase inhibitors modulators of ABC transporter-mediated drug resistance. Drug Resistance Updates, 2012. 15(1): P.70-80.

[38] Styczynski J , Wysocki M, Debski R, Czyzewski K, Kolodziej B, Rafinska B, Kubicka M, Koltan S, Koltan A, Pogorzala M, et al. Predictive value of multidrug resistance proteins and cellular drug resistance in childhood relapsed acute lymphoblastic leukemia. J Cancer Res Clin Oncol 2007; 133:875-93; PMID: 17671794; http://dx.doi.org/10.1007/s00432-007-0274-1.

[39] Swerts K, De Moerloose B, Dhooge C, Laureys G , Benoit Y and Philippé J: Prognostic significance of multidrug resistance-related proteins in childhood acute lymphoblastic leukemia. Eur J Cancer 42: 295-309, 2006.

[40] Teuffel O, Stanulla M, Cario G, Ludwig WD, Zimmermann M, Schrappe M, et al. Anemia and survival in childhood acute lymphoblastic leukemia. Haematological. 2008;93(11):1652-7.

[41] Wulf GG, Modlich S, Inagaki N, Reinhardt D, Schroers R, Griesinger F et al. ABC transporter ABCA3 is expressed in acute myeloid leukemia blast cells and participates in vesicular transport. Haematologica 2004; 89: 1359-1397.

[42] Yamano G, Funahashi H, Kawanami O, Zhao LX, Ban N, Uchida Y et al. ABCA3 is a lamellar body membrane protein in human lung alveolar type II cells. FEBS Lett 2001 ; 508: 221-225.

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