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Original Article

Is PDGFRB expression level has effective role in Iranian pediatric acute lymphoblastic leukemia biogenesis?

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ABSTRACT

Correlation between gene Expression and recurrence as a treatment failure in pediatric patients with Acute Lymphoblastic Leukemia (ALL) is an unsolved problem in scientific associations. We evaluate predictive value of PDGFRB expression level for estimating recurrence in Iranian Pediatric Patients with ALL and its MRD after chemotherapy. ALL MRD refers to the presence of residual Leukemia cells following the achievement of complete remission. MRD can be crucial with genetic novel biomarkers for risk stratification moreover It has effective role at target therapy and patient's survival rate management. Real-time Polymerase Chain Reaction reacting was done with GAPDH for expressing PDGFRB gene. Gathered data, analyzed with SPSS version 22 and REST 2009 software. Peripheral Blood (PB) of Iranian pediatric patients with approved ALL enrolled in this study. PDGFRB gene expressing was analyzed by RT-PCR. The results showed that PDGFRB gene expression was significantly up regulated in new diagnosis patients (Median, $p<0.001$) and relapse phases (Median, $p<0.005$) compared to the control group (Median, $p<0.001$). PDGFRB gene expression in induction phase was significantly lower than its level at new diagnosis stage ($p<0.001$). Moreover, PDGFRB gene was significantly up regulated in relapse phase compared to the new diagnosis. One hundred ALL samples (50: new case, 30: controls, and 20: Relapsed) enrolled. Our study revealed that up-expression of PDGFRB is a novel remarkable biomarker in pediatric B-ALL and it may play a critical role in leukemogenesis. The authors suggest of designing a multiple childhood malignancy center project to evaluate this pattern in a cohort study.

Keywords: Acute Lymphoblastic Leukemia, PDGFRB, MRD, Relapse, Prognostic marker

Introduction

Network analysis

Based on available data in biological networks, and ALL effective genes-protein interactions, top ten genes with greater effect and role in ALL biogenesis and progression were identified; PDGFRB was also observed in this division. Known Interactions from curated databases and experimentally determined and predicted Interactions, gene neighborhood, gene fusions, gene co-occurrence, text mining, co-expression, protein homology had been evaluated. It is the fifth effective gene in pediatric ALL based on molecular and cellular function and transcription factors moreover. PDGFRB has interaction with ABL1, ACT1, IL3, PDGFRA genes based on text mining and experimentally determined, co-expression and databases, PIK3CA, PIK3CG, EBF1 in the field of experimentally, text mining, co-expression, and FIP1L1 and ACTB based on text mining moreover **(Figure 1)**. After this assessment in dry lab framework; this gene effectiveness in this patients' groups had been confirmed and then we started second phase of our study in laboratory (Wet lab) [1, 2].

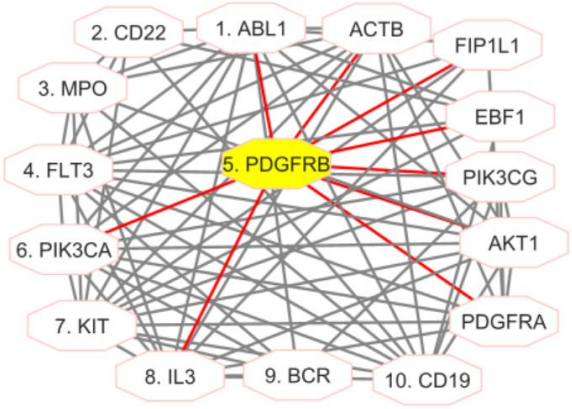

Figure 1. PDGFRB role in Pediatric ALL Biogenesis (Cystoscope Analysis)

Pediatric ALL outcome

Cancer is second leading cause of death in most countries, its incidence has increased in recent years. Cancer treatment management is one of the important priorities of Iranian health sector. Accurate and reliable information in the field of diagnosis and treatment is the most importance pillar of cancer management. According to WHO Iran is at moderate risk of cancer; and about 8% of death in Iran have been caused by cancer; Report of national cancer registration program of the ministry of health indicates that, the most common cancers among men in Iran is Gastric, Skin, Prostate, Colorectal, Bladder, Lung, Leukemia and in women Breast, Colorectal, Skin, Gastric, Thyroid, and Leukemia. Cancer prevention and treatment is the key to success in personalize medicine promotion.

Acute Lymphoblastic Leukemia (ALL) is the most common hematologic malignancy among pediatric patients, but it can also occur in adults with weaker prognosis. ALL is classified in two main subgroups: T cell and B cell, based on the world Health organization (WHO) classification and patient's molecular and pathogenesis changes. ALL risk factors are previous cancer treatment, exposure to radiation, genetic disorder, and epigenetic alterations. Accordingly, it would be possible to perform promotion in the diagnosis and treatment of malignancies by investigating the progression of the disease at the molecular level. Chemotherapy has improved the treatment of this disease; it is better to provide novel therapeutic approaches due to the possibility of high recurrence and severe toxicity however [3].

Molecular investigating the progression of this disease, provide novel therapeutic approaches and perform progress in diagnosis and treatment. The genes which have role at biological pathways in the field of epigenetic, immune system, transcription factors, tyrosine kinase, and apoptosis are effectors in leukemia biogenesis. ALL has high survival rate in childhood and is more common in 0-14 years old boys. About 90% of patients have complete remission; despite that, large percentage of patients, don't response to treatment and even died in some cases [4]. Pediatric clinical trials have improved 5-year event-free survival above 85% and 5-year overall survival above 90% in B-cell acute lymphoblastic leukemia (ALL) in many study groups, whilst outcomes for T-cell ALL are still lagging behind by 5–10% in most studies [5]. According to the GLOBOCAN statistics in 2022, ALL has high survival rate in childhood and is more common in 0-14 Years old boys [6].

Transcriptional profiles investigation, provides a better insight in to pathogenesis and biology of ALL and determines treatment goals association with prognosis and result in personalized approach to medicine with greater efficacy and less toxicity [7].

MRD importance

The Measurable Residual Disease (MRD) is used to monitor the response to the treatment, prognosis, risk stratification, and outcome management in childhood ALL. MRD had been evaluated since 2001 by blasts counting in patient's Bone Marrow samples after induction. MRD monitoring is performed by precise methods, such as quantitative polymerase chain reaction (qPCR)-based techniques and multi-parametric flow cytometry (MFC). qPCR is the gold standard method on the 15th or 33rd days after treatment. Up to 2/3 of children have detectable MRD after induction therapy depending on the biological subtype and detection method. Patients with detectable MRD, have an increased likelihood of relapse and MRD has emerged as the strongest independent predictor of individual patient outcome and is crucial for risk stratification [3, 8].

Leukemic blasts exhibit a different gene expression pattern after genetic mutations, which differed from normal hematopoietic progenitors. MRD is carried out on post-induction and this evaluation is gold standard time point for its assessment [9]. Measuring MRD by sensitive and quantitative techniques will lead to better sensitivity detection capacity of one blast cell in a background of one million cells [14].

It is a critical diagnostic tool that predicts disease progression and is increasingly used as an important end point to monitor disease status and evaluate novel treatments for leukemia. MRD has a major impact on clinical decision making in patients with ALL. MRD status influences treatment regimens and predicting outcomes in patients with ALL. MRD technologies rapidly evolve toward sequencing-based assays, but machine learning and new flow cytometers also innovate cell-based assays [10].

Relapse is the most common treatment failure through chemotherapy of pediatric with ALL and is indicator for poor prognosis and outcome [11]. Certain subgroups such as infants, adolescents and young adult and patients who have relapse; have an inferior outcome and ALL will be counted one of the principal causes of pediatric cancer related death for these mentioned reasons [12].

According to various results from published studies, still the correlation between gene expression and treatment failure or prognosis in pediatric patients with ALL is an unsolved problem [13]. Treatment outcome has improved significantly in the last 40 years as a result of treatment intensification. Pediatric risk of relapse at diagnosis, low and intermediate risk patients have 5-year event free survival (EFS), rate of 90% and patients with

high-risk features can attain an 80% survival with augmented therapy [14].

Difference types of MRD assessment

To detect residual disease, the Peripheral Blood (PB) and Bone Marrow cell morphology method is adopted where the number of residuals must be at least less than 5% of bone marrow cells' population. At this stage, the patient is considered to be in complete remission. Since 50-90% of patients with B-ALL precursor (BCP-ALL) and almost all of the patients with T-ALL had unusual Immunophenotype during diagnosis, they were good candidates for detection of residual leukemic cell [15].

Biology of Relapsed ALL has considerable genetic heterogeneity, both at chromosomal and at single gene level. Different driver mutations and/or chromosomal aberrations and deregulated signaling interactions between leukemia cells and the immune microenvironment have been implicated in the development of T-cell acute lymphoblastic leukemia (T-ALL). Therapy expression profiles of the immune cells revealed significant changes. Residual blast cells in MRD+ EOI samples Differences in cellular communication were noted in the presence of residual disease in T cell and hematopoietic stem cell compartments in the bone marrow. These results provide critical first insights into the pediatric T-ALL transcriptional landscape at single cell resolution and identify potential therapeutic targets to improve outcomes for children with T-ALL [16, 17].

B-ALL biology

ALL is a malignancy characterized by an expeditious Increase in immature B- (in ~85% of cases) and T-(in ~15% of cases). Lymphocytes in the PB and BM Chemotherapy continues to be the main treatment for ALL; there is considerable evidence for superior outcome from multiple rounds of highly intensive chemotherapy. Although novel therapies have been developed, their effectiveness is greatly enhanced when used in combination with chemotherapy. Relapsed and refractory ALL continue to be a major concern, increasing the current understanding of the molecular mechanisms by which chemotherapy resistance develops. Extensive genetic heterogeneity in B- and T-acute lymphoblastic leukemia precursor cells indicates a range of biomarkers that promote disease development and recurrence [18].

The tyrosine kinase inhibitor (TKI), Imatinib Mesylate, has played a significant role in the treatment of Philadelphia chromosome-positive ALL (Ph + ALL). The achievement of durable and sustained therapeutic success remains a challenge due to the development of TKI resistance during the clinical course. Targeting ALL and its Ph + subtype by identifying and addressing differentially expressed genes to identify possible repurposable drugs that target identified hub proteins. Subset of drugs, specifically Glipizide for Ph + ALL, and Maytansine and Isoprenaline for ALL, have been identified as potential candidates for therapeutic intervention. Clinical trials are recommended to validate the promising results obtained through drug repositioning strategies [19].

Childhood ALL has seen significant advances in treatment, yet children classified as high-risk still face challenging outcomes. A deeper understanding of specific biological markers—such as molecular profiles, Genetic variations, and immune system characteristics—has become crucial. Pediatric ALL sub-groups represented in **Table 1**. These markers indicators of how it may progress and respond to treatment. Drugs like tyrosine kinase inhibitors can be used to target high-risk leukemia with certain genetic mutations. Hope for better management of this complex disease. While the National Cancer Institute (NCI) criteria have traditionally guided risk stratification based on initial clinical information, recent advances highlight the pivotal role of biological markers in shaping the prognosis of childhood ALL. Understanding of high-risk childhood ALL, focusing on molecular, cytogenetic, and immunophenotypic markers offering insights into its molecular landscape and clinical intricacies in the hope of contributing to future targeted and tailored therapies. Continuing to accelerate improvements in their outcomes [20].

Table 1. Pediatric ALL Sub-types (Molecular Genomic, Cytogenetic, Immunophenotype)

#	Sub-type	Recurrence	Outcome
1	B-ALL with BCR: ABL Fusion	2-5 % in Pediatric ALL	Poor Prognosis
2	B-ALL with BCR-ABL1Like Fusion	10% in Pediatric ALL	Survival Rate less than 30%
3	B-ALL with KMT2A(MLL) Rearrangements	5% in Pediatric ALL	Good Prognosis
4	B-ALL with MEF2D Rearrangements	2-3% in Pediatric ALL	Good Prognosis
5	B-ALL with TCF3: HLF Fusion	Less than 1% in Pediatric ALL	Poor Prognosis
6	Hypodiploid ALL (Less than 44 Chromosomes)	1% in Pediatric ALL	Adverse Prognosis
7	Intrachromosomal Amplification of RUNX1	2% in Pediatric ALL	Adverse Prognosis
8	Early T-cell Precursor ALL (Molecular Heterogeneity)	15% in Pediatric ALL	Favorable Prognosis
9	Mixed Phenotype ALL	2-5 % in Pediatric ALL	Favorable Prognosis

Stratification for T-ALL vs B-ALL

Pediatric clinical trials have improved 5-year event-free survival above 85% and 5-year overall survival above 90% in B-ALL in many study groups, but outcomes for TALL are still lagging behind by 5–10% in most studies. Clinical, Immuno-phenotypic, Genetic, Biology and treatment response are the most differences between B and T ALL sub groups due to main factors effectiveness. T-ALL Patients age, most favorable genetic subtypes in B-ALL, Blast resistance in T-ALL sub-group, and patients with B-cell ALL are more amendable to available targeted therapies. ETV6-RUNX 1 is most common fusion gene in B-ALL patients and has favorable outcome while Transcription Factor Oncogenes such as TAL1 are aberrantly expressed in 60%

of T-ALL patients and lead to poor prognosis outcome. BCR–ABL1 fusions are common in B-cell ALL, and in T-cell ALL (25% adult ALL, 3-5% Pediatric ALL), and ABL class B-ALL have response to treatment only with tyrosine kinase inhibitors.

In contrast to B-cell ALL, no consensus genetic classification with prognostic or therapeutic implications has been reached for T-cell ALL. The majority of relapses among patients with T-cell ALL actually occur in those classified to have favorable (low-risk) leukemia based on minimum residual disease, and the prognosis for relapsed T-cell ALL is poor. Minimum residual disease is particularly useful for identifying patients with T-cell ALL with persistent minimum residual disease after remission induction and early intensification therapies who might benefit from more intensive chemotherapy or allogeneic transplantation.

Over the past 5 years, a shift has occurred in the treatment of patients with relapsed and refractory B-cell ALL, and a number of targeted therapies and immunotherapies have shown remarkable efficacy, and improved overall survival for this disease. Unfortunately, neither targeted therapies nor immunotherapies have been successful in the treatment of T-cell ALL. The use of TKIs, including imatinib and dasatinib, has markedly improved survival for patients with Philadelphia chromosome-positive B-cell ALL. Children with Philadelphia chromosome-like B-cell ALL might also benefit from targeted therapy. Although patients with T-cell ALL have historically fared worse than those with B-cell ALL, the difference in outcome has narrowed with modern chemotherapy approaches. Improved understanding of ALL biology and the integration of novel therapies are continuing to influence the field and will hopefully continue to improve survival outcomes for both T-cell and B-cell ALL. For T-cell ALL in particular [21].

PDGFRB role in ALL biogenesis and risk assessment

Iranome is a database of normal population in Iran; 800 people from 8 Iranian races over the age of forty, without any disease have been sequenced in this database. We reviewed Iranome and research results in the field of Leukemia in last ten years; and accordingly designed this study for evaluation of new biomarker which has effective role at the biogenesis of leukemia for treatment strategies management (PDGFRB).

This project was planned because of rare report with focus on the expression of PDGFRB gene in Iranian pediatric patients with ALL. The most important controversial challenge in this research, was whether the considered profile could have the predictive value for estimating MRD amount and relapse in mentioned patients.

PDGFRB (5q32), the protein encoded by this gene is a cell surface tyrosine kinase receptor for members of platelet-derived growth factor family. These growth factors are mitogens for cells of mesenchymal origin and normally repressed in lymphocytes. PDGFRB is a common fusion partner in leukemia especially in B and Ph-like ALL. PDGFRB genetic alterations, block tyrosine kinase pathway and these patients' group, reach to treatment only with tyrosine kinase pathway inhibitors. High expression of this gene caused tumor progression, angiogenesis and metastasis [22].

The PDGFRB signaling pathway is involved in the development of Blood vessels and hamatopoietic cells in the bone marrow, The autocrine activity of this messenger pathway has beeen implicated in glioma, sarcoma, and leukemia, and its paracrine activity has been reported in epithelial cancers. It plays a role in tumor formation and growth, angiogenesis, and metastasis. High expression level of this gene in B and Ph-like ALL patients is an indicator for poor prognosis [22].

So far, more than 30 gene fusions involving the PDGFRB have been reported, and their accurate identification is necessary for early diagnosis of the disease targeted treatment, and increasing the survival rate of patients [23]. High expression of PDGFRB, is novel prognostic marker in B and Ph-like ALL sub-groups especially [24].

We evaluated expression profiling of PDGFRB among pediatric patients with ALL at three time points (New case, Post-Induction, Relapse) compare to control normal children at same age group and the results shows that Iranian children PDGFRB expression profiling is similar to recent sudied in other world geographical regions. We suggest this evaluation in larger patients group to increase results validation, correct microbial algorithm, and genetic profiling in the field of pediatric ALL.

Materials and Methods

Sample specifications

Peripheral Blood (PB) specimens were collected from ALL patients (n=50) admitted to the Mofid Children's Hospital Research Center, and Children's Medical center Tehran, Iran. All patients gave informed written consent for sample collection. This experimental study was approved by the Ethics Committee of College of Sciences-University of Tehran (IR.UT.SCIENCE.REC.1401.001). New case ALL patients received standard induction chemotherapy consisting of vincristine, glucocorticoids, and L-asparaginase, with/without daunorubicin. Patient's treatment were follow-up and sampling of them were also done (n=50) at post-Induction term; after confirmation of complete remission with para-clinic tests evaluation **(Figure 4)** besides that, PB samples were received from patients which were at relapse phase (n=20). Peripheral blood samples from healthy individuals (n=30) were analyzed as the control group furthermore.

Specimen collection

Case patient's peripheral Blood samples collected after diagnosis confirmation by paraclinical tests. Among our Patients, 38.9% did not have known sub-group, 24.1% B-ALL, 18.5% Ph-Like ALL, and 18.5% were at T-ALL subgroup. 28% had WBC less than 3000 and in 18% WBC was 9000(K/uL). In 42% Blast was more than 20, and in 16% it was more than 35. In the examination of CD markers by flow cytometry, CD 34 and CD45 showed a higher expression than normal range in 44.7%

of cases, and CD markers 19, 22 and 10 in 41.3 % of B-ALL cases, and 5, 7, 2, and 4 in 48.5 % of T-ALL cases had expression more than normal range in addition **(Figure 2)**.

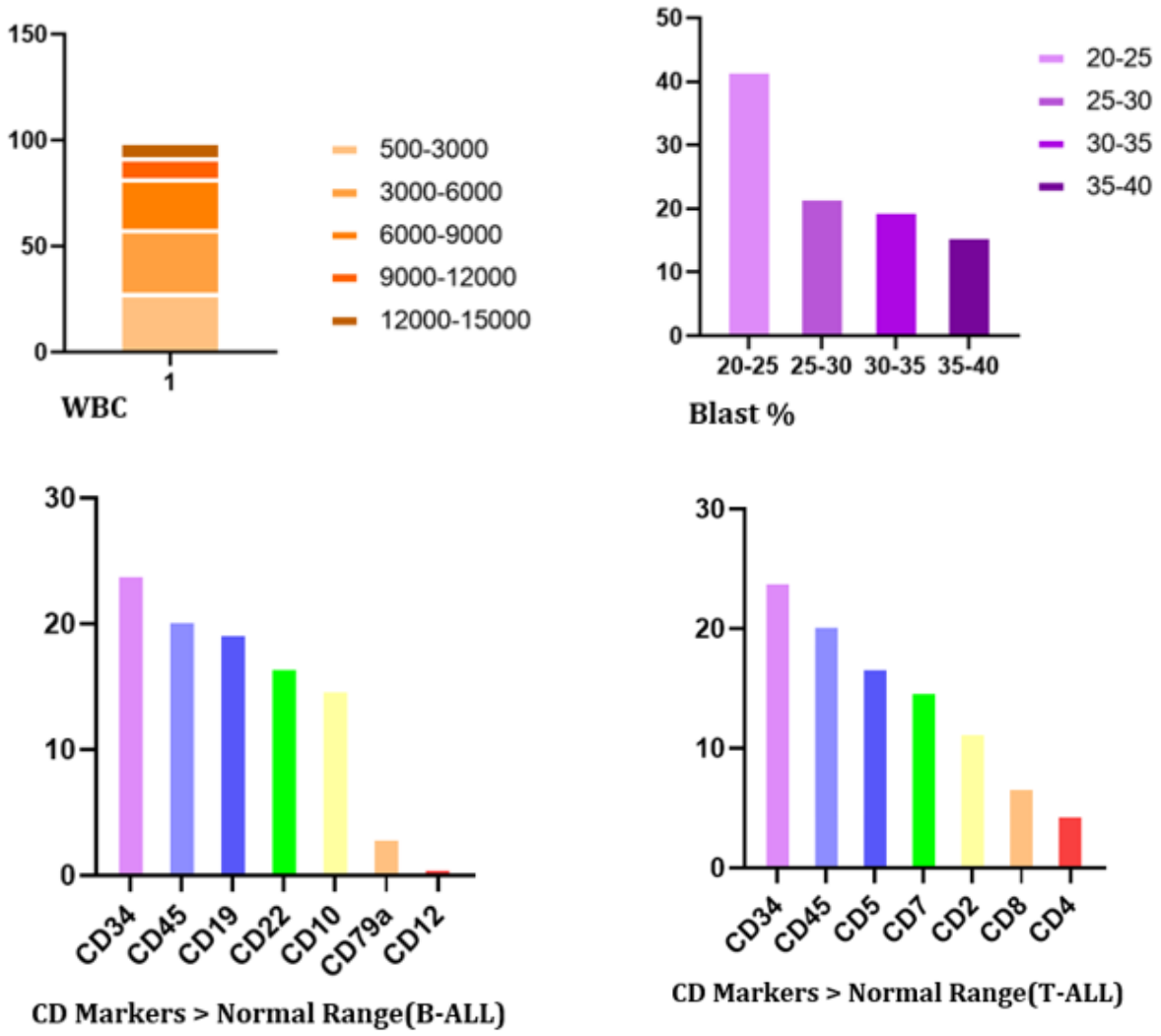

Figure 2. Paraclinical Tests Results (New Case Time Point)

Peripheral Blood samples were collected in EDTA containing tubes. The sample tubes were stored in freezer at -20˚C. After samples collection period, they taken out of freezer and then washing twice using phosphate-buffered saline (PBS, pH=7.4, 0.15 M, Gibco, UK), the cell pellets were directly lysed in TriPure Isolation Reagent (Roche, Germany).

Cytogenetic analysis

Patient's Bone Marrow aspirated samples were directly cultured and harvested following standard cytogenetic methods. G-banding and FISH (if necessary) were undertaken on each bone marrow sample to detect all cytogenetic abnormalities. The chromosomal aberrations in this study were interpreted according to International System of Human Cytogenomic Nomenclature (ISCN 2020) guidance [25]. The identified chromosomal abnormalities were listed at **Table 2**.

Table 2. Cytogenetic Analysis Results. (New case and Post-Induction Time Point)

#	Chromosomal Abnormality	Percentage (New Case)	Percentage (Post-Induction)
1	t (12;21)(p13;q22)/ETV6-RUNX1	13.3%	7
2	t (4;11) (q21; q23)/KMT2A-AFF1	10%	6
3	+Philadelphia Chromosome	8.3%	5
4	t (1;19) (q23; p13)/TCF3-PBX1	6.7%	3
5	t (9;22) (q34; q11)/BCR-ABL	6.7%	4.7
6	t (1;7) (p32;q34)/BTF3L-RAF	5.2%	1.3
7	del (11) (q23)/MLL	5.2%	2.3
8	t (7;10) (q34; q24)/TRB-HOX11	3.35%	2.7
9	t (5;14) (q35;q11)/RANBP17-TRD	3.35%	2.5
10	t (7;14) (p15; q32)/TRC-TCL1A	3.3%	1.4

11	t (8;14) (q24; q32)/MYC-IGH	1.6%	0.6
Total	Abnormal Karyotype	67%	36.5
#	Normal Karyotype	Percentage	
1	46, XX or 46, XY	33%	63.5

Total RNA extraction and cDNA synthesis

Total RNA was isolated from Frozen PB using RNX-Plus Isolation Reagent according to the manufacturer's procedure (RNX-Plus/Sinaclon/Iran) [26]. To determine the quantity and quality of RNA samples, absorbance at 260/280 nm wavelengths was measured by using the NanoDrop spectrophotometer. The SMOBIO First Strand cDNA Synthesis kit (SMBIO technology, Belarus) [27], was used to reverse transcribe 1 µg of total RNA into complementary DNA (cDNA). The synthesized cDNA was stored at -20°C for further analysis.

Quantitative Real-Time Polymerase Chain Reaction

To assess the relative quantity of mRNA transcripts, RT-PCR was undertaken in a StepOne Plus Real-Time PCR System (Bio-Rad, USA) by using the SYBR Green assay in duplicate. The cycling conditions were an initial denaturation step at 95°C for 10 minutes, followed by 40 cycles of 95°C for 15 seconds and 61°C (Combined Annealing/Extension) for 1 minutes. Ultimately a melting curve was generated to ensure primer specificity for target gene. A standard curve was also generated using a serial dilution (5-fold dilutions) of cDNA samples to determine the efficiency of Real Time polymerase chain reactions (RT-PCR). All reactions were conducted in a final volume of 20 µl comprising 10 µl qPCR Master Mix (SMBIO), 2 µl (200 ng/µl) of cDNA, 1 µl of each primer and 6 µl of ddH_2O. Expression levels of PDGFRB were normalized with GAPDH, a housekeeping gene recommended for such analysis by American Society of Clinical Oncology (ASCO), [28]. Relative quantification was undertaken with the $2^{-\Delta\Delta Ct}$ method [29]. The primers were designed using the publicly available Primer 3 software [30]. Details of the primers used are shown in **Table 3**.

Table 3. The list of Primers

	PDGFRB
Forward	CCAATGAGGGTGACAACGAC: 20
Reverse	ATTCAGGGTGGAGCTGGCTA: 20
	GAPDH
Forward	CGGATTTGGTCGTATTGGGC: 20
Reverse	TTCTCAGCCTTGACGGTGCCATG: 23

Statistical analysis

Mann-Whitney U test was used to compare PDGFRB and GAPDH expression levels between the healthy and ALL patient groups at beginning of treatment and post-Induction time point. This test was also used to compare the two subgroups of ALL patients (B and T) and the patient groups which were at relapse phase likewise with respect to the expression levels of PDGFRB and GAPDH. A $P<0.005$ was considered statistically significant. All statistical analyses were implemented in the statistical package for social sciences (SPSS), version 20 (SPSS, Chicago, IL, USA) and REST 2009 software.

Patients Follow-Up

The follow-up of the treatment process was done by direct communication between the patients and their parents. All patients participated in this study after genetic counseling. Then they read and approve the consent letter. As much as we could, we accompanied them and helped them financially and psychologically. All the children who accompanied us in this research got acquainted with the alphabet of genetics in simple language and understood the importance of this study. We made dolls for all of them and every Saturday, they had gifts from us. So that they can experience an easier healing time. After the induction phase, samples were taken again. In addition, and molecular tests were performed to assess genetic MRD.

Results and Discussion

New case time point

Para-clinical tests

This study was run to evaluate the relation between expression profiling and it's MRD after Induction, in pediatric ALL patients. After hematologist screening, patient's PB samples, collected and examined with para-clinical test and their leukemia was confirmed. Performed paraclinical tests were reported in previous section (Specimen collection).

Cytogenetic analysis

Patient's BM samples were taken after cultured, harvest, and G-banding chromosomal analysis was done, chromosomal rearrangements were identified according to **Table 2**, t (12;21) (p13; q22) had the highest frequency with 13.3% and t (8;14) (q24; q32) had the lowest with 1.6% in these patients and these rare sub-group of T-ALL had poor outcome; The patient was diagnosed with a case of T-ALL/LBL with t(9;22);BCR-ABL1 (Ph+). The addition of tyrosine kinase inhibitors to intensive chemotherapy has greatly improved the outcomes of Ph+ ALL patients [32].

About 33% of patients had normal karyotype **(Table 2)**. Based on the result of chromosomal analysis patients were classified in to B and T sub-groups. The average age of them was 8 years and 40% were girls and 60% were boys. Gender was not effective in this research according to our results and prediction and change in expression levels of the target and reference gene, was not difference between boys and girls. These tests, performed patient's leukemia and their sub-group. In order to complete our

research, the expression of PDGFR gene was evaluated by RT-PCR techniques, its results will be explained in next section.

RT-PCR

Expression profiling was evaluated in terms of GAPDH and TAL1 by RT_PCR. The used primers, were reported in **Table 3**; Primer 3 Software [30], and the NCBI Primer Blast [31] were used for designing the described primers. The expression results are shown in **Figure 4**.

Post-Induction time point

Patients' follow-up treatment was done, After Induction (28-33 days) PB was collected from them again. By repeating Para-clinic tests and chromosomal analysis their complete remission phase was confirmed from hematological point of view. This evaluation results, were showed that the WBC amount was 6000-15000(K/uL). And the average of Blast was 2-5 %. In the examination of CD markers by flow cytometry, CD 34 and CD45 showed an expression in the range of normal; in both B and T patient's sub-groups **(Figure. 3)**. t (12;21) (p13; q22) had the highest frequency with 7% and t (8;14) (q24; q32) had the lowest with 0.6% in these patients; about 63.5% of patients had normal karyotype **(Table.2)**. Based on the result of chromosomal analysis patients were classified in to B and T sub-groups.

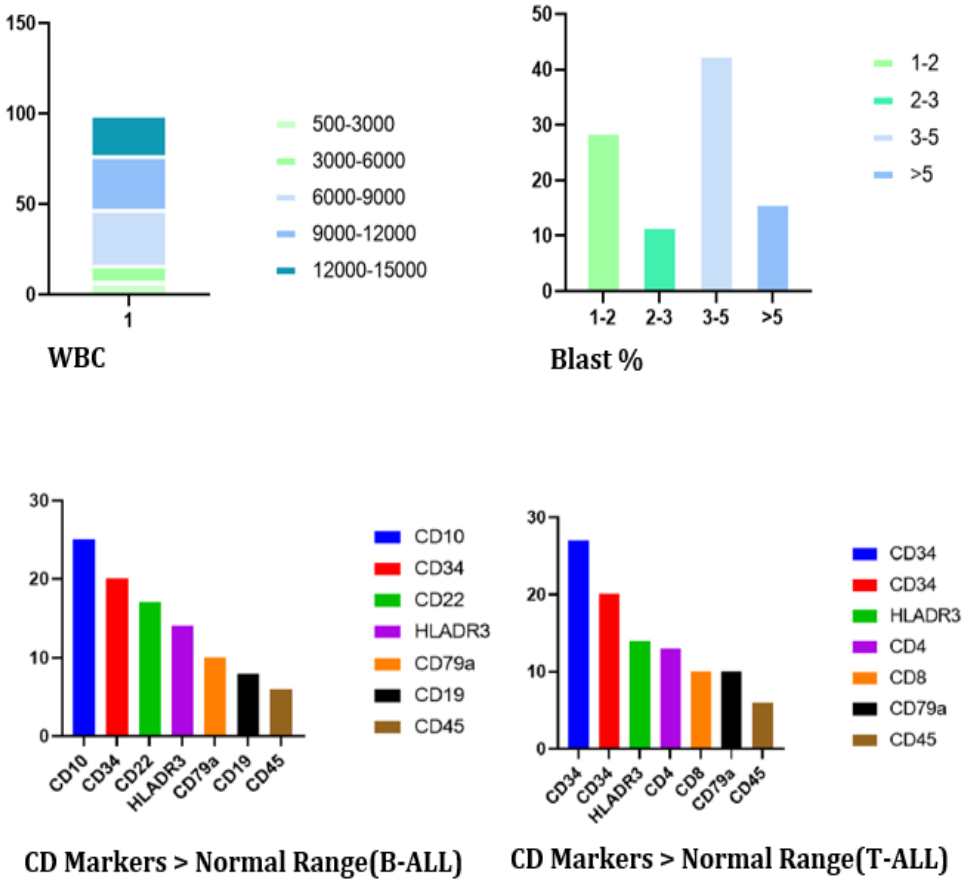

Figure 3. Paraclinical Tests Results (Post-Induction Time Point)

MRD assessment

After that, we evaluated MRD in patient's PB from genetic point of view and assessed Expression level of GAPDH (Normal Control), and PDGFRB by RT-PCR again. These assessment results, were reported in **Figure 4**. Difference in these genes' expression profiling between patients and with regarded to the difference in their sub-groups and compared to the beginning of their treatment, showed that in B-subgroup, PDGFRB expression level at beginning of treatment time point, was higher than control group; moreover, at post-Induction time point, this level decreased because the use of Tyrosine Kinase Inhibitors and was near to control normal groups. Therefore, this gene expression is effective in B-ALL biogenesis and has diagnostic and prognostic value as a novel biomarker and for MRD evaluation at post-Induction time point more specifically. We recommended this assessment in Pediatric ALL especially in B sub-group patients in both mentioned time points (Beginning of treatment: Diagnosis and Post-Induction: MRD).

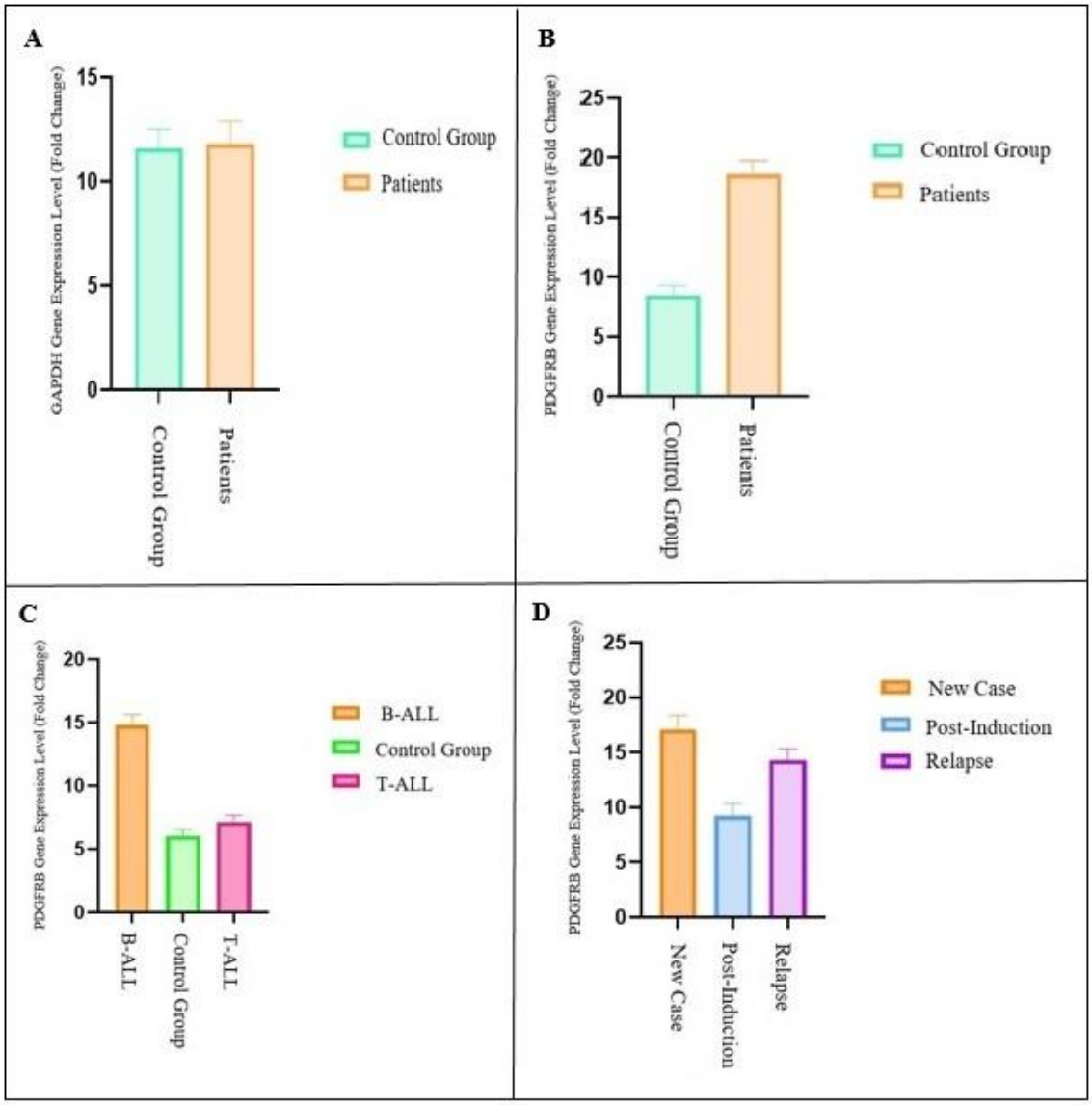

Figure 4. Expression level of PDGFRB and GAPDH (Fold Change)
(**A**. Control and Patient. GAPDH, **B.** Control and Patient. PDGFRB, **C.** B and T-ALL. PDGFRB, **D.** Different Time Points. PDGFRB).

Based on these results, the expression level of PDGFRB in T-ALL patients, was not significantly different compared to the normal control group (P=0.08); and in order to prove the effect of this gene up-expression in these patients' groups, this study should be completed with a larger number of patients more over **(Figure 4C)**; By MRD evaluation in these patients and treatment protocol improvement, treatment time point and cost decreased in contrast.

Leukemia is the fifth most common cancer in Iran, it is increasing during last decade. Diagnostic and prognostic methods with more accuracy, are effective in its management. Epigenetic and Immune response factors have important role in its prevalence in Iran. By reviewing recurrent researches, Genetic profiling of cancer in Iran is identified and these population-based profile can be as a prognostic biomarker with diagnostic citation capability consequently [33].

Leukemia caused by incomplete maturation and differentiation of blood cells in blood and bone marrow. It is classified based on type of involved cells. ALL is more common in children and AML is more specific to adults. Molecular biology of this disease, determined the molecular function of genetic changes and lead to new sub groups classification. Many genes have been reported in the biogenesis of ALL up to now and classified based on their function. Tumor suppressors, Oncogenes and fusions which are created by chromosomal translocations, are in this category; they have role at early detection.

Environmental factors and genetic alterations, have role in cancer biogenesis and progress. Change in life style and its place, lead to change in epidemiology and microbiome and have important role in cancer prevalence. Recent researches, identified Genetic profile of blood cancer among Iranian population; these are prognostic marker and have role in survival rate promotion [34]. We tried to collect samples from different geographical areas with different prevalence rate; So that a more investigation to be done about these samples microbiome role and ALL biogenesis in the future.

Change in normal Bone Marrow cells DNA, cause them to become leukemic. certain genes that help cells grow, divide or stay alive which is called oncogenes; and the genes that keep cell

grow and division under control or make die at right time, which are called tumor suppressor have effective role in biogenesis of leukemia furthermore, when they have mutations. These modifications, stop BM cells from maturing the way they normally would or help cells grow out of control and therefore they will be leukemic cells; many mutations have effective role in leukemia biogenesis but specific rearrangements are common and have the role at prognosis evaluation. t (12;21) (p13; q22), t (4;11) (q21; q23), t (1;19) (q23; p13), +Ph are at these lists **(Table 2)** Despite that about 25-30% of cases doesn't established abnormality [35].

Finance burden of cancer on health-care systems is at top of attention of health policies at developed countries; estimate direct and indirect cost of cancer treatment, especially ALL among children, based on BFM protocol in Iran; specifies total direct medical cost per patient for a complete treatment period. Health system administrators should devise an appropriate strategy to reduce direct medical costs which have more economic burden special for hospitalization days and chemotherapy costs bases on findings. Total cost of childhood ALL management is very high and it is high for patient and society as a whole. Diagnosis and treatment have also been identified as significant drivers of rising cost of cancer. Targeted therapy due to the use of drugs which have the mechanism with inhibition effect on target genetic modification, can decrease these cancer treatment costs. MRD evaluation by its role on treatment strategy, can reduce time point of treatment which lead to cost management.

Diagnostic tests in the field of MRD assessment, in terms on specificity and sensitivity were investigated and this evaluation have important role in target therapy. In the case of achieving target treatment; MRD will be negative and in the relapse situation, MRD will be positive. The evaluation of MRD in new case patients by flow cytometry and RT-PCR, is a prognostic marker and its time point has importance role in relapse detection. This measurement factors increased in both count and sensitivity elements and will increased cut off and lead to personalized medicine. About 30% of patients are at the risk of relapse and increase in diagnostic factors for MRD evaluation led to target treatment at these patients' group [36].

About 30-40% 0f B-ALL cases and 10-20% of T-ALL patients, have chromosomal rearrangement. Cytogenetic analysis remains the backbone of B-ALL diagnosis in many countries as chromosome banding analysis recurrent chromosomal abnormalities detectable in B-ALL patients and propose guidelines regarding their detection. Referring in parallel to the more general molecular classification approach, offer a diagnostic framework useful in a broad clinical genetic setting. t (12;21) (p13; q22), t (4;11) (q21; q23), t (1;19) (q23; p13), +Ph are at these lists and in B-ALL Primary chromosomal abnormalities list too. Translocation t (9;22) (q34; q11) [BCR: ABL1] is Secondary chromosomal abnormalities and complexity in B-ALL and lead to ABL1 overexpression**.** Despite that about 25-30% of cases doesn't established abnormality [37].

MRD evaluation at different time points have different results and its results shows treatment process. Negative MRD lead to good prognostic and complete remission [38]. According to latest European guidelines, patients with ALL after induction have to be evaluated from amount of MRD by appropriate biomarkers. Post-Induction is the best time point for this assessment. New therapeutic methods such as monoclonal antibodies and engineered T cells at patients with ALL are common, and evaluation of MRD at these patients' group is essential for reach to personalized medicine. ALL patients have complex genetic rearrangements and about 40% of them have chromosomal instability.

The evaluation of MRD with genetic biomarkers have higher diagnostic value and assessment of expression profiling of novel hub genes and novel fusions have been recommended in pediatric patients even after clinical symptoms improvement. Suitable biological sample for this assessment is PB and suitable tests are Flow cytometry with 10^{-4} RT-PCR with 10^{-5} and NGS with 10^{-7} diagnostic sensitivity [39].

B-cell precursor acute lymphoblastic leukemia (ALL), molecular subgroups are less well defined in T-lineage ALL. Currently, molecular characteristics are rarely considered for risk stratification. T-ALL patients characterized on transcriptome, and partly on DNA methylation and gene mutation level in correlation with clinical outcome .Adult T-ALL patients characterized by transcriptome sequencing with meaningful clinical follow-up. Risk classification based on molecular subgroups might emerge and contribute to improvements in outcome [40].

In this study, a more comprehensive and accurate evaluation of MRD in ALL patients was performed with the use of RT-PCR technique and with novel biomarker (PDGFRB). PDGFRA and PDGFRB are classical proto-oncogenes that encode receptor tyrosine kinases responding to platelet-derived growth factor (PDGF). Recently, mutations in PDGFRB were linked to several cancerous and noncancerous disease and lead to gain of function in proto-oncogenes. PDGFRB related fusions are common at B-ALL and also T-group patients, these changes distribution is depend on patient age, sex, the amount of white blood cells, and Immuno-phenotyping. Functional analysis of these variants has led to the preclinical validation of tyrosine kinase inhibitors targeting PDGF receptors, such as Imatinib, as a treatment for some of these conditions.

PDGFRB (5q32), the protein encoded by this gene is a cell surface tyrosine kinase receptor for members of platelet-derived growth factor family. These growth factors are mitogens for cells of mesenchymal origin and normally repressed in lymphocytes. PDGFRB is a common fusion partner in leukemia especially in B and Ph-like ALL. These genetic alterations block tyrosine kinase pathway and these patients' group, reach to treatment only with tyrosine kinase pathway inhibitors. High expression of this gene caused tumor progression, angiogenesis, and metastasis [15].

Ph-like ALL, have no BCR-ABL fusion. Reported 10-15% at childhood group, 20% at adult and 30% at young adult patients' group. These patients have lower survival rate compared to

another sub-groups [41, 42]. High expression level of this gene in B-ALL and Ph-like ALL patients lead to poor prognosis, Ph-like ALL characterizing by gene expression profile similar to Ph-positive B-ALL at lacking BCR-ABL Fusion. The molecular pathogenesis of Ph-Like B-ALL, is heterogeneous and involves aberrant genomics receptor over expression. Kinase fusions and mutations leading to kinase signaling activation in 91% of patients, Leukemogenic cellular proliferation and differentiation blockade. Tyrosine kinase inhibitors at this sub-group, will lead to targeted therapy [43, 44].

The PDGFRB signaling pathway is involved in the development of Blood vessels and hamatopoietic cells in the bone marrow, The autocrine activity of this messenger pathway has beeen implicated in glioma, sarcoma, and leukemia, and its paracrine activity has een reported in epithelial cancers. It plays a role in tumor growth, angiogenesis, migration of tumor cells and metastasis. Clinical studies on the fuction of this gene show that the expression of this gene plays a significant role in organogenesis, inflammation, and tumor formation and development [36].

Rearrangements in PDGFRB define a subtype of myeloid or lymphoid neoplasia that is sensitive to tyrosine kinase inhibitors. These genetic changes have been reported and introduced in the Ph-like ALL subtype. These rearrangements can be identified by classical cytogenetic techniques. The fusion of PDFRB gene with EBF1 gene, Which is a tumor suppressor, cause a decrease in the expression of this gene and increase in the expression of the PDGFRB gene and induces leukemia in B cells [45, 46].

So far, more than 30 fusion genes involving the PDGFRB gene have been reported, and their accurate identification is necessary for early diagnosis of the disease, targeted treatment, and increasing the survival rate of patients [34]. A new subtype in the B-ALL named Ph-like ALL and these patients show a high risk of relapse and have resistance to common treatments. They have complete remission if treated with tyrosine kinase inhibitors [47, 48]. Ph-like ALL subgroup is reported in 10 to 15% in children, 20% in adults and 30% in young adult. This subgroup of BCP ALL lacks the BCR-ABL fusion genes and will show a high risk of relapse. Many of patients at Ph-like subgroup, have genetic rearrangements related to tyrosine kinase pathway, they have poor prognosis and low survival rate and have response to tyrosine kinase suppressors indeed. These patients will have a positive MRD if treated with common chemotherapies and will reach to complete remission if treated with tyrosine kinase inhibitors [49].

Therefore, identifying rearrangements of the PDGFRB gene in this sub-group of paitients will lead to targted treatment [50, 51]. High expression of PDGFRB, is prognostic marker and affect the migration of leukemic cells and metastasis [52, 53].

We examining the expression of PDGFRB in patients compared to control normal samples, at new case time point; we follow their treatment protocol too. Cancer cells in th PB and BM, will destroyed by systemic chomotherapy and we evaluated their genetic MRD after that in post-Induction time point. Our results were consistent with results of recent international studies in the field of leuckemia biogenesis novel biomarkers [3, 44, 46, 50, 51, 52].

MRD evaluation by the use of RT-PCR and with novel biomarkers, lead to treatment protocol and financial resources of leukemia management; our main goal is health economics promotion and diagnostic and therapeutic financial resources maitenance among leukemia patients in Iranian society. We provide an update on our knowledge on B-ALL pathogenesis, on the opportunities for the introduction of targeted therapy and on the challenges that are still ahead, and suggest such evaluation for leukemia patients in other world regions.

Conclusion

In conclusion, this study results emphasizes that up-expression of PDGFRB had effective role in biogenesis of Iranian pediatric B-ALL and it can be considered as a diagnostic and prognostic biomarker. We identified the high expression of this gene compared to control normal and it has critical role in this patient's group and the authors suggest that in order to improve treatment strategies management, MRD had been evaluated by this novel biomarker at post-Induction time point. It has proposed potential target for therapeutic investigations.

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