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Plasmacytoma Variant Translocation-1 as Prognostic Markers in Pediatric Acute Lymphoblastic Leukemia

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ABSTRACT

Introduction and Aim: Acute lymphoblastic leukemia (ALL) is the commonest form of childhood leukemia. Molecular studies can provide diagnostic and prognostic information with direct impact on the patient. Recent micro RNAs (miRNAs) studies showed that aberrant expression can be used as signatures of ALL with different subtypes and predict drug resistance. The present work aimed to evaluate the expression of Plasmacytoma Variant Translocation-1 (PVT1) in Childhood ALL patients.

Methodology: The study included 55 children from 2 months to 15 years old; 45 patients with ALL before receiving any treatment, and 10 control children. Long non-coding RNA PVT1 expression in plasma cells was detected by reverse transcription quantitative polymerase chain reaction.

Results: Lnc_PVT1 expression is upregulated by twelve-folds in ALL patients. At optimum cut-off value of 22.0, the biomarker has a sensitivity of 72% and 82% specificity to discriminate ALL patients from controls. Correlating the mean values of miR_1204 gene fold expression with different subgroups concerning prognostic criteria, there was no statistical differences between age groups, gender, ALL phenotypes, cytogenetic abnormality, total leukocytes count, hemoglobin concentrations and platelet count. In contrast, statistical significance was detected with bone marrow blasts percentage, clinical response, and minimal residual disease.

Conclusion: A higher expression of lnc-PVT1 was observed in ALL patients, and it had a diagnostic and prognostic potential. In addition, miR-1204 was down-regulated in pediatric ALL and associated with higher risk.

Keywords: Plasmacytoma; Variant; Translocation 1; Pediatric; Leukemia.



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INTRODUCTION

Childhood acute lymphoblastic leukemia (ALL) is the most common cancer among children under 14 years of age ⁽¹⁾.

Despite remarkable improvements in survival (up to 85.0%, 5 year event-free survival rates), non-responding ALL children still represent one of the most frequent causes of death from cancer in pediatrics ⁽²⁾.

Childhood ALL comprises a wide range of molecular subtypes with distinctive somatic genetic alterations such as aneuploidy, rearrangements of chromosomes, and point mutations ⁽³⁾.

These alterations contribute to leukemogenesis by altering key regulatory processes, subverting normal proliferation control, blocking differentiation, and promoting resistance to death signals ⁽⁴⁾.

Despite this understanding of the molecular basis of this disease, accurate patient risk stratification is an ongoing challenge in chronic ALL (cALL) treatment and the development of innovative therapies ⁽⁵⁾.

Recently, a new class of non-coding RNAs, designated as long non-coding RNAs (lncRNAs) have been described ⁽⁶⁾.

lncRNAs are expressed in most cell types and at most stages of development and play regulatory roles in various biological processes, including cell pluripotency and tumorigenesis ⁽⁷⁾.

lncRNAs can exert their effects through many cellular processes ⁽⁸⁾, such as spatial conformation of chromosomes, chromatin and DNA modifications, RNA transcription, pre-mRNA splicing, mRNA degradation, and mRNA translation ⁽⁹⁾.

Plasmacytoma variant translocation-1 (PVT1), being a miRNA host gene, is an lncRNA involved in the pathogenesis of hematological malignancies ⁽¹⁰⁾.

It is located in chromosome region 8q24.21, relatively close to the transcription factor c-Myc. Translocations within c-Myc or PVT1, which cause the overexpression of these two oncogenes compared to healthy cells, are characteristics associated with B-cell malignancies including Burkitt Lymphoma (BL), Non-Hodgkin lymphoma, mouse plasmacytoma (Pct) and multiple myeloma (MM) ^(10, 11).

Furthermore, up-regulation of PVT1 contributes to tumor survival and chemo-resistance while down-regulation inhibits cell proliferation and induces a strong apoptotic response ⁽¹²⁾.

In addition, it has been proposed that PVT1 regulates c-Myc expression but also that PVT1 is regulated by c-Myc ⁽¹³⁾. However, some authors suggest that Myc and PVT1 contribute to cancer by different mechanisms ⁽¹⁴⁾.

The PVT1 gene is transcribed to several mature RNAs by alternative splicing, including a cluster of seven miRNAs, six of them annotated in the miR Base as miR-1204, miR-1205, miR-1206, miR-1207-5p, miR-1207-3p, and miR-1208. The function of these miRNAs is unknown with the exception of

miR-1204 ⁽¹¹⁾. miR-1204 has been involved in different roles related to development, differentiation and senescence ⁽⁹⁾.

On one hand, miR-1204 has been described as increasing p53 levels and causing cell death ⁽¹⁵⁾.

In fact PVT1 expression is induced in response to p53 ⁽¹⁶⁾. On the other hand, miR-1204 has been shown to activate Myc and cell proliferation in mouse pre- B cell lines ⁽¹⁵⁾. Thus, the aim of the present study was to evaluate the expression of PVT1 in Childhood ALL patients, and to find out regulatory axis of lnc-RNA PVT1 gene on miRNA-1204 in pathogenesis of ALL in these patients. In addition, we aim to evaluate the impact of lnc-RNA PVT1 on regulation of c-Myc, P53 target genes, and find out the association between the lnc-RNA PVT1 and the Childhood ALL Phenotype together with disease course, prognosis and clinic pathological features.

PATIENT AND METHODS

The current study was conducted on 55 individuals aged from 2 months to 15 years. They were divided into two groups. Group I included 45 patients selected from the Ain-Shams pediatric hospital, hematology department in the period from August 2017 to December 2018. Patients were diagnosed as childhood acute lymphoblastic leukemia before receiving any treatment in form of chemotherapy or radio-therapy. They were diagnosed on the basis of complete blood count, morphological bone marrow (BM) examination, Immuno-phenotyping and cytogenetic analysis.

Patients suffered from other hematological, immunological, cancer or genetic diseases were excluded. Group II included 10 children selected from the Clinic of Ain Shams University Hospital in the period from January 2018 to March 2018. Control group was matched for age and sex with the patients group.

After taking the approval of the Research Ethics Committee of Faculty of Medicine, Ain Shams University (FMASU 1568/2013), a written informed consent was obtained from each patient parent after informing him or her about the steps of the procedure and the expected effects.

Sample collection and preparation:

A volume of 1-3 ml of peripheral blood was collected from the patient into EDTA-containing labeled tubes. The anticoagulated blood was diluted with an equal volume of phosphate-buffered saline (PBS). A volume of 1 ml diluted blood was layered carefully down the side of a tube containing 750 µl of Ficoll-Hypaque solution (Sigma-Aldrich, USA) and the tube was then centrifuged at room temperature at 400 xg for 40 min. Differential migration of cells during centrifugation resulted in the formation of layers containing different cell types. The mononuclear cells were carefully removed from the interface and suspended in 3 volumes of PBS, gently mixed by pipetting up and down, then centrifuged at 100 xg at room temperature for 10 min. The supernatant was removed and the leukocytes pellet was washed once with PBS, then centrifuged.

Extraction and Purification of total RNA:

Total RNA was isolated from polymorphnuclear leucocytes

according to the initial silica extraction method originally described by Boom et al. (17) using the miRN easy Serum/Plasma kit (Qiagen, Hilden, Germany) (Cat. no. 217184).

Principle: The miRN easy Serum/Plasma kit was used for extraction and purification of total RNA through adsorption of nucleic acids to silica membranes in the presence of high concentrations of a chaotropic salt (a substance which disrupts the three-dimensional structure in macromolecules, such as proteins, DNA or RNA and denature them).

Procedure: Frozen samples were thawed at room temperature (18-25 °C). Five volumes (500 µl) QIAzol Lysis Reagent was added to 100 µl of the sample, then mixed by vortex. Then, the tube containing the lysate was placed on the benchtop at room temperature (15–25°C) for 5 min. One hundred µl of Chloroform was added to the tube containing the lysate and capped securely, then mixed by vortexing for 15 seconds. The tube containing the lysate was centrifuged for 15 min at 12,000 x g at 4°C. After centrifugation, the sample was separated into 3 phases: an upper, colorless, aqueous phase containing RNA; a white interphase; and a lower, red, organic phase. The upper aqueous phase was transferred to a new collection tube. One and half volumes of 100% ethanol were added and mixed thoroughly by pipetting up and down several times. Then, seven hundred µl of the sample was pipeted up into an RNeasy MinElute spin column in a 2 ml collection tube. The lid was closed gently and centrifuged at 8000 x g (10,000 rpm) for 15 seconds at room temperature (15–25°C). The flow-through was discarded. Seven hundred µl Buffer RWT was added to the RNeasy MinElute spin column. The lid was closed gently and centrifuge for 15 seconds at 8000 x g (10,000 rpm) to wash the column. Subsequently, the five hundred µl Buffer RPE was pipetted onto the RNeasy MinElute spin column. The RNeasy MinElute spin column was placed into a new 2 ml collection tube. The lid of the spin column was opened and centrifuged at full speed for 5 min to dry the membrane. The collection tube was discarded with the flow-through. Then, the RNeasy MinElute spin column was placed in a new 1.5 ml collection tube. Fourteen µl of RNase-free water was added directly to the center of the spin column membrane. The lid was closed gently and centrifuged for 1 min at full speed to elute the RNA. Finally, the purified RNA sample was stored at -80°C till further analysis.

Reverse transcription of mRNAs and miRNAs:

Total RNAs were reversibly-transcribed using a miScript II RT kit (Qiagen, Hilden, Germany) (Cat. no. 218161).

Principle: Unlike mRNAs, miRNAs are not polyadenylated in nature. During reverse transcription reactions, mature miRNAs are selectively converted to cDNA. Mature miRNAs are polyadenylated by poly (A) polymerase and then reverse transcribed into cDNA using oligo-dT primers and miScript reverse transcriptase enzyme.

Procedure: The reverse-transcription master mixture was prepared on ice for the first strand cDNA synthesis by mixing: 4 µl 5x miScript HiFlex buffer, 2 µl 10x miScript dNTP mixture, 2 µl miScript reverse transcriptase mixture and 7 µl RNase-free water.

Exactly 5 µl of RNA sample were added and each tube was mixed gently by pipetting up and down. The tubes were briefly centrifuged (to spin down the contents and eliminate any air bubbles), and then loaded into the Thermal Cycler (Biometra, Germany). Reverse transcription and first strand cDNA synthesis was performed for one cycle (37 °C for 60 min and 95 °C for 5 min). The cDNA product of the reverse transcription was stored at -20 °C until amplification.

Quantitative real time polymerase chain reaction (qRT-PCR):

qPCR amplification of miR-1204gene: Relative miRNA expression of the candidate miRNA (miR-1204) was analyzed by using miScript SYBR Green PCR Kit (Qiagen, Germany) (Cat. no. 218300) and miScript Primer Assay (Hs_miR-1204 and Hs_SNORD68_11 as a housekeeper gene (Qiagen, Germany). All samples were analyzed using the 5-plex Rotor-Gene Real-Time PCR Analyzer (Qiagen, Germany).

Principle: For the detection of mature miRNA, the cDNA prepared in the previous step acts as a template for real-time PCR analysis using a miRNA-specific miScript Primer Assay and the miScript SYBR Green PCR kit, which includes a universal primer (reverse primer) and QuantiTect SYBR Green PCR Master Mix

Procedure: The following master mix was prepared for a final volume of 18 µl per well as follows; 10 µl 2xQuantiTect SYBR Green PCR master mix, 2 µl 10x miScript universal reverse primer, 2 µl 10x miScript primer assay and 4 µl RNase-free water. Exactly 2 µl template cDNA were added and each well was mixed gently by pipetting up and down. The PCR plate was tightly sealed with the adhesive sheet. The real-time PCR cyclor was adjusted as follows: initial denaturation at 95°C for 15 min and 40 cycles (denaturation at 94°C for 15 sec, annealing at 55°C for 30 sec and extension at 70°C for 30 sec). Melting curves were performed by rapid heating to 95°C for 15 secs to denature the DNA, followed by cooling to 60°C to assure the purity and specificity of amplified products.

Calculations: The relative expression of miRNA-181a was normalized to an internal control (miRNA SNORD68-11) and relative to a calibrator (miRNA-34a from normal polymorphonuclear leucocyte sample) and was calculated according to Livak and Schmittgen (2001) as follows:

Relative quantitation (RQ) = $(2)^{-\Delta\Delta CT}$, Where $\Delta\Delta CT = (CT_{miRNA} - CT_{miRNA\ SNORD68-11})_{patient} - (CT_{miRNA} - CT_{miRNA\ SNORD68-11})_{control}$. Using the $(2)^{-\Delta\Delta CT}$ method, the data were presented as the fold change in miRNA level normalized to an endogenous reference miRNA and relative to controls .

qPCR amplification of mRNA (TP53 and c-Myc):

Gene expression analysis by real time PCR:

P53gene expression level was amplified from mRNA using QuantiTect primer assay Hs_TP53_1_SG QuantiTect Primer Assay, Hs_c-Myc_1_SG (Qiagen, Germany) and QuantiTect SYBR Green PCR Kit cat no: 204141 (Qiagen, Germany) and Hs_ACTB_1_SG QuantiTect Primer Assay cat no: 249900 as housekeeper gene. All samples were analyzed using the 5 plex Rotor Gene PCR Analyzer (Qiagen, Germany).

Procedure: x QuantiTect SYBR Green PCR Master Mix, 10x QuantiTect Primer Assay, template cDNA, and RNase-free water were thawed at room temperature (15–25°C). Then, the reaction mix was prepared for a final volume 18 µl per well reaction volume as following: 10 µl of 2x QuantiTect SYBR Green PCR Master Mix, 2 µl 10x t Universal Primer, 2 µl 10x Quantitect Primer Assay and 4 RNase-free water. The reaction mix was mixed thoroughly but gently, and dispensed appropriate volumes into the Rotor-Disc wells then 2 µl template cDNA was added, to reach 20 µl as final volume. Carefully, tightly the disc was sealed with Rotor-Disc Heat-Sealing Film. Consequently, the real-time cycler Initial was programmed as: activation step 15 min 95°C for HotStarTaq DNA Polymerase activation. Three -step cycling: denaturation 15 s 94°C, annealing 30 s and 55°C, extension 30 s 70°C, for 40 cycles. Moreover, the expression levels were normalized to B-actin levels as a reference gene. The relative expression level (fold change) for c-Myc was then calculated using the equation $2^{-\Delta\Delta Ct}$ test control as follow:

qPCR amplification of Lnc-RNA (PVT1) gene:

PVT1 gene expression level was amplified from mRNA using RT2 LncRNA PCR primer assay; Lnc-PVT1 Primer Assay (cat no: 330701) (Qiagen, Germany) and QuantiTect SYBR Green PCR Kit cat no: 204141 (Qiagen, Germany) and Hs_ACTB_1_SG QuantiTect Primer Assay cat no: 249900, ID: QT00095431 as housekeeper gene. All samples were analyzed using the 5 plex Rotor Gene PCR Analyzer (Qiagen, Germany).

Procedure: The RT2 SYBER Green Mastermix, RT2 LncRNA qPCR, and cDNA synthesis reaction were briefly centrifuged for (10-15 s) to bring the contents to the bottom of the tubes. The PCR components were then prepared in a nuclease-free tube .

Statistical analysis: The collected data were analyzed using statistical package for social sciences version 19 (SPSS Inc., Chicago, USA), running on IBM compatible computer. Testing for normal distribution was done by Kolmogorov-Smirnov and Shapiro-Wilk tests. For comparison between two groups, the independent samples (t) test or Mann-Whitney tests were used. For comparison between three groups, Kruskal Wallis Test (non-parametric) was used. For comparison between categorical

groups, the Chi square (χ^2) test was used. For all tests, P values < 0.05 were considered significant ⁽¹⁸⁾.

RESULTS

The ALL group (n=45) were classified according to WHO-2012 phenotypic classification into three subgroups (Pre-B-ALL represents 21(47%), T-ALL 13(29%) and biphenotypic ALL 11(24%)) table (1). The expression of Lnc_PVT1 biomarker; was compared in children with ALL versus the healthy controls. We found a significant difference in the expression of Lnc-PVT1 ($p < 0.01$). Lnc_PVT1 expression is upregulated by twelvefold in ALL patients (median: 19.3; range: 3.2 – 36) compared to control group (median: 0.8; range: 0.2 – 5.5) (Table 2). When comparing the expression level of Lnc_PVT1 in ALL patients with healthy controls; we found that Lnc_PVT1 is a good diagnostic biomarker in ALL; at optimum cut-off value of 22.0; the biomarker has a sensitivity of 72% and 82% specificity to discriminate ALL patients from controls The AUC was 0.9 and $p = 0.001$. Data are illustrated in Table 3. Correlating the mean values of miR_1204 gene fold expression with different subgroups concerning prognostic criteria; it was found that no statistical difference ($p > 0.05$) was found between (favorable / unfavorable) age groups; gender (male/female); ALL phenotypes (pre-B ALL, T-ALL, biphenotypic ALL, Cytogenetic abnormality(Favorable / Unfavorable); total leukocytes count ($\leq 50 / > 50$); hemoglobin level ($> 6 / \leq 6$) /gm/dl; platelets count ($\leq 10,000 / > 10,000$)/ccm;. In contrast, statistical significance ($p \leq 0.05$) was detected with childhood ALL patients at diagnosis and control group gene expression levels; bone marrow blasts percentage ($\leq 25 / > 25$); Clinical Response (remission / relapse) and minimal residual disease ($\leq .01 / > .01$) as shown in table (4). Finally, significant correlation was observed between TP53 with Lnc-PVT1 as well as miR-1204 ($r = 0.5, -0.4$), respectively. The correlation is weak positive with Lnc-PVT1 ($r = 0.4$); high expression of TP53 is associated with increase in Lnc-PVT1 expression. As well as positive correlation was observed between TP53 and miR-1204 ($r = -0.5$). These findings are presented in Table (5).

Table (1): Patient characteristics, comorbid disease and clinical manifestations among study groups

Variable	Group A (n=12)	Group B (n=12)	Test	P value
Age (years)	45.89 ± 3.88	46.27 ± 3.86	0.946	0.528
Sex (n,%)	Male	11(91.67%)	1.987	0.108
	Female	2(16.67%)		
Comorbidity (n,%)	Smoking	4 (33.33%)	0.001	1.00
	Diabetes	5 (41.67%)	1.84	0.12
	Hypertension	3 (25%)	1.79	0.13
	Ischemic HD	0 (0%)	1.76	0.14
Symptoms (n,%)	Discharge	12 (100%)	0.001	1.0
	Pruritis	6 (50%)	0.001	1.0
	Anal pain	4 (33.33%)	1.81	0.13
Symptoms duration (months)	5.35 ± 1.39	5.83 ± 1.67	1.11	0.375
Type of recurrent fistula (n, %)	Intersphincteric	7 (58.33%)	1.84	0.122
	Low transsphincteric	3 (25%)		
	Subcutaneous	3 (25%)		

Table (2): Operative and postoperative data among study groups

	Group A (n = 12)	Group B (n = 12)	Test	P value	
Operative time (minutes)	27.08 ± 1.40	38.75 ± 1.63	5.314	< 0.001*	
Duration of wound discharge (weeks) (median (IQR))	3 (3 – 4)	2 (2 – 3)	4.126	0.019*	
Wound size (cm ³)	3.31 + 0.54	2.11 + 0.37	3.789	0.033*	
Wound healing time (weeks) (median (IQR))	6 (6 – 8)	5 (4 – 6)	5.008	0.005*	
Postoperative VAS median (IQR))	2 (2 – 5)	2 (2 – 4)	1.964	0.268	
Postoperative complications (n,%)	SSI (surgical site infection)	1 (8.33%)	0 (0%)	1.766	0.142
	Bleeding	0 (0%)	0 (0%)	0.001	1.00
	Urine retention	2 (16.67%)	1 (8.33%)	1.784	0.158
	Minor incontinence	1 (8.33%)	0 (0%)	1.766	0.142
One-year recurrence rates	1 (8.33%)	0 (0%)	1.766	0.142	

DISCUSSION

Pediatric leukemia is unique for its clinical and biological properties: response to treatment, cure rate, and immune-phenotype. The development of leukemia relates not only to chromosome abnormalities and gene mutations, but is also closely associated with epigenetic characteristics and miRNA expression profiles⁽¹⁹⁾. Previous studies showing that miRNAs can modulate the expression of target genes at the post-transcriptional level and that miRNA expression is also subject to epigenetic regulation contributed to the clinical application of miRNAs⁽²⁰⁾.

MicroRNA (miRNA) is a non-coding single stranded RNA, which plays an important role in cell proliferation, differentiation, apoptosis and tumor formation and development. Studies have shown that miRNA is intimately associated with the susceptibility of tumor cells to chemotherapeutic drugs⁽²¹⁾.

In the present study, molecular analysis using (RQ-PCR) technique was applied on 45 peripheral blood (PB) samples of children with ALL at diagnosis (before starting treatment) and 10 healthy children as control group; to assess the expression level of Inc_PVT1 and miR-1204 as an indicators to monitor disease prognosis and outcome. In addition, we find out the correlation between Inc_PVT1 and the c-Myc as proliferative gene, as well the TP53 as apoptotic gene.

Among studied subjects, it was found that the level of the Inc-PVT1 gene expression was increased by 12 folds in pediatric ALL compared to control group. A high significant difference was observed between ALL patients and controls. In consistence with previous study, it was found that IncRNA PVT1 expression level was measured in peripheral blood cells from 28 patients with de novo acute promyelocytic leukemia (APL), and it was significantly upregulated in APL patients compared with healthy donors⁽²²⁾.

Also, the Inc-PVT1 was significantly associated with lower expression of MYC. In addition, the study was focused on the role of Inc-PVT1 in APL, they observed that the higher PVT1 expression levels in APL cell line induces cell cycle arrest and stop. Moreover, the knockdown of c-Myc in APL cell line decreases the Inc-PVT1 expression, in the other side, the knockdown of PVT1 by short interference RNA decreases the

c-Myc expression and inhibits cell proliferation. These finding have strongly supported the role of Inc-PVT1 in the pathogenesis of APL by promoting the cell proliferation.

Moreover, the aberrant expression of IncRNA PVT1 has been observed in several types of cancers including Hepatocellular carcinoma⁽²³⁾, Non-small cell lung cancer⁽²⁴⁾, pancreatic adenocarcinoma⁽²⁵⁾, and gastric cancer⁽²⁶⁾, and it has been functionally linked to cancer tumorigenesis. Cumulative evidences showed that the Inc_PVT1 act as host gene for several miRNAs⁽¹¹⁾. Although there are a few reports demonstrating the role of Inc-PVT1 in the pathogenesis of cancer, the cross link between the Inc-PVT1 and c-Myc have been reported in specific subtypes of Acute Myeloid Leukemia and its expression was repressed during All trans Retinoic Acid (ATRA)-induced differentiation⁽²⁷⁾. The relation between Inc-PVT1 and c-Myc was explained by the fact that both genes are located on 8q24, and c-Myc is a well-known transcriptional activator gene which promotes cell proliferation, therefore, an increase in c-Myc activity may result in increase in number of copies of Inc-PVT1. These findings suggest that the elevated PVT1 expression may also result from MYC protein activation in APL cells and vice versa⁽¹⁴⁾. More importantly, a recent study had demonstrated that the involvement of PVT1 by regulating c-Myc in cancer was firmly established to play a role in pathogenesis of cancer⁽²⁸⁾.

The marker that had been used in this study was microRNA-1204 (miR-1204). It is small RNAs consisting of 21–25 nucleotides; located on chromosome 8 in Homo sapiens that bind to partially complementary sequences in the 3'untranslated region of mRNA (3'UTR) and provide negative post-transcriptional regulation. It was recognized that the role of miRNA in cancer pathogenesis has fueled interest in the development of clinical trials and preclinical studies targeting specific miRNAs⁽²⁹⁾. These genes frequently appear to be functionally related, being involved in the activation of specific oncogenic or tumor suppressor genes pathways in cancer in different pathways. There is increasing evidence that miR-1204 play an important role in development or differentiation and that their deregulated expression may be a contributing factor in a variety of cancer as: there is a study of the pathophysiology of ovarian and breast cancer, it has been shown that MYC and PVT1 act independently, this action is mediated through apoptosis. Furthermore, this action may be

associated specifically with amplification of the 8q24 region. Induction of apoptosis by over-expression of miR-1204 and perhaps the other miRNAs in the miR-1204 -1208 cluster as well suggests an anti-proliferative role for the miRNAs independent of MYC⁽³⁰⁾.

Our results revealed a high significant difference for the expression of miR-1204 between child (pediatric) acute Lymphoblastic leukemia and healthy control group ($p < 0.01$) and it is significantly associated with clinical response and blast percentage as well as high risk group patients, additionally a receiving operating characteristic curve analysis was conducted to evaluate the diagnostic and prognostic potential of miR-1204 in pediatric acute lymphoblastic leukemia. We observed that miR-1204 has a diagnostic and prognostic value to discriminate acute lymphoblastic leukemia from health group (control) and between high and low risk groups.

We observed in the expression level of miR-1204 was lower in pediatric ALL than the control with highly statistical significance ($p < 0.01$). miR-1204 was up-regulated by 2 folds in acute lymphoblastic leukemia (median: 11.5), (range: 3.6 – 28) compared to control group (median: 4.8) (range: 0.7 - 17). Up to date there is no evidence which proves the promotion of miR-1204 in expression in case of ALL but there are several studies that agreed with this study about the expression of miR-1204.

As an attempt to evaluate the prognostic value of biomarker miR-1204 in pediatric ALL, comparative analysis was conducted between different high and low risk groups. A statistical significant difference was observed between acute lymphoblastic leukemia with high blast count verse low blast count, between patients who achieved complete remission verse those who relapsed and between minimal residual disease < 0.01 verse > 0.01 ($p < 0.01$). On the other hand, no significant associated level of miR-1204 and other risk groups including: age, gender, total leucocytes count, hemoglobin concentration, platelet count, phenotypic type and cytogenetic abnormalities ($p > 0.01$). According to **Peng et al.**⁽³¹⁾, they have proved that miR-1204 is related to high risk group in nasopharyngeal carcinoma in both in vitro and in vivo studies cells to paclitaxel. They have established that paclitaxel-resistant CNE-1/Taxol, HNE-2/Taxol have high expression level on a variety of miRNAs except for miR-1204 which was down-regulated in both in vitro and in vivo studies done with paclitaxel on nasopharyngeal carcinoma which proves that miR-1204 can be used as a prognostic biomarker in nasopharyngeal carcinoma. Another study done by **Nowicka et al.**⁽³²⁾ that showed in there study that miRNA-1204 have potential prognostic value to serve as screening tool for head and neck squamous cell carcinoma (HNSCC) by performing meta-analysis to construct a protein-protein interaction network, which was used to identify targets of the miRNAs and potential drugs. This study viewed relationship between miRNA expression and prognosis of HNSCC.

In order to evaluate the diagnostic and prognostic potential

of miR-1204 in pediatric acute lymphoblastic leukemia, receiving operating characteristic curve was conducted, the sensitivity and specificity of the biomarker was calculated, our results revealed that miR-1204 can discriminate between acute lymphoblastic leukemia and control with a sensitivity of (73) and specificity of (80) when the cut of value was adjusted at (8.2). In addition a high significance was achieved for miR-1204 in discriminating high risk of acute lymphoblastic leukemia from standard risk group ($p < 0.01$). The biomarker can differentiate between different risk groups. According to **Egawa et al.**⁽³³⁾, they have proved that miR-1204 have play an important role as a diagnostic biomarker in bladder cancer by the downregulation of it by targeting of KRT7 and altering its oncogenic function in addition to its role as a biomarker in glioblastoma by knocking down the expression of p27Kip1 and inhibition of apoptosis and invasion therefore this proves that miR-1204 has a promising diagnostic role of cancers.

Conclusions

We can conclude that the higher expression level of Inc-PVT1 is observed in ALL patients, and it has a diagnostic and prognostic potential. In addition, miR-1204 is down-regulated in pediatric acute lymphoblastic leukemia and compared to healthy control and associated with high risk group of patients. Moreover, it has a diagnostic and prognostic value for pediatric acute lymphoblastic leukemia. Finally, the Inc-PVT1 was significantly linked to miR-1204 and both c-Myc and TP53 in ALL. This could open the era to use this marker as a therapeutic target in the future. The limitation of this study was related to small sample size and minor heterogeneity of pediatric acute lymphoblastic leukemia. Therefore, we recommend extension of this study on large sample size and more homogenous subgroups.

Conflict of interest: none

Financial disclosure: none to be disclosed

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