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Predictive Value of miRNA-181a in Pediatric Acute Lymphoblastic Leukemia

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Abstract

Background: Acute lymphoblastic leukemia (ALL), a common pediatric malignant neoplasia, showed high relapse rate after induction therapy. Some miRNAs have been shown to regulate normal hematopoiesis and their disruption could contribute to leukemogenesis: Recently, specific miRNA, including miR-181a, was shown to be involved in the pathogenesis of ALL, serving as a biomarker for diagnosis and relapse of ALL. **Aim of the Study:** To evaluate miR-181a expression level as a predictive marker for children with acute lymphoblastic leukemia. **Patients and Methods:** 40 pediatric ALL patients were included in this study. miR-181a expression was assessed at diagnosis before start of treatment. Samples were either peripheral blood or bone marrow aspirate sample. Patients were evaluated clinically and laboratory after the induction therapy. **Results:** The remission rate was significantly higher in patients with high miR-181a expression compared to those with low expression ($p < 0.001$). **Conclusion:** The expression level of miR-181a was significantly higher in remission group than in non-remission group was and predict good response to induction therapy.

Keywords

miR-181a, Micro-RNA, ALL

1. Introduction

Acute lymphoblastic leukemia (ALL) is considered the most common pediatric cancer, one in four of all malignancies in children and approximately 75% of all pediatric leukemia. ALL is characterized by clonal proliferation of B-cell precur-

sors and/or T-cell precursors and results in accumulation of lymphoblasts in bone marrow (BM) and various extra-medullary sites [1]. Despite the improvement in survival of childhood ALL, non responding or relapsing patients still represent one of the most common causes of death in children. Therefore, early treatment response can predict the risk of relapse and help assigning patients to subsequent risk-adapted therapy [2].

miRNAs are small (typically 18 - 25 nucleotides), single stranded non-coding RNAs that negatively regulate gene expression at the post-transcriptional level. According to the target mRNA, miRNAs serve as tumor suppressors or oncogenes [3]. Recently, specific miRNAs have been reported to be involved in the pathogenesis of leukemia [4]. Many studies have been published on either an individual miRNA or a panel of miRNAs as a diagnostic biomarker in childhood ALL and many of these studies were carried out to find miRNAs as a non-invasive prognostic biomarker [5] [6] [7] [8] [9].

miRNA-181 (miR-181) family encoded by three different transcripts is located on three different chromosomes. MiR-181a and miR-181b cluster together on chromosome 1 and miR-181a2 and miR-181b2 are located on chromosome 9. The miR-181c and miR-181d are on chromosome 19 [10].

miR-181 is known to play a crucial regulatory role in leukocyte cell differentiation and function. It has been shown that miR-181 has a key role in T-cell maturation, particularly at the $CD4^+$ $CD8^+$ stage of thymocyte development. miR-181 inhibits the expression of genes involved in positive selection and T-cell maturation such as BCL2, CD69, and TCR. These findings support that future studies should focus on miR-181 family in the management of ALL [11]. miR-181a and miR-181b have been reported to be highly expressed in childhood ALL. The sensitivity & specificity of miR-181a were 86.5% & 93.3%, respectively [12].

In this study, we aim to evaluate the utility of on miR-181a expression as predictive factor, risk stratification and to correlate its expression to the response after induction therapy in childhood ALL.

2. Patients and Methods

This prospective cohort study was carried out in Oncological Clinical Pathology Department, South Egypt Cancer Institute (SECI), Assiut, Egypt and included 40 newly diagnosed pediatric acute lymphoblastic leukemia patients. Their ages ranged from 2.5 to 16 years with a mean of 8.6 ± 3.4 years. All cases were collected from pediatric oncology clinic of SECI from January 2018 to May 2019.

Diagnosis of ALL patients was established through full history taking, clinical examination, complete blood picture, bone marrow aspirate (BMA) examination, flow cytometric and cytogenetic analysis. All the cases met the ALL WHO 2016 diagnostic criteria. Patients have been evaluated for the response after induction therapy clinically, by complete blood picture and bone marrow aspirate examination. We divided pediatric ALL cases after induction therapy to:

- Remission cases (complete and incomplete) in which they are clinically free, normal CBC and normal BMA after induction phase (blast cells less than 10%).
- Non remission cases include: Relapsed cases in which (clinically show persistent or relapsed organomegaly or lymphadenopathy, persistent or relapsed cytopenia and BMA shows blast cell 10% or more) and died cases include cases died during or just after induction phase.

Written informed consent was taken from all patients and study protocols were approved by the ethical committee, South Egypt Cancer Institute, Assiut University, Egypt.

2.1. Specific Laboratory Investigation

Before start of treatment and after established diagnosis, miR-181a expression is measured in peripheral blood or bone marrow aspirate sample by real time PCR.

2.2. Measurement of miR-181a

2 ml of bone marrow aspirate or peripheral venous blood sample were withdrawn in sterile vacationer tubes containing EDTA under complete aseptic conditions. miRNA was extracted from samples by miRNeasy Mini kit (Qiagen, Germany) extraction kit. Using TaqMan® MicroRNA Reverse Transcription (RT) Kit and TaqMan® Small RNA Assay (applied biosystems, USA), specific miRNA is accurately detected and converted to cDNA. Then, Real-time PCR (q-PCR) was performed with TaqMan® Universal Master Mix (Thermo Fisher Scientific, USA) and TaqMan® specific Assay using 7500 Fast Real-Time PCR System (Thermo Fisher Scientific, USA).

The relative expression of miR-181a was presented as the fold change using $2^{-\Delta\Delta CT}$ method, normalized to endogenous housekeeping RNU6B gene and relative to the normal control subject included in the experiment.

3. Statistical Analysis

Qualitative data were described using number and percent and quantitative data using mean ± SD. All analyses were conducted using SPSS software version 21. Univariant factors were analyzed using the chi-square test for categorical variables except variables with less than 5 in one cell in which fisher exact test was done for them. Mann Whitney test was done for quantitative variable. In all statistical tests, level of significance of 0.05 was used, below which the results are considered statistically significant.

4. Results

4.1. Clinical and Laboratories Features of the Study Group

The study was done on 40 newly diagnosed pediatric ALL patients, which were evaluated for miR-181a expression relative to normal healthy, same sex and age children. Clinical and laboratories features of the study group are seen in **Table 1**.

Table 1. Clinical and laboratories features of the cases. Mean ± SD or Number (%).

Parameter	Finding
Number	40
Age (year)	6.8 ± 3.4
Gender	
Male	22 (55%)
Female	18 (45%)
Hepatosplenomegaly	
Yes	14 (35%)
No	26 (65%)
Generalized lymphadenopathy	
Yes	15 (37.5%)
No	25 (62.5%)
WBCs	
less than 50,000/mm^3	11 (27.5%)
Equal or more than 50,000/mm^3	29(72.5%)
Immunophenotyping	
B-ALL	33 (82.5%)
T-ALL	7 (17.5%)
Philadelphia chromosome BCR-ABL1/t (9; 22) (q34; q11)	
Positive	20 (50%)
Negative	20 (50%)

ALL = Acute lymphoblastic leukemia; B-ALL = B-lineage acute lymphoblastic leukemia; T-ALL = T-lineage acute lymphoblastic leukemia.

Among 40 newly diagnosed ALL, there are 22 (55%) males and 18 (45%) females. Also, 14 cases (35%) have hepatosplenomegaly and 15 cases (37.5%) have generalized lymphadenopathy.

As regard to WBCs count, 11 (27.5%) show WBCs count less than 50,000/mm^3 and 29 (72.5%) show WBCs count more than or equal to 50,000/mm^3.

Among 40 cases of pediatric ALL, 33 (82.5%) cases are B cell phenotype, 7 (17.5%) cases are T cell phenotype by flow cytometry. As regard to Philadelphia chromosome t (9; 22) (q34; q11) BCR-ABL1 cytogenetic analysis by FISH, there are 20 (50%) cases positive and 20 (50%) cases are negative.

4.2. Expression of miR-181a in 40 Newly Diagnosed ALL Cases

We found that 33 patients (82.5%) show over expression of miR-181a with RQ (57.6 ± 170.5) and 7 (17.5%) of patients show under expression of miR-181a with RQ (0.43 ± 0.24) (cut off = 1) (Table 2).

Table 2. Expression of miR-181a in 40 newly diagnosed ALL cases.

Parameter	Finding
Number	40
miR-181a expression	
Over expression	33 (82.5%)
Under expression	7 (17.5%)
miR-181a (RQ)	
Over expression	
Mean ± SD	57.6 ± 170.5
Min - max	1.2-987
Under expression	
Mean ± SD	0.43 ± 0.24
Min - max	0.11 - 0.78

RQ: relative quantification or fold change by RT-qPCR.

4.3. Association between miR-181a Expression and Clinic-Laboratories Features of Cases

No significant difference between high and low expresser of miR-181a as regard to age, sex, hepatosplenomegaly, generalized lymphadenopathy and WBCs in these patients. Neither were the significant difference between high and low miR-181a expresser as regard to immunophenotyping and t (9; 22) (q34; q11) BCR-ABL1 in these patients (Table 3).

4.4. Outcome of Cases after Induction Therapy and Its Association with miR-181a Expression

We evaluated cases after induction therapy and found that 26 (65%) of the patients were in remission after the induction therapy and 14 (35%) of cases were not in remission (Table 4, Figure 1).

We then asked whether miR-181a could serve as the biomarkers for prediction of leukemia remission and found that there is a significant association between over expression of miR-181a and the remission after induction therapy ($p < 0.001$).

5. Discussion

miR-181a was reported to be over expressed in childhood ALL. miR-181a is known to play a regulatory role in leukocyte cell differentiation and function [9]. These findings support the suggestion miR-181a can be used as a predictive factor in the management of ALL.

We conducted this study to assess the expression of miR-181a in childhood ALL, its clinical association with clinic-pathological features of cases and the response after induction therapy.

Table 3. Association between miR-181a expression and clinic-laboratories features of 40 ALL cases.

Parameter	Over expression	Under expression	P
Number	33	7	
Age (year)			
Mean ± SD	6.9 ± 3.6	6.1 ± 2.5	0.76
Gender			
Male	19 (57.6%)	3 (42.9%)	0.68
Female	14 (42.4%)	4 (57.1%)	
Hepatosplenomegaly			
Yes	12 (36.4%)	2 (28.6%)	0.99
No	21 (63.6%)	5 (71.4%)	
Generalized lymphadenopathy			
Yes	13 (39.4%)	2 (28.6%)	0.7
No	20 (60.6%)	5 (71.4%)	
WBCs			
less than 50,000/mm^3	22 (66.7%)	7 (100%)	
Equal or more than 50,000/mm^3	11 (33.3%)	0 (0%)	0.16
Immunophenotyping			
B-ALL	28 (84.8%)	5 (71.4%)	0.58
T-ALL	5 (15.2%)	2 (28.6%)	
Philadelphia chromosome BCR-ABL1/t (9; 22) (q34; q11)			
Positive	16 (48.5%)	4 (57.1%)	0.99
Negative	17 (51.5%)	3 (42.9%)	

Significant $p < 0.05$.

Table 4. Outcome of cases after induction therapy and its association with miR-181a expression.

Parameter	Over expression	Under expression	P
Number	33	7	
Outcome after induction therapy			
Remission	26 (78.8%)	0 (0%)	<0.001*
Non remission	7(21.2%)	7 (100%)	

*Very Significant $p < 0.005$.

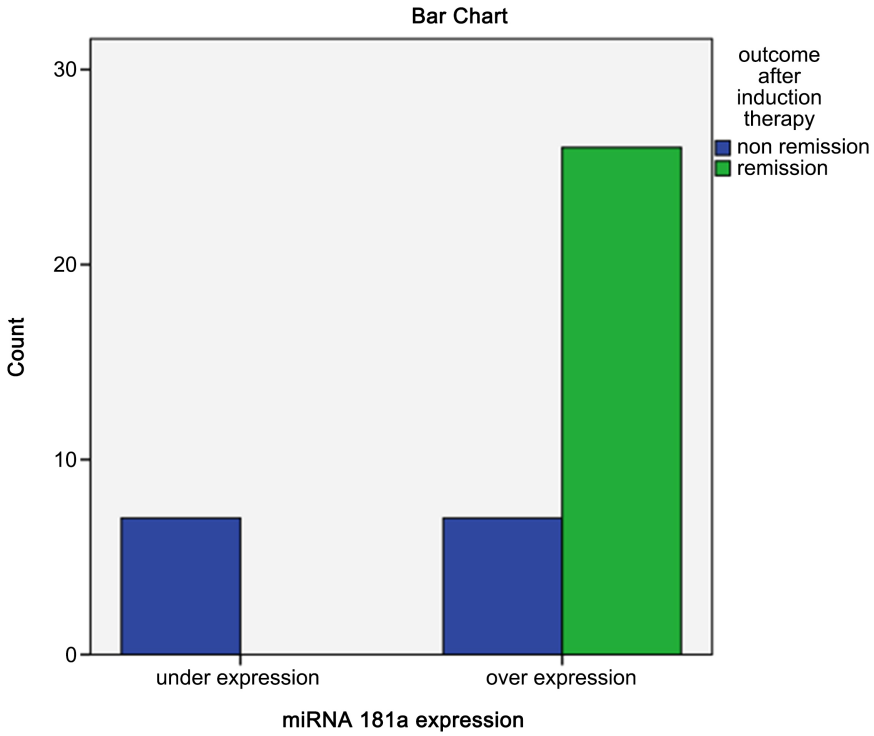

Figure 1. Association between miR-181a expression and the outcome after induction therapy.

We found no significant difference between high and low expresser of miR-181a as regard to sex, hepatosplenomegaly, WBCs level and generalized lymphadenopathy in these patients. Neither was the significant difference between high and low miRNA 181 expresser as regard to immunophenotyping and t (9; 22) in these patients.

These results agreed with El-Khazragy *et al.* [13] study that concluded no significant correlation between miR-181a expression and each of the following: age, sex, WBCs count and immunophenotyping in pediatric ALL patients.

Yan *et al.* [14] disagreed with our result, reported that miR-181a expression was upregulated in T-ALL.

As regard to response after induction therapy, we found significant relationship between expression of miR-181a and the outcome after induction therapy in which patient with over expression of miR-181a developed remission and had a good response to induction therapy.

This finding is in parallel with Wang JJ &Yu JP [15] study proved that miR-181a over expression inhibited leukemia cell proliferation, induced apoptosis, and reduced Adriamycin resistance. Also, our result is supported by the previous meta-analysis. Lin *et al.* [16] reported that high expressed miR-181a could prolong overall survival (OS) in patients with hematological malignancies including ALL. Also, our result is in line with Li *et al.* [17] and Bai *et al.* [18] concluded that over expression of miR-181a sensitizes leukemia cells to daunorubicin and cytarabine treatment, respectively.

El-Khazragy *et al.* [13] could not find a link between the expression of miRNA-181a in pediatric ALL and the prognosis.

In the contrary, Zhu *et al.* [19] found that the expression of miR-181a in favorable prognosis group was significantly lower than in poor prognosis group. Also our result disagreed with Han *et al.* [20] who proved that miR-181a was upregulated with higher fold changes in the relapse samples compared with complete remission samples in pediatric ALL case and Wang *et al.* [21] who determined that increased expression of miR-181a was associated with a poor outcome.

6. Conclusion

We conclude that miR181 over expression is associated with good response to the induction therapy and hence good outcome, which may help provide new insights into the disease monitoring and treatment response of childhood ALL. This also will help clinicians to divide patient to high and low risk group with different treatment strategies according to miR181 expression.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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