Gene Reports 24 (2021) 101237

Contents lists available at ScienceDirect

Gene Reports

journal homepage: www.elsevier.com/locate/genrep

The role of miRNA-196a2 genotypes in the susceptibility of acute lymphoblastic leukemia in Egyptian children

Dalia M Abd El Hassib, Nevein A Abdulhafeez, Ola M. Atef, Seham G. Ameen *

Chemical and Clinical Pathology Dept., Faculty of Medicine, Benha Univ., Benha, Egypt

ARTICLE INFO

Keywords:
Acute lymphoblastic leukemia
miRNA-196a2
Polymorphism

ABSTRACT

Background: Different types of miRNA were discovered to have a big role now in many diseases and cancer.
Objectives: This study assessed the association between the miRNA-196a2 (rs 11614913) polymorphism and acute lymphoblastic leukemia (ALL) in children.
Design: Retrospective case-control study.
Settings: This study was done in Benha University Hospital Pediatric Department & Benha Specialist Hospital for Children from May 2019 to February 2020.
Subjects and methods: Blood DNA samples from cases and control children were studied for the miRNA-196a2 (rs11614913) polymorphism using Polymerase Chain Reaction-Restriction Fragment-Length Polymorphism (PCR-RFLP) approach.
Sample size: 100 childhood ALL patients and 60 apparently healthy children as control were recruited in our study.
Results: The frequency of miRNA-196a2 (rs 11614913) C allele was 56.7% in control compared to 54% in cases. In addition there was no significant association between CC or TT genotypes and susceptibility of pediatric acute lymphoblastic leukemia (P = 0.74 and 0.36). There was statistically highly significant increase in TT genotype in males compared to females and significant increase in mean of LDH and ESR in TT genotype compared to CC genotype.
Conclusion: We concluded there was no association between miRNA-196a2 genotypes and pediatric acute lymphoblastic leukemia in Egypt.
Limitations: Small patient sample size.

1. Introduction

Acute lymphoblastic leukemia (ALL) is known to be a hematological heterogeneous disease which is characterized by the multiplication of immature lymphoid cells within the bone marrow, peripheral blood, and further organs. Its incidence represents 75%–80% of acute leukemias in children (Brown et al., 2020).

Childhood ALL is the commonest malignancy found in children and adolescents and is characterized by a wide range of clinical and biological heterogeneity which is widely sustained by a diverse background of disease initiating and maintaining frequent structural and/or numerical genetic mutations acquired by the leukemic clone (Stanulla et al., 2020).

Etiology of ALL is not yet known, but a lot of factors are involved in the causality of ALL. When we talk about risk factor, pediatric ALL is related to some genetic syndromes, ionizing radiation, and genetic susceptibility. Environmental exposure factors play a part in the accumulation of somatic mutations in children (Farokhian et al., 2020).

microRNAs (miRNAs) is a small non-coding RNAs (ncRNAs) of about 22 nucleotides in size, it plays significant roles in gene regulation. Their dysregulation is involved in human diseases including cancer, miRNAs could play roles in the cancer development, contribute in the process of tumor immunity and can be used as biomarkers for the diagnosis and prognosis (He et al., 2020). Multiple studies have shown association of miRNA SNPs with cancer and other diseases (Dzikiewicz-Krawczyk, 2015).

Abbreviations: ALL, acute lymphoblastic leukemia; miRNAs, microRNAs; ncRNAs, non-coding RNAs; PCR-RFLP, Polymerase Chain Reaction-Restriction Fragment-Length Polymorphism; SNPs, single nucleotide polymorphisms.

* Corresponding author.
E-mail address: seham.ameen@fmed.bu.edu.eg (S.G. Ameen).

https://doi.org/10.1016/j.genrep.2021.101237
Received 21 January 2021; Received in revised form 26 March 2021; Accepted 1 June 2021
Available online 9 June 2021

Multiple evidences in the recent years have shown that miRNA-196a2 (rs11614913) may play a role in the development of many cancer types, like lung, gastric, breast, colorectal, gallbladder cancer and head and neck cancer and prostate cancers (Chen et al., 2020; Liu et al., 2018). As for childhood ALL, there are only few published studies, which show that variant C allele contributes to an increased risk of childhood ALL (Chen et al., 2020; Rakmanee et al., 2017; Tong et al., 2014).

miRNA-196a gene is located at a region between HOXC10 and HOXC9 on chromosome 12 (12q13.13). The miRNA-196a2 SNP rs11614913 located in the mature sequence of miRNA-196a2 and negatively influence endogenous processing of miRNA precursor to its mature form (Deghady et al., 2019).

An updated meta-analysis concluded that the rs11614913 polymorphism conferred a reduced susceptibility to lung cancer (homozygote comparison, recessive model) and hepatocellular carcinoma (allelic contrast, homozygote comparison, recessive model) or an increased susceptibility to HNC (allelic contrast, homozygote comparison), also may contribute to the development of cancer, a candidate marker for the diagnosis of these cancers, and could also be a potential protective factor for cancer risks (Liu et al., 2018).

miRNA-196a2 T allele is associated with decreased cancer risk in overall population and studies on Asian populations. It is also associated with a decreased risk of gynecological cancers, ovarian, breast and hepatocellular cancer (Choupani et al., 2019).

Unfortunately, to date, the association of miRNA-196a2 polymorphism and acute lymphoblastic leukemia is still unclear (Farokhian et al., 2020). Therefore, this study aims to investigate the association between miRNA196a2 polymorphism and acute lymphoblastic leukemia in children.

2. Subjects and methods

2.1. Study subjects

This research was 160 case-control study including 100 childhood ALL patients their age ranged from 3 to 15 years old with no other hematological diseases or therapy related malignancy recruited from pediatric department in Benha University Hospital and Benha Children Specialist Hospital and 60 apparently healthy children as control for cases with matched age and sex recruited from surrounding siblings and relatives from the same city (Benha City) from May 2019 to February 2020. Patients were assessed by initial white blood cell count, all cases were morphologic, immunologic and cytogenetically proven, also clinical and demographic data including age and sex were retrospectively studied.

All participants' parents/guardians signed an informed written consent before joining the study for which approval of the Medical Ethical Committee for Human Research in Benha University was granted.

2.2. Sample collection

Venous blood was withdrawn from each participant under complete aseptic conditions, then each blood sample was divided into 3 parts: 1st part in K3-EDTA tube then divided into 2 aliquots one used for complete blood count and immunophenotyping and the other was stored at −20 °C for subsequent DNA extraction, 2nd part in sodium citrate tube for ESR and 3rd part in a plain tube, were left for clotting at 25 °C for 30 min to be then centrifuged at 1500 rpm for 15 min at room temperature for clinical chemistry tests.

2.3. DNA extraction

Genomic DNA was extracted from peripheral whole blood on EDTA tube, using G-Spin™ Total DNA Extraction Kit (iNtRON, cat. no. 17045, lot. no. 15250850; Korea) following the instructions of the manufacturer.

2.4. Genotyping of the miRNA196a2 (rs11614913) polymorphism

The single nucleotide polymorphism of miRNA-196a2 (rs11614913) (T>C) was performed by Polymerase Chain Reaction Restriction Fragment Length Polymorphism (PCR-RFLP) analysis. Genotyping of miRNA-196a2 (rs11614913) (T>C) polymorphism was done using forward primer 5′ CCC-CTT-CCC-TTC-TCC-TCC-AGA-TA 3′ and reverse primer 5′ CGA-AAA-CCG-ACT-GAT-GTA-ACT-CCG 3′. Extracted DNA was amplified by 2xEasy Taq PCR SuperMix (Transgenbiotech, cat. no. AS111; lot #M31009, Beijing, China).

The amplification of DNA was done in a 50 μl mixture containing 5 μl of DNA, 2 μl of forward primer, 2 μl of reverse primer, 25 μl of 2xEasyTaq®PCR Super Mix and 16 μl nuclease free water. The PCR was performed in Thermal cycler (applied biosystems-Model#9902-Singapore). The PCR protocol was the initial denaturation of 94 °C for 5 min followed by 35 cycles of 94 °C for 1 min, 55 °C for 1 min, 72 °C for 2 min with a final extension at 72 °C for 10 min.

The PCR product were detected before digestion by *MspI* by electrophoresis on 2% agarose gel, stained with ethidium bromide and then visualized using Ultra-Violet Light Transillumination to ensure presence of DNA.

2.5. Polymerase Chain Reaction-Restriction Fragment Length Polymorphism (PCR-RFLP)

The amplified targeted DNA was digested by (*MspI*) restriction enzyme (BioLabs, New England) (lot no: 10026225). Products were separated by gel electrophoresis on 2% agarose gel and stained with ethidium bromide.

The product presented three different patterns as in Fig. 1; the different genotypes were identified as follows:

1. A single 149 bps fragment indicated the wild-type TT-genotype.
2. Two products of 125 and 24 bps indicated the homologous variant CC genotype.
3. Three products of 149, 125 and 24 bps indicated the heterozygous variant CT genotype.

2.6. Statistical analysis

These data were tabulated and analyzed using the computer program SPSS (Statistical package for social science) version 26 (SPSS Inc., Chicago, IL, USA) the significance of difference was tested using one of the following tests: Student's *t*-test: Used to compare mean of two groups of quantitative data, inter-group comparison of categorical data was performed by using Chi square test (X^2-value) and Z test: used to compare proportion between two groups of qualitative data. A P value <0.05 was considered statistically significant (*) while >0.05 statistically insignificant P value <0.01 was considered highly significant (**) in all analyses.

3. Results

The present study included 100 childhood ALL patients and 60 apparently healthy children as control groups. Mean age of studied group was 6.97 ± SD2.93 years, while age of matched control group was 7.21 ± SD2.81 years. Among ALL group 74% had splenomegaly, their immunophenotyping showed 82% with pre-B ALL and 18% with T-ALL, while 88% with normal karyotyping, 8% had translocation and 4% with hyperdiploidy as their karyotyping results. The data of follow up showed 90% with remission and 10% had relapse.

Concerning laboratory data in our study ALL group had significantly higher increase in the mean of WBCS (mean ± SD) (16,258.0 ± 12,641.5 $\times 10^9$/L) ($P < 0.001$), and the mean of SGPT (mean ± SD) (33.5 ± 19.45 U/ml) ($P < 0.001$), SGOT (mean ± SD) (41.8 ± 22.5 U/ml) ($P < 0.001$), ESR (mean ± SD) (88.32 ± 16.21 mm/h) ($P < 0.001$), LDH (mean ± SD)

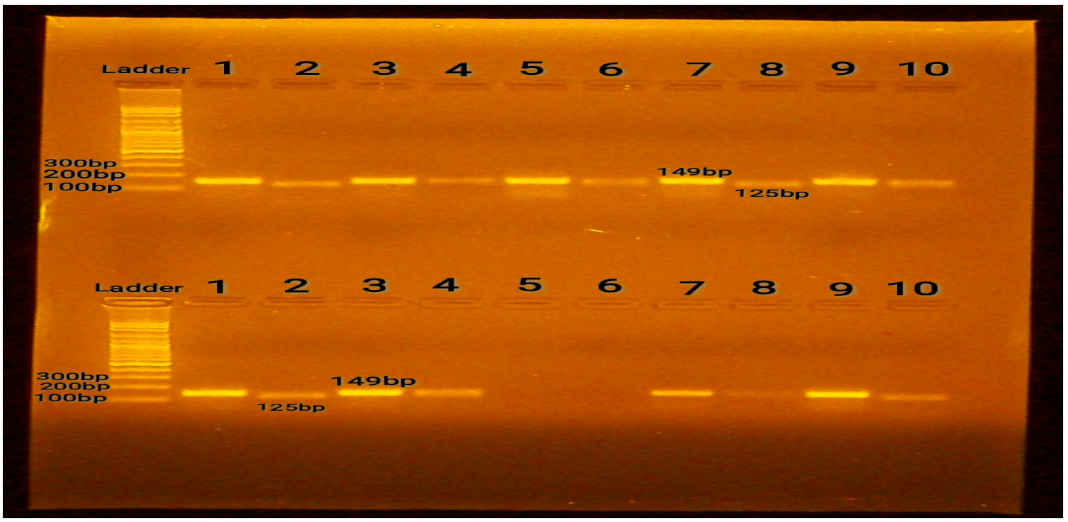

Fig. 1. Analysis of miRNA-196a2 (rs116114913) polymorphism.
Agarose gel electrophoresis showing PCR-RFLP analysis of miRNA-196a2 gene after addition restriction enzyme (*Msp*I). In the upper half of lanes 1, 3, 4, 5, 6, 7, 9 and 10 (TT) band(149); and lanes 2, and 8 (CC) band (125 and 24 bp).

(1900.42 ± 86.36 U/L) (P < 0.001), creatinine (mean ± SD) (0.88 ± 0.66 mg/dl) (P = 0.025)& we found significantly decrease in the mean of PLT count (mean ± SD) (36.28 ± 27.95 × 10^9/L) (P < 0.001), Hb (mean ± SD) (8.3 ± 1.85 g/dl) (P < 0.001) when compared to control group (Table 1).

3.1. The miRNA-196a2 polymorphism and acute lymphoblastic leukemia

The genotypic frequencies for miRNA-196a2 (rs11614913) genotypes were established among Egyptian ALL children and are shown in Table 2. The TT and CC proportions were 44 and 54%, in the childhood ALL patient group, and 36.7 and 56.7% in the control group respectively.

Based on statistical analysis the distribution of miRNA-196a2 genotypes was not differentially distributed between the childhood ALL case and control groups (P > 0.05). The homologous variant CC and the wild type TT variant of miRNA-196a2 genotypes were close in the control group and the case group (P = 0.74 and 0.36, respectively) (Table 2). The allele frequencies of miRNA-196a2 were analyzed and the results are presented in Table 2. The results showed that the variant C allele in pediatric ALL cases was 54% compared with 56.7% in the controls. There was no relation found between miRNA-196a2 C allele and susceptibility to childhood ALL (P = 0.38), also frequency of T allele was 44% in childhood ALL cases compared to 36.7% in control group, this conclude that there were no statistically significant differences between the studied groups regarding miRNA-196a2 polymorphism (P > 0.05) or allelic frequency (P = 0.38).

Table 2
miRNA-196a2 genotypes distribution and allelic frequency in studied groups.

	Group I patients (100)		Group II control (60)		Statistical Z test	P value
	No	%	No	%		
miRNA-196a2						
CC	54	54.0	34	56.7	0.33	0.74
TT	44	44.0	22	36.7	0.91	0.36
Trouble shooting	2	2.0	4	6.7	1.5	0.13
miRNA-196a2						
C	108	54.0	68	56.7		0.38
T	88	44.0	44	36.7		
Trouble shooting	2	2.0	4	6.7		

3.2. Association between miRNA-196a2 polymorphism and clinic pathological data in childhood ALL patient group

We observed in our study that there were highly significant increase of TT genotype in males compared to females in ALL group (P = 0.002), while was significant increase in the mean of age in TT group compared to CC group (P = 0.04) (Table 3).

As for our ALL group clinical data there was significant increase in CC genotype in patients with fever comparing to TT genotype. In laboratory data there was significant increase in the mean of LDH in TT group compared to CC group (P = 0.02) while significant increase in the mean of ESR in CC group compared to TT group (P = 0.04).

In this study there was no association between miRNA-196a2

Table 1
Laboratory data of all studied group.

	Group I patients (100)		Group II control (60)		Statistical test (st t)	P value
	Mean	±SD	Mean	±SD		
WBCs (×10^9/L)	16,258.0	12,641.5	7440.0	1489.3	5.37	<0.001**
Hb (g/dL)	8.3	1.85	13.13	1.11	18.38	<0.001**
PLT (×10^9/L)	36.28	27.95	283.0	74.21	29.94	<0.001**
SGOT (U/ml)	41.8	22.5	23.1	5.18	6.33	<0.001**
SGPT (U/ml)	33.5	19.45	22.67	5.42	4.21	<0.004**
ESR (mm/h)	88.32	16.21	5.93	1.16	39.26	<0.001**
Creatinine (mg/dl)	0.88	0.66	0.60	0.16	3.25	0.025*
LDH (U/L)	1900.42	86.36	146.37	24.48	8.84	<0.001**
Bone marrow blast %	86.36	12.80				

Table 3
Association between demographic data and miRNA-196a2 genotype in ALL group.

Gender	TT group (44)		CC group (54)		Statistical test (x^2)	P value
	No	%	No	%		
Male	36	81.8	28	51.9	9.61	0.002
Female	8	18.2	26	48.1		

	TT group (44)		CC group (54)		St *t*-test	P value
	Mean	±SD	Mean	±SD		
Age	7.71	3.23	6.52	2.52	2.05	0.04

polymorphism and immunophenotyping, karyotyping or follow up results.

Logistic regression analysis was conducted for prediction of CC using ESR, LDH, age, sex, and fever as covariates we found that the most predictor of CC was ESR.

4. Discussion

miRNAs are 19–22 ribonucleotide long noncoding RNA. They intermediate posttranscriptional regulation of many human genes and therefore have preclinical and clinical research applications in many diseases. With recent technologies that is used for gene expression analysis, molecular profiling of new microRNAs in childhood ALL, has been reachable (Rashed et al., 2019).

Acute lymphoblastic leukemia is classified into B-ALL and T-ALL. B-ALL is the most common type of childhood ALL, and its prognosis is better than T-ALL. Immunotyping for T subtype is one of the intermediate risk indicators. The prognosis of T subtype is worse than that of B subtype (Li, 2020).

microRNAs can be categorized as oncogenic or tumor suppressive, their expression levels are increased and decreased in patients with cancer. MicroRNAs are involved in cancer cell processes such as cell proliferation, differentiation, metastasis, apoptosis and tumorgenesis. In the recent years one of the hotspots of research is the investigations of miRNAs in ALL in children (Chen et al., 2020).

Single nucleotide polymorphisms (SNPs) are common genetic variations within the human genome. SNPs in protein-coding genes can affect the functions of proteins and successively influence susceptibility to cancer. Lately, several miRNA polymorphisms are shown to have a big role in different types of cancers (Farokhizadeh et al., 2019).

Hence the aim of our study was to examine the association between miRNA-196a2 (rs 11614913) polymorphism and childhood ALL in Egypt.

In the current study, we provide evidence showing that miRNA-196a2 (rs 11614913) genotypes were not significantly associated with susceptibility to childhood ALL in Egyptian children ($P > 0.05$) (Table 2).

The allele frequency of miRNA-196a2 in our study was 44% for T allele in cases compared to 36.7% in control, while C allele was 54% in cases compared to 56.7% in control.

Chen et al. found that the distribution of miRNA-196a2 (rs11614913) genotypes were not differentially distributed between the childhood ALL case and control groups (P value = 0.8107) with no statistically significance, and found that T allele was in 57.7% in ALL cases compared to 56% in control and C allele was 42.3% in ALL cases compared to 44% in control (Chen et al., 2020).

Rakmanee et al. found that miRNA-196a2 (rs11614913) variant CC, TC heterozygote and CC/TC genotypes were significantly associated with increase childhood ALL susceptibility compared with TT wild type in Thailand, and found that T allele was 33% in cases compared to 51% in control and C allele was 67% in cases compared to 49% in control (Rakmanee et al., 2017).

However Tong et al. found that T allele was 54.9% in cases compared to 58% in control and C allele was 45.1% in cases compared to 42% in control, and that TC heterozygote and CC/TC were associated with an increased risk of pediatric ALL in Chinese ($P = 0.007$) (Tong et al., 2014).

In our study we observed that the most common clinical presentation was splenomegaly with 74% this go in line with Jaime-Pérez et al. who found 63% splenomegaly (Jaime-Pérez et al., 2019) and was nearly similar to Hashemi M et al. who reported organomegally in 78.7% of ALL cases (Hashemi et al., 2014), while Kakaje et al. found lymphadenopathy was the most common presentation with 82.9% (Kakaje et al., 2020).

Liver function tests in our study were as follows (mean ± SD) of SGOT (41.8 ± 22.5 U/ml) and SGPT (mean ± SD) (33.5 ± 19.45 U/ml). This is in agreement with Islam et al. who found mean serum SGOT is (mean ± SD) (47.46 ± 15.00 U/ml) and serum SGPT was (mean ± SD) (38.00 ± 7.34 U/ml) (Islam et al., 2020). Alawad et al. have similar results (Alawad et al., 2016), while Ahmed et al. found there was no statistically significant difference between children with leukemia compared to healthy control children regarding liver enzymes (Ahmed et al., 2017).

Regarding ESR in our study it was found in ALL group to be statistically highly significant compared to control group (mean ± SD) (88.32 ± 16.21 mm/h) ($P < 0.001$), and there was a statistically highly significant increase in mean of LDH in ALL group compared with control group LDH (mean ± SD) (1900.42 ± 86.36 U/L) ($P \leq 0.001$).

Louvigné et al. who found ALL patients ESR statistically highly significant ($P < 0.001$) (Louvigné et al., 2020), while Hamad et al. & Tahir et al. who found statistically significant increase in mean of LDH in ALL cases compared to control group ($P \leq 0.001$) (Hamad et al., 2019; Tahir et al., 2017).

We found that there was statistically significant increase in mean of LDH and ESR in TT group compared to CC group with ($P = 0.02$, 0.04 respectively), while there was no statistically significant differences between different laboratory data including white blood count, Hb, SGOT, SGPT, creatinine and BM blast % and different miRNA196a2 genotypes in ALL group.

We demonstrated in our work that there was no association between miRNA-196a2 polymorphism and immunophenotyping, karyotyping and follow up results of ALL group.

As for childhood ALL, there are only few published studies. First, in 2014 Tong and her colleagues in China worked on 570 childhood ALL cases and 673 matched control and found that the TC heterozygote and CC/TC genotypes were associated with increased childhood ALL risk (Tong et al., 2014). Then, in 2017, Rakmanee and his colleagues recruited 104 childhood ALL cases and 180 control and showed that the variant C allele contributed to an increased risk of childhood ALL in Thailand (Rakmanee et al., 2017). Then in 2020 Chen and his colleagues collected 266 childhood ALL cases and 266 control and showed miRNA-196a2 rs11614913 polymorphism was not significantly associated with susceptibility to childhood ALL in Taiwan (Chen et al., 2020).

In this study the case control ratio was 1:0.6 the low control number in our study was mainly because of financial hurdles.

There are few studies that have results different from ours and few agree with our present study in that there was no association present between miRNA-196a2 polymorphism and childhood ALL and they explained that difference in results may be due to different ethnic groups and the small sample size.

5. Conclusion

We found that there was significant increase in mean of LDH and ESR in TT group compared to CC group, while there was no statistically significant differences between different laboratory data including white blood count, Hb, SGOT, SGPT, creatinine and BM blast % and different miRNA196a2 genotypes in ALL group. Finally we observed

there was no association between miRNA-196a2 (rs11614913) polymorphism and the susceptibility of ALL in Egyptian children.

CRediT authorship contribution statement

N.A. Abdulhafeez: Designed the study and analyzed the data, contribute to the final version of the manuscript. **D.M. Abd El Hassib:** Designed the study and analyzed the data, contribute to design & implementation of the research to the analysis of the results and aided in interpreting the results. **S.G. Ameen:** Designed the study and analyzed the data, contribute to design & implementation of the research to the analysis of the results and aided in interpreting the results. **O.M. Atef:** Contribute to sample preparation &worked on the manuscript.

All authors discuss the results & commented on the manuscript.

Declaration of competing interest

The authors declare that they have no competing interests.

References

Ahmed, S., Amer, S.M., Allam, N.G., El-Alfy, M.S., 2017. Clinical chemistry studies in Egyptian children with acute lymphoid and myeloid leukemia. Int. J. Chem. Biomed. Sci. 3 (2), 10–17.

Alawad, M.A., Elmahdi, S.A., Ahmed, S.A., Abdrabo, A.A., 2016. Assessment of liver functions among Sudanese leukemic patients in Khartoum State. J. Biomed. Res. 2 (2), 11–15.

Brown, P., Inaba, H., Annesley, C., Beck, J., Colace, S., Dallas, M., Larrier, N., 2020. Pediatric acute lymphoblastic leukemia, version 2.2020, NCCN clinical practice guidelines in oncology. J. Natl. Compr. Cancer Netw. 18 (1), 81–112.

Chen, C.C., Hsu, P.C., Shih, L.C., Hsu, Y.N., Kuo, C.C., Chao, C.Y., Pei, J.S., 2020. MiR-196a-2 genotypes determine the susceptibility and early onset of childhood acute lymphoblastic leukemia. Anticancer Res. 40 (8), 4465–4469.

Choupani, J., Nariman-Saleh-Fam, Z., Saadatian, Z., Ouladsahebmadarek, E., Masotti, A., Bastami, M., 2019. Association of mir-196a-2 rs11614913 and mir-149 rs2292832 polymorphisms with risk of cancer: an updated meta-analysis. Front. Genet. 10, 186. https://doi.org/10.3389/fgene.2019.00186.

Deghady, A., Elwafa, R.A., Lsorady, M.A.E., 2019. MicroRNA-196a2 single nucleotide polymorphism rs11614913 in Egyptian patients with chronic lymphocytic leukemia. Hematol. Transfus. Int. J. 7 (1), 17–20.

Dzikiewicz-Krawczyk, A., 2015. MicroRNA polymorphisms as markers of risk, prognosis and treatment response in hematological malignancies. Crit. Rev. Oncol. Hematol. 93 (1), 1–17.

Farokhian, F., Beyzaei, Z., Ramzi, M., Geramizadeh, B., 2020. Association between genetic polymorphism of XRCC7 (G6721T) and risk of acute lymphoblastic leukemia. Egypt. J. Med. Hum. Genet. 21 (19), 1–4.

Farokhizadeh, Z., Dehbidi, S., Geramizadeh, B., Yaghobi, R., Malekhosseini, S.A., Behmanesh, M., Karimi, M.H., 2019. Association of MicroRNA polymorphisms with hepatocellular carcinoma in an Iranian population. Ann. Lab. Med. 39 (1), 58–66.

Hamad, M.N., Kamal, M., Saeed, M.A., Suliman, M.A., 2019. Assessment of serum ferritin levels in Sudanese patients with acute lymphoblastic leukemia. Health Sci. 8 (7), 92–96.

Hashemi, M., Sheybani-Nasab, M., Naderi, M., et al., 2014. Association of functional polymorphism at the miR-502-binding site in the 3′ untranslated region of the SETD8 gene with risk of childhood acute lymphoblastic leukemia, a preliminary report. Tumor Biol. 35 (10), 10375–10379.

He, B., Zhao, Z., Cai, Q., Zhang, Y., Zhang, P., Shi, S., Wang, X., 2020. miRNA-based biomarkers, therapies, and resistance in cancer. Int. J. Biol. Sci. 16 (14), 2628.

Islam, T., Rahman, A.S., Hasan, M.K., Jahan, F., Mondal, M.C., Mondal, M.C., Tohura, S., 2020. Liver function tests in patients of acute leukemia before and after induction chemotherapy. J. Biosci. Med. 8 (02), 110.

Jaime-Pérez, J.C., García-Arellano, G., Herrera-Garza, J.L., Marfil-Rivera, L.J., Gómez-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. Hematol. Transfus. Cell Ther. 41 (1), 57–61.

Kakaje, A., Alhalabi, M.M., Ghareeb, A., Karam, B., Mansour, B., Zahra, B., Hamdan, O., 2020. Rates and trends of childhood acute lymphoblastic leukaemia: an epidemiology study. Sci. Rep. 10 (1), 1–12.

Li, L., 2020. Analysis of prognostic factors in children with acute lymphoblastic leukemia. Survival 25, 17.

Liu, Y., He, A., Liu, B., Zhong, Y., Liao, X., Yang, J., Mei, H., 2018. rs11614913 polymorphism in miRNA-196a2 and cancer risk: an updated meta-analysis. OncoTargets Ther. 11, 1121. https://doi.org/10.2147/OTT.S154211.

Louvigné, M., Rakotonjanahary, J., Goumy, L., Tavenard, A., Brasme, J.F., Rialland, F., Jean, S., 2020. Persistent osteoarticular pain in children: early clinical and laboratory findings suggestive of acute lymphoblastic leukemia (a multicenter case-control study of 147 patients). Pediatr. Rheumatol. 18 (1), 1–8.

Rakmanee, S., Pakakasama, S., Hongeng, S., Sanguansin, S., Thongmee, A., Pongstaporn, W., 2017. Increased risk of Thai childhood acute lymphoblastic leukemia with the MiR196a2 T> C polymorphism. Asian Pac. J. Cancer Prev. 18 (4), 1117.

Rashed, W.M., Hamza, M.M., Matboli, M., Salem, S.I., 2019. MicroRNA as a prognostic biomarker for survival in childhood acute lymphoblastic leukemia: a systematic review. Cancer Metastasis Rev. 38 (4), 771–782.

Stanulla, M., Cavé, H., Moorman, A.V., 2020. IKZF1 deletions in pediatric acute lymphoblastic leukemia: still a poor prognostic marker? Blood 135 (4), 252–260.

Tahir, I.M., Iqbal, T., Jamil, A., Saqib, M., 2017. Association of BCL-2 with oxidative stress and total antioxidant status in pediatric acute lymphoblastic leukemia. J. Biol. Regul. Homeost. Agents 31 (4), 1023–1027 (Oct-Dec, PMID: 29254309).

Tong, N., Xu, B., Shi, D., Du, M., Li, X., Sheng, X., Wu, D., 2014. Hsa-miR-196a2 polymorphism increases the risk of acute lymphoblastic leukemia in Chinese children. Mutat. Res. Fundam. Mol. Mech. Mutagen. 759, 16–21. https://doi.org/10.1016/j.mrfmmm.2013.11.004.