ELMER PRESS

Original Article

J Hematol. 2024;13(6):278-284

Methionine Synthase Reductase A66G Variant in Pediatric Acute Lymphoblastic Leukemia Patients

Elrashed B. Yasin[a, d], Haitham M.H. Qutob[a], Raed Alserihi[b, c]

Abstract

Background: Methionine synthase reductase, which is encoded by the methionine synthase reductase (*MTRR*) gene, plays a crucial role in the methylation reactions and the production of DNA and its epigenetic processes. There was a correlation between the *MTRR* (A66G) polymorphism and the likelihood of developing acute lymphoblastic leukemia (ALL). This study was carried out to investigate the correlation among pediatric ALL cases.

Methods: Within the participant population of this case-control study, there were 86 individuals who had been diagnosed with ALL, and there were also 150 healthy persons who acted as the control group. To determine the *MTRR* (A66G) polymorphism, DNA was first extracted and then observed through the use of real-time polymerase chain reaction.

Results: The results of the flow cytometry analysis showed that the prevalence of B-cell ALL (B-ALL) was much higher than that of T-cell ALL (T-ALL), which accounted for only 20 cases (23.3%). Upon comparing the hematological parameters of ALL subtypes in patients with T-ALL, it was discovered that there was a statistically significant higher mean total white blood count ($P < 0.0005$) and mean blast percentage ($P = 0.050$). Upon examination, it was discovered that both of these figures were much higher than the average. In accordance with the results of the molecular analysis, the occurrence of the *MTRR* homozygous GG genotype was found to be considerably lower in the patients' group (4.65%) than in the control group (20.67%). However, the *MTRR* homozygous AA and heterozygous AG were nearly similar in the two groups. The risk of acute lymphoblastic leukemia and *MTRR* genotypes, on the other hand, exhibited a correlation that was not statistically significant ($P = 0.082$).

Conclusions: The study's findings showed that among pediatric ALL patients, the *MTRR* A66G polymorphism was not linked to an increased risk of ALL.

Keywords: ALL; Methionine synthase reductase; *MTRR* A66G polymorphism

Manuscript submitted October 15, 2024, accepted November 8, 2024
Published online December 2, 2024

[a]Department of Medical Laboratory Technology, Faculty of Applied Medical Sciences, King Abdulaziz University, Rabigh, Saudi Arabia
[b]Department of Medical Laboratory Sciences, Faculty of Applied Medical Sciences, King Abdulaziz University, Jeddah, Saudi Arabia
[c]Hematology Research Unit (HRU), King Fahad Medical Research Center, King Abdulaziz University, Jeddah, Saudi Arabia
[d]Corresponding Author: Elrashed B. Yasin, Department of Medical Laboratory Technology, Faculty of Applied Medical Sciences, King Abdulaziz University, Rabigh, Saudi Arabia. Email: eyasin@kau.edu.sa

doi: https://doi.org/10.14740/jh1360

Introduction

The malignant condition, known as acute lymphoblastic leukemia (ALL), affects white blood cells (WBCs) and is brought on by the uncontrollably high proliferation of immature lymphoid cells in bone marrow, blood, and other organs, which are prevented from differentiating too soon [1]. A variety of genetic anomalies, such as chromosome translocations, mutations, and aneuploidies in genes regulating the cell cycle and lymphoid cell development, are responsible for the illness [2].

Researchers have discovered that 75% of cases may be traced back to B-cell lineage progenitors, with malignant T-cell precursors accounting for the remaining cases [3, 4]. Both B-cell ALL (B-ALL) and T-cell ALL (T-ALL) subtypes are capable of presenting in adults and children, making up 20% of adult cases and about 80% of all juvenile leukemia cases [3]. Children with this condition have over 90% 5-year survival rates, whereas rates for teens and young adults range from 75% to 85% [4]. In Saudi Arabia, the incidence for men is roughly 2.71 per 100,000, whereas it is 1.86 per 100,000 for women [5].

The pathogenesis of ALL is complex, with multiple components (including immunological, environmental, genetic, and drug-related variables) working at different levels. It also involves a close and intricate interplay among these components. The pathophysiology of ALL is characterized by its monoclonal origin, unchecked cell proliferation brought on by growth receptors' constant self-stimulation, an inability to react to inhibitory signals, and a reliance on reduced apoptosis for cell survival [6]. Fetal development is the time when leukemia genetic changes occur [7]. Sometimes these mutations result in changes to chromosomal structure, which can lead to fusion genes where one or both partners express transcription factors. Detrimental mutations can occasionally be small changes like deletions or point mutations. A transcription factor necessary for cell development in several hematopoietic lineages is usually less significant after mutations [6, 7].

The formation of malignant cells in the hematopoietic (lymphoid) system begins with genetic changes of genes involved in DNA synthesis or DNA methylation [8]. Because folate provides a methyl group for regular DNA synthesis and is essential for the methylation process of DNA through homocysteine in the methionine cycle, it is imperative to maintain a sufficient concentration of folate (tetrahydrofolate-THF) [4]. Genetic variations in the genes encoding these enzymes can affect the availability or activity of the enzymes involved in the folate pathway, which can lead to changes in folate metabolism [4]. The amount of folate available in cells can be affected by genetic variations in genes encoding essential folate cycle regulatory and transport enzymes, such as methylenetetrahydrofolate dehydrogenase, cyclohydrolase and formyltetrahydrofolate synthetase 1 (MTHFD1), methionine synthase reductase (MTRR), methionine synthase (MTR), methylenetetrahydrofolate reductase (MTHFR), dihydrofolate reductase (DHFR), gamma-glutamyl hydrolase (GGH), and replication factor C subunit 1 **(**RFC1). These variations may also affect the susceptibility to ALL (ALL) and the response to therapy [8].

MTRR, also known as 5-methyltetrahydrofolate-homocysteine methyltransferase reductase, is an enzyme that is part of the MTRR family and is important for the metabolism of homocysteine and methionine that is dependent on folate. It may also be a member of the electron transferase family [9]. It also has a major impact on maintaining appropriate amounts of activated cobalamin, a methyl carrier that is used in the process of remethylating homocysteine to methionine [9]. Consequently, it is in charge of catalyzing the change of MTR's inactive state.

Numerous epidemiological and case-control studies have been conducted to thoroughly study and record the potential link between the *MTRR* gene and an increased risk of leukemia, other cancers, and other diseases and disorders [9-14]. People with the *MTRR* 66GG mutation had a significantly higher incidence of ALL by a factor of 2.15 (95% confidence interval (CI): 1.06 - 4.39; P = 0.032), according to the connection between *MTRR* polymorphism and susceptibility to ALL [15]. ALL and the *MTRR* 66 GG genotype had an odds ratio (OR) of 1.77 with a 95% CI ranging from 0.96 to 3.26 [16].

There are no published studies that address the connection between these single nucleotide polymorphisms (SNPs) and ALL in Saudi Arabia. Moreover, information about metabolic enzymes and their function in acute lymphocytic leukemia is highly lacking. The aim of this study was to investigate the potential association between acute lymphocytic leukemia hypersensitivity and the *MTRR* polymorphism. Thus, the study's goal is to look at the relationship between childhood ALL and polymorphisms in the folate metabolic *MTRR* gene.

Materials and Methods

Study subject

These research protocols and permission forms were approved by a unit of the Biomedical Ethics Research Committee, King Abdulaziz University (reference number: 698-20). All patients have signed written informed consent forms. This study was carried out in accordance with the principles outlined in the Declaration of Helsinki. The present case-control study involved the recruitment of 236 participants from King Abdulaziz University, located in Jeddah, Saudi Arabia. The sample of 236 individuals was split into 86 pediatric ALL cases and 150 controls. Using cytogenetics and histology, the laboratory verified the diagnosis of patients with ALL. ALL was diagnosed using bone marrow tests, a complete blood count, and flow cytometry. To validate the results, cytogenetics and fluorescence *in situ* hybridization (FISH) testing were employed. Exclusion criteria for ALL instances included patients with secondary malignancies with unsigned consent forms. The inclusion criteria for the healthy controls dictated that they had not received a diagnosis of any form of cancer or other medical condition.

Hematological analysis

An automatic blood counter (Sysmex KX 21) and a peripheral blood image were used to measure hematological biomarkers. ALL was found by looking at the shape of blood cells and the number of WBCs at different levels. The finding was then confirmed by using antigen-specific antibodies to analyze different groups of immune cells with a Coulter EPICS X-Mcl TM flow cytometer (Miami, Florida, USA).

Analysis of genomic DNA and polymerase chain reaction (PCR)

Isolating genomic DNA from peripheral leucocytes was accomplished through the utilization of the spin-column extraction technique. The Nanodrop (NanoDrop™ Lite-UV Spectrophotometer, Thermo Fisher Scientific) was utilized in order to determine the concentration and purity of the DNA. The TaqMan real-time PCR (qTOWER3G, Anlitka Je-na, Germany) was utilized to analyze the MTRR A66G polymorphism. The sequences of the primers and probes used were as follows: the forward MTRR primer: AGG CAA AGG CCA TCG; the reverse MTRR primer: ATC CAT GTA CCA CAG CT; and the MTRR probes are: G- CAG AAG AAA TGT GTG A, and A- CAG AAG AAA TAT GTG A. Using the absolute analysis method, the reaction mixture was subjected to a total of 40 cycles, during which it was heated to a temperature of 95 °C for 10 min, 95 °C for 15 s, and 60 °C for 1 min.

Statistical analysis

The Statistical Package for the Social Sciences (SPSS), version 25, was used to conduct the statistical analysis. We used a Chi-square test to look at how the genotypes were distributed throughout the groups, and we ran a regression to see how the

Table 1. Subtypes of ALL and Gender in the Pediatric Samples

Variable	Children	P value
Subtypes		
B-ALL	66 (76.7%)	0.001
T-ALL	20 (23.3%)	
Gender		
Male	57 (66.3%)	0.001
Female	29 (33.7%)	

ALL: acute lymphoblastic leukemia; B-ALL: B-cell ALL; T-ALL: T-cell ALL.

polymorphism affected the likelihood of developing ALL.

Results

Demographic data

Out of a total of 236 participants, 86 were identified as having pediatric ALL by flow cytometry. The control group included of 150 healthy-looking individuals who were matched for age and sex. There were 57 men (66.3% of the total) and 29 women (33.7% of the total) in the research groups. The age range for both groups was 2 to 16 years, with a mean ± standard deviation of 9.7 ± 1.83.

Immunophenotyping

Flow cytometry was used for immunophenotyping, which supported the diagnosis of ALL and showed that B-ALL was more common (66 cases, or 76.7% of the total) than T-ALL (20 cases, or 23.3% of the total). The prevalence of B-ALL was higher in men compared to women (Table 1).

Hematological data

When looking at the hematological parameters among the different forms of ALL, we found that T-ALL in children had a considerably higher mean total WBC count and blast percentage compared to B-ALL. Mean red blood cell (RBC), hemoglobin (Hb), and platelet (PLT) counts were not significantly different (Table 2).

Table 3. Distribution of *MTRR* (A66G) Genotypes Among Children

Genotype	Patients	Control	P value
AA	40 (46.51%)	59 (39.33%)	
AG	42 (48.84%)	60 (40.00%)	0.082
GG	4 (4.65%)	31 (20.67%)	

MTRR: methionine synthase reductase.

Genotyping of MTRR (A66G) polymorphism

There was no evidence of a non-normal distribution in the *MTRR* A66G genotype distribution (P > 0.05). Both the research and control groups showed a higher frequency of the *MTRR* heterozygous AG genotype than the homozygous AA or GG genotypes. Patients were more likely to have the homozygous GG or AA genotypes compared to the control group, while the control group was more likely to have the heterozygous AG genotype, according to the comparison of genotype distributions among the research groups. Table 3 shows that there was no statistically significant distribution of genotypes among the study groups. Risk of ALL was not significantly associated with *MTRR* polymorphism in the regression analysis (OR: 1.179, 95% CI: 0.745 - 1.865, P value = 0.082).

The mean RBC count was not statistically different between ALL patients with wild-type (AA) and mutant-type (AG + GG) alleles, although the P value was 0.058. On the other hand, there were no statistically significant changes in the mean Hb, total WBC, PLT, or blast (%) counts (Table 4).

Discussion

ALL is the predominant form of pediatric malignancy, affecting individuals across all age groups, with the highest incidence observed between 2 and 5 years of age. Consequently, age is considered a significant factor in disease initiation [17]. While the etiology of ALL remains incompletely understood, multiple hypotheses propose that environmental and genetic factors play a role in initiating leukemogenesis [18]. The pro-

Table 2. Comparison of the WBCs and Blast Cells Counts According to ALL Subtypes

Parameter	B-ALL, mean ± SD	T-ALL, mean ± SD	P value
Total WBCs count ($\times 10^9$/L)	41.1 ± 32.4	173.1 ± 41.8	< 0.0005
RBCs count ($\times 10^{12}$/L)	3.9 ± 0.7	3.9 ± 0.7	0.814
Hemoglobin (g/dL)	8.4 ± 5.6	8.3 ± 1.9	0.624
PLT count ($\times 10^9$/L)	43.5 ± 5.1	54.5 ± 37.6	0.354
Blast (%)	59.1 ± 15.8	72.1 ± 12.9	0.039

ALL: acute lymphoblastic leukemia; B-ALL: B-cell ALL; T-ALL: T-cell ALL; WBC: white blood cell; RBC: red blood cell; PLT: platelet; SD: standard deviation.

Table 4. Comparison of Hematological Parameters in ALL Patients With Wild and Mutant Type *MTRR* Alleles

Parameter	Wild (n = 39), mean ± SD	Mutant (n = 47), mean ± SD	P value
Total WBCs count (× 10^9/L)	58.8 ± 11.7	69.9 ± 10.3	0.606
RBCs count (× 10^{12}/L)	3.6 ± 0.9	3.8 ± 0.8	0.058
Hemoglobin (g/dL)	8.5 ± 6.7	8.6 ± 3.5	0.727
PLT count (× 10^9/L)	45.6 ± 4.43	55.8 ± 6.43	0.188
Blast (%)	61.4 ± 21.4	69.1 ± 19.2	0.241

MTRR: methionine synthase reductase; ALL: acute lymphoblastic leukemia; WBC: white blood cell; RBC: red blood cell; PLT: platelet; SD: standard deviation.

tective role of SNPs against oxidative stress and their crucial role in detoxifying and, in rare cases, inactivating carcinogens make them an intriguing genetic variable. The possibility that many gene variants affect the risk of having ALL has piqued a great deal of interest in these variations [19]. This study explores the significance of the *MTRR* (A66G) gene polymorphism related to folate metabolism in the context of various environmental pollutants, positing it as a potential risk factor for ALL.

Based on the data, it appears that there were more male patients than female, with a ratio of about 2:1. This conclusion is in agreement with what Jawaid et al (2017) found in Pakistan, where they also found a higher incidence of males than females [20]. Similarly, a 2:1 male-to-female ratio was identified in the ALL populations studied in Pakistan by Sultan et al (2016) and Shahab et al (2014) [21-23]. The results corroborate those of the American Cancer Society and the Saudi Cancer Registry (2010) [24, 25].

This study's results show that 57.3% of the patients were under the age of 16. This aligns with previous reports from the American Cancer Society (2008) and Ebrahim et al (2017) in Sudan, where the majority of cases were found to be between the ages of 6 and 16 [26, 27]. A complete blood count (CBC) was conducted on all patients, indicating leukocytosis, anemia, thrombocytopenia, and an elevated blast proportion. The results aligned with those of several studies conducted in Sudan, Mexico, Iran, Egypt, and Pakistan [28-32]. The research indicated that B-ALL was more prevalent than T-ALL, consistent with many studies conducted in Jordan, Brazil, Italy, Egypt, and Mexico, all of which demonstrated a higher prevalence of B-ALL compared to T-ALL [21, 33-37]. Nonetheless, these findings are inconsistent with two investigations conducted in Iran and Pakistan, which demonstrated that the incidence of T-ALL surpasses that of B-ALL [38]. This discrepancy suggests that the disease behaves differently in different populations, likely due to the interaction of biological and external factors.

According to the findings of the study, individuals who were diagnosed with T-ALL exhibited significantly higher mean total WBC counts and blast percentages in comparison to those who were diagnosed with B-ALL. On the other hand, there was no statistically significant difference between the two groups in terms of the means of other hematologic parameters, such as the number of RBCs, the number of PLT, and the concentration of Hb. At the time of diagnosis, patients with T-ALL had considerably higher total WBC counts and blast percentages than patients with B-ALL, according to a study conducted in China by Dai et al (2021) [39]. These findings are in line with the findings of that study, which also examined the same phenomenon [39]. Furthermore, our findings are consistent with those of independent investigations carried out in Brazil by De Sousa et al (2015) [40] and in Iraq by Jaafar et al (2018) [41]. Both of these studies indicated a significant increase in the total WBC counts in patients with T-ALL when compared to patients with B-ALL [40, 41].

During the course of the inquiry, it was discovered that the homozygous genotypes AA and GG of the *MTRR* (A66G) polymorphism were identified in a greater proportion of patients than they were in the control group. In contrast, the heterozygous genotype AG was shown to be less prevalent in the patients as compared to the group that served as the control. On the other hand, the connection between the *MTRR* (A66G) polymorphism and the risk of ALL was not statistically significant across all age demographics. This finding is consistent with the findings of a number of other investigations, which indicated that there were no significant differences in susceptibility to ALL in relation to the *MTRR* (A66G) polymorphism [42, 43].

In a study that was carried out in Germany by Gast et al (2007), it was discovered that there were no statistically significant differences between patients and controls in terms of the allele and genotype frequencies that were associated with *MTRR* polymorphisms [44]. Similar to the previous study, two investigations that were carried out in Korea and Western Europe came to the conclusion that there was no significant link between the polymorphism and susceptibility to ALL [45, 46]. There have also been a number of research investigations conducted in Russia, the UK, and Italy that have found that *MTRR* polymorphism may reduce the risk of ALL [15, 47-49].

On the other hand, one study conducted in China found that the *MTRR* 66 GG mutation was related with a 2.15-fold increased risk for ALL [15]. In Korea, Kim et al (2009) identified a slightly elevated risk for ALL associated with the *MTRR* 66 GG genotype [50]. The contradictory findings on the association between *MTRR* polymorphism and the likelihood of developing ALL indicate that the polymorphism's effect on susceptibility to ALL may differ among groups and may be impacted by interactions with particular environmental factors.

The results of the study show that the mean RBC count, Hb concentration, total WBC count, PLT count, and blast percentage did not differ statistically significantly between ALL patients with wild-type and mutant *MTRR*(A66G) variations. Additionally, there is a dearth of published information com-

paring the hematological parameters of patients with different polymorphisms of the *MTRR*.

Conclusions

The current study's findings show that there were no statistically significant changes between ALL patients with wild-type and mutant *MTRR*(A66G) variations in terms of mean RBC count, Hb concentration, total WBC count, PLT count, and blast percentage. It is also important to note that there are currently no published data comparing the hematological parameters of patients with different *MTRR* polymorphisms.

Acknowledgments

The project was funded by the Deanship of Scientific Research (DSR) at King Abdulaziz University, Jeddah, under grant number (GPIP: 1406-415-2024). The authors, therefore, acknowledge DSR for the technical and financial support.

Financial Disclosure

This research was funded by the Deanship of Scientific Research (DSR) at King Abdulaziz University, Jeddah, under grant number (GPIP: 1406-415-2024).

Conflict of Interest

The authors declare no conflict of interest.

Informed Consent

Informed consent was obtained from all subjects, as well as from their parents or guardians.

Author Contributions

Conceptualization: EBY; methodology: HMQ; software: RA; validation: EBY, RA, and HMQ; formal analysis: EBY, RA, and HMQ; investigation: EBY, RA, and HMQ; resources: EBY, RA, and HMQ; data curation: EBY, RA, and HMQ; writing - original draft preparation: EBY, RA, and HMQ; writing - review and editing: EBY and HMQ; visualization: EBY, RA, and HMQ; supervision: EBY; project administration: EBY; funding acquisition: EBY. All authors have read and agreed to the published version of the manuscript.

Data Availability

The data supporting the findings of this study are available from the corresponding author upon reasonable request.

References

1. Brady SW, Roberts KG, Gu Z, Shi L, Pounds S, Pei D, Cheng C, et al. The genomic landscape of pediatric acute lymphoblastic leukemia. Nat Genet. 2022;54(9):1376-1389. doi pubmed
2. Mai H, Li Q, Wang G, Wang Y, Liu S, Tang X, Chen F, et al. Clinical application of next-generation sequencing-based monitoring of minimal residual disease in childhood acute lymphoblastic leukemia. J Cancer Res Clin Oncol. 2023;149(7):3259-3266. doi pubmed
3. Ajuba IC, Madu AJ, Okocha C, Ibegbulam OG, Okpala I, Nna OE. Frequency and clinical impact of ETV6/RUNX1, AF4-MLL, and BCR/ABL fusion genes on features of acute lymphoblastic leukemia at presentation. Niger J Clin Pract. 2016;19(2):237-241. doi pubmed
4. Kakaje A, Alhalabi MM, Ghareeb A, Karam B, Mansour B, Zahra B, Hamdan O. Rates and trends of childhood acute lymphoblastic leukaemia: an epidemiology study. Sci Rep. 2020;10(1):6756. doi pubmed
5. Jastaniah W, Essa MF, Ballourah W, Abosoudah I, Al Daama S, Algiraigri AH, Al Ghemlas I, et al. Incidence trends of childhood acute lymphoblastic leukemia in Saudi Arabia: Increasing incidence or competing risks? Cancer Epidemiol. 2020;67:101764. doi pubmed
6. Jabbour E, O'Brien S, Konopleva M, Kantarjian H. New insights into the pathophysiology and therapy of adult acute lymphoblastic leukemia. Cancer. 2015;121(15):2517-2528. doi pubmed
7. Onyije FM, Olsson A, Baaken D, Erdmann F, Stanulla M, Wollschlager D, Schuz J. Environmental risk factors for childhood acute lymphoblastic leukemia: an umbrella review. Cancers (Basel). 2022;14(2):382. doi pubmed
8. Ma LM, Yang HP, Yang XW, Ruan LH. Methionine synthase A2756G polymorphism influences pediatric acute lymphoblastic leukemia risk: a meta-analysis. Biosci Rep. 2019;39(1):BSR20181770. doi pubmed
9. Milne E, Greenop KR, Scott RJ, Haber M, Norris MD, Attia J, Jamieson SE, et al. Folate pathway gene polymorphisms, maternal folic acid use, and risk of childhood acute lymphoblastic leukemia. Cancer Epidemiol Biomarkers Prev. 2015;24(1):48-56. doi pubmed
10. Wang P, Li S, Wang M, He J, Xi S. Association of MTRR A66G polymorphism with cancer susceptibility: Evidence from 85 studies. J Cancer. 2017;8(2):266-277. doi pubmed
11. Lv H, Hu SY, Du ZZ, Zhai Z, Cao L, Sun YN, Lu J, et al. Gene polymorphisms in the folate metabolic pathway and risk of pediatric acute lymphoblastic leukemia: a case-control study in a Chinese population. Int J Clin Exp Pathol. 2018;11(3):1724-1731. pubmed
12. Wei L, Niu F, Wu J, Chen F, Yang H, Li J, Jin T, et al. Association study between genetic polymorphisms in folate metabolism and gastric cancer susceptibility in Chinese Han population: A case-control study. Mol Genet Genomic Med. 2019;7(5):e633. doi pubmed
13. Elderdery AY, Tebein EM, Alenazy FO, Elkhalifa AME, Shalabi MG, Abbas AM, Alhassan HH, et al. Impact

of methionine synthase reductase polymorphisms in chronic myeloid leukemia patients. Genes (Basel). 2022;13(10):1729. doi pubmed
14. Farasani A. Experimental study of A66G-single nucleotide polymorphism in the MTRR gene and acute myeloid leukemia. Journal of King Saud University-Science, 2023;35(1):102439.
15. Yang L, Liu L, Wang J, Qiu L, Mi Y, Ma X, Xiao Z. Polymorphisms in folate-related genes: impact on risk of adult acute lymphoblastic leukemia rather than pediatric in Han Chinese. Leuk Lymphoma. 2011;52(9):1770-1776. doi pubmed
16. Kwak SY, Kim UK, Cho HJ, Lee HK, Kim HJ, Kim NK, Hwang SG. Methylenetetrahydrofolate reductase (MTHFR) and methionine synthase reductase (MTRR) gene polymorphisms as risk factors for hepatocellular carcinoma in a Korean population. Anticancer Res. 2008;28(5A):2807-2811. pubmed
17. Johnston WT, Lightfoot TJ, Simpson J, Roman E. Childhood cancer survival: a report from the United Kingdom Childhood Cancer Study. Cancer Epidemiol. 2010;34(6):659-666. doi pubmed
18. Healy J, Richer C, Bourgey M, Kritikou EA, Sinnett D. Replication analysis confirms the association of ARID5B with childhood B-cell acute lymphoblastic leukemia. Haematologica. 2010;95(9):1608-1611. doi pubmed
19. Brisson GD, Alves LR, Pombo-de-Oliveira MS. Genetic susceptibility in childhood acute leukaemias: a systematic review. Ecancermedicalscience. 2015;9:539. doi pubmed
20. Jawaid A, Arif K, Amjad N. Clinical presentations of acute leukemia in pediatric emergency department of Pakistan. Bone. 2017;29(28.4):27.7-3.3.
21. Hussen MMA. Association of cytochrome P450 2 E1 (C1053T) and NADPH quinone oxide reducatase 1 (C609T)(C 465T) genes polymorphism with acute lymphoblastic leukemia in Sudanese patients. Sudan University of Science & Technology. 2019.
22. Sultan S, Irfan SM, Parveen S, Mustafa S. Acute lymphoblastic leukemia in adults - an analysis of 51 cases from a tertiary care center in Pakistan. Asian Pac J Cancer Prev. 2016;17(4):2307-2309. doi pubmed
23. Shahab F, Raziq F. Clinical presentations of acute leukemia. J Coll Physicians Surg Pak. 2014;24(7):472-476. pubmed
24. Bazarbashi S, Al Eid H, Minguet J. Cancer incidence in Saudi Arabia: 2012 data from the Saudi Cancer Registry. Asian Pac J Cancer Prev. 2017;18(9):2437-2444. doi pubmed
25. CO, C., Facts & Figures 2014-2016. 2014.
26. Society AC. Cancer facts & figures. 2008: the Society.
27. Ebrahim J, et al. Immunophenotypic features of T cell-acute lymphoblastic leukemia in Sudan. 2017.
28. Hanna J. Expression of CD95 in acute lymphocytic leukemia (ALL) in Egyptian children before and after treatment. J Blood Disord Transfus. 2015;6:1.
29. Barakat M, Elkhayat Z, Kholoussi N, Elnady H, Ismail M, Raafat J. Monitoring treatment response of childhood acute lymphocytic leukemia with certain molecular and biochemical markers. J Biochem Mol Toxicol. 2010;24(6):343-350. doi pubmed
30. Mahmood N, Shahid S, Bakhshi T, Riaz S, Ghufran H, Yaqoob M. Identification of significant risks in pediatric acute lymphoblastic leukemia (ALL) through machine learning (ML) approach. Med Biol Eng Comput. 2020;58(11):2631-2640. doi pubmed
31. Jaime-Perez JC, Garcia-Arellano G, Herrera-Garza JL, Marfil-Rivera LJ, Gomez-Almaguer D. 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. 2019;41(1):57-61. doi pubmed
32. Moussavi F, et al. The first CBC in diagnosis of childhood acute lymphoblastic leukemia. International Journal of Medical Investigation. 2014;3(1).
33. Aljaafreh L. Immunophenotypic profile of acute leukemia cases using multicolor flow cytometry, three year experience at King Hussein medical center. JRMS. 2015;22(3):53-58.
34. Shrestha S, et al. Immunophenotypic study of acute leukemia by flow cytometry at BPKMCH. Journal of pathology of Nepal. 2013;3(5):345-350.
35. Spinelli O, Tosi M, Peruta B, Guinea Montalvo ML, Maino E, Scattolin AM, Parolini M, et al. Prognostic significance and treatment implications of minimal residual disease studies in Philadelphia-negative adult acute lymphoblastic leukemia. Mediterr J Hematol Infect Dis. 2014;6(1):e2014062. doi pubmed
36. Terwilliger T, Abdul-Hay M. Acute lymphoblastic leukemia: a comprehensive review and 2017 update. Blood Cancer J. 2017;7(6):e577. doi pubmed
37. Gallegos-Arreola M, et al. Pathophysiology of acute lymphoblastic leukemia. INTECH. 2013;3(1):43-60.
38. Pahloosye A, et al. Presenting clinical and laboratory data of childhood acute lymphoblastic leukemia. Iranian Journal of Pediatric Hematology and Oncology. 2011;1(3):71-77.
39. Dai Q, Zhang G, Yang H, Wang Y, Ye L, Peng L, Shi R, et al. Clinical features and outcome of pediatric acute lymphoblastic leukemia with low peripheral blood blast cell count at diagnosis. Medicine (Baltimore). 2021;100(4):e24518. doi pubmed
40. Lustosa de Sousa DW, de Almeida Ferreira FV, Cavalcante Felix FH, de Oliveira Lopes MV. Acute lymphoblastic leukemia in children and adolescents: prognostic factors and analysis of survival. Rev Bras Hematol Hemoter. 2015;37(4):223-229. doi pubmed
41. Jaafar FH, Kadhom AE. Expression of CD45, CD34, CD10, and human leukocyte antigen-DR in acute lymphoblastic leukemia. Iraqi Journal of Hematology, 2018;7(1):14-19.
42. Koppen IJ, Hermans FJ, Kaspers GJ. Folate related gene polymorphisms and susceptibility to develop childhood acute lymphoblastic leukaemia. Br J Haematol. 2010;148(1):3-14. doi pubmed
43. Edris MT, et al. Association of methionine synthase reductase (MTRR A66G) polymorphism with susceptibility to acute lymphoblastic leukemia. Italian Journal of Medicine. 2023;17(2).

44. Gast A, Bermejo JL, Flohr T, Stanulla M, Burwinkel B, Schrappe M, Bartram CR, et al. Folate metabolic gene polymorphisms and childhood acute lymphoblastic leukemia: a case-control study. Leukemia. 2007;21(2):320-325. doi pubmed
45. de Jonge R, Tissing WJ, Hooijberg JH, Jansen G, Kaspers GJ, Lindemans J, Peters GJ, et al. Polymorphisms in folate-related genes and risk of pediatric acute lymphoblastic leukemia. Blood. 2009;113(10):2284-2289. doi pubmed
46. Gra OA, Glotov AS, Kozhekbaeva Z, Makarova OV, Nasedkina TV. [Genetic polymorphism in GST, NAT2, and MTRR and susceptibility to childhood acute leukemia]. Mol Biol (Mosk). 2008;42(2):214-225. pubmed
47. Vijayakrishnan J, Studd J, Broderick P, Kinnersley B, Holroyd A, Law PJ, Kumar R, et al. Genome-wide association study identifies susceptibility loci for B-cell childhood acute lymphoblastic leukemia. Nat Commun. 2018;9(1):1340. doi pubmed
48. Gemmati D, Ongaro A, Scapoli GL, Della Porta M, Tognazzo S, Serino ML, Di Bona E, et al. Common gene polymorphisms in the metabolic folate and methylation pathway and the risk of acute lymphoblastic leukemia and non-Hodgkin's lymphoma in adults. Cancer Epidemiol Biomarkers Prev. 2004;13(5):787-794. pubmed
49. Sepe DM, McWilliams T, Chen J, Kershenbaum A, Zhao H, La M, Devidas M, et al. Germline genetic variation and treatment response on CCG-1891. Pediatr Blood Cancer. 2012;58(5):695-700. doi pubmed
50. Kim HN, Kim YK, Lee IK, Yang DH, Lee JJ, Shin MH, Park KS, et al. Association between polymorphisms of folate-metabolizing enzymes and hematological malignancies. Leuk Res. 2009;33(1):82-87. doi pubmed