Cukurova Medical Journal
ÇUKUROVA ÜNİVERSİTESİ TIP FAKÜLTESİ

Cukurova Med J 2023;48(2):377-384
DOI: 10.17826/cumj.1221593

RESEARCH

DNA polymerase delta (POLD1 and POLD2) gene expression in pediatric acute lymphoblastic leukemia patients and its relationship with prognosis

Pediatrik akut lenfoblastik lösemi hastalarında DNA polimeraz delta (POLD1 ve POLD2) gen ekspresyonu ve prognoz ile ilişkisi

Ahmet Yöntem[1], İbrahim Bayram[2], Gülay Sezgin[2], Serhan Küpeli[2], Ayşe Özkan[2], Atila Tanyeli[2]

[1]Çukurova University Faculty of Medicine, Department of Pediatrics, [2]Division of Pediatric Oncology and Pediatric Bone Marrow Transplantation Unit, Adana, Turkey

Abstract

Purpose: This study aimed to investigate the status of DNA polymerase delta (POLD1 and POLD2) gene expression at the time of diagnosis in pediatric acute lymphoblastic leukemia (ALL) patients, compared with the normal population, and its relationship with prognosis and other clinical findings.

Materials and Methods: Seventy-three patients diagnosed with ALL between January 2008 and November 2015 and 29 healthy control subjects were included in the study. Gene expression profiling of peripheral blood samples was performed using Real-time PCR.

Results: The mean value of POLD1 gene expression was found to be significantly higher in ALL patients at the time of diagnosis than the control group (376.5± 685.8 and 17.9± 19.8, respectively), but there was no difference in POLD2 gene expression (511.5± 898.1 and 125.4± 132.7, respectively). POLD1 and POLD2 gene expressions were found to be low in patients with relapse and exitus, but the results were not statistically significant. Patients with low levels of POLD1 expression had lower survival rates in the 5th year than those with high levels of expression (54% and 68%, respectively), and similarly, patients with low levels of POLD2 expression had lower survival rates in the 5th year compared to those with high levels of expression (58% and 68%, respectively).

Conclusion: Lower POLD1 and POLD2 expressions at the time of diagnosis in ALL patients may adversely affects the prognosis.

Keywords:. Acute lymphoblastic leukemia, childhood, DNA polymerase enzymes, POLD1 and POLD2 expression

Öz

Amaç: Bu çalışmada pediatrik akut lenfoblastik lösemi (ALL) hastalarında tanı anında DNA polimeraz delta (POLD1 ve POLD2) gen ekspresyonunun normal popülasyona göre durumu, prognoz ve diğer klinik bulgularla ilişkisinin araştırılması amaçlandı.

Gereç ve Yöntem: Çalışmaya Ocak 2008 ile Kasım 2015 tarihleri arasında ALL tanısı almış 73 hasta ve 29 sağlıklı kontrol olgu dahil edildi. Periferik kan örneklerinde gen ekspresyon profili Real-time PCR yöntemi kullanılarak yapıldı.

Bulgular: POLD1 gen ekspresyonunun ortalama değeri, ALL hastalarında tanı anında kontrol grubuna göre anlamlı olarak yüksek bulundu (sırasıyla 376,5± 685,8 ve 17,9± 19,8), ancak POLD2 gen ekspresyonunda fark yok idi (sırasıyla 511,5± 898,1 ve 125,4± 132,7). POLD1 ve POLD2 gen ekspresyonları, relaps ve exitus olan hastalarda düşük saptandı ancak sonuçlar istatitiksel olarak anlamlı değildi. Düşük POLD1 ekspresyonu olan hastalarda yüksek olanlara göre 5. yılda sağkalım oranları daha düşük saptandı (sırasıyla %54 ve %68), benzer şekilde düşük POLD2 ekspresyonu olan hastalarda yüksek olanlara göre 5. yılda sağkalım oranları daha düşük saptandı (sırasıyla, %58 ve %68).

Sonuç: Akut lenfoblastik lösemi hastalarında tanı anında POLD1 ve POLD2 ekspresyonlarının düşük olması prognozu olumsuz etkileyebilir.

Anahtar kelimeler: Akut lenfoblastik lösemi, çocukluk çağı, DNA polimeraz enzimleri, POLD1 ve POLD2 ekspresyonu

Address for Correspondence: Ayşe Özkan, Çukurova University Faculty of Medicine, Pediatric Oncology and Pediatric Bone Marrow Transplantation Unit, Adana, Turkey E-mail: drayseozkan79@yahoo.com.tr
Received: 20.12.2022 Accepted: 16.04.2023

INTRODUCTION

Leukemia accounts for approximately 25%-30% of all childhood cancers, while 97% of leukemia takes an acute form[1]. In children under 15 years of age, acute lymphoblastic leukemia (ALL) is approximately five times more common than acute myeloid leukemia (AML)[2]. In a 2021 study conducted with children under the age of 15, Siegel et al. reported a 5-year survival rate of ALL patients of 91%[3]. Although the cause of leukemias is not fully known, familial, environmental factors, chromosomal abnormalities, inherited cytopenias and hereditary single-gene mutations are thought to play a role in their occurrence[4].

The primary function of DNA replication is the continuation of the lineage of the parents' genetic information. The enzymes responsible for DNA replication are DNA polymerase enzymes, which allow the DNA chains to be elongated, processed and corrected. DNA polymerases are divided into seven families, in which DNA polymerase delta (Pol δ) plays an essential role in genome stability through its effects on DNA replication and repair. The p125 catalytic subunit of Pol δ is encoded by the POLD1 gene in human cells, while POLD2 is the second small subunit of DNA polymerase δ. Mutations occur because of the faulty replication, movement or repair of DNA, and today, it is clear that most diseases and cancers develop due to the effects of such mutations[5]. In many studies, defects in the polymerase or exonuclease activity of DNA polymerase delta have been shown to increase the rate of mutation and cancer incidence[6-11].

In this study, we aimed to investigate the status of DNA polymerase delta (POLD1 and POLD2) gene in patients newly diagnosed with ALL, compared with the normal population, and its relationship with prognosis and other clinical findings.

MATERIALS AND METHODS

Study design and patient selection

Patients with acute leukemia aged between 1 month and 18 years who were newly diagnosed and followed-up at the Division of Pediatric Oncology/Stem Cell Transplantation Unit, Cukurova University, Balcali Research Hospital, were included in the study. Of the 168 patients diagnosed with acute leukemia between January 2008 and November 2015, 28 diagnosed with AML, 12 from Syria, five who were followed-up by another center after diagnosis and 50 lacking sufficient RNA were excluded from the study. The remaining 73 patients with ALL were included in the study. Furthermore, 29 children whose physical examinations and complete blood counts were normal and who were admitted to the General Pediatric Outpatient Polyclinic were included as the control group.

The morphological, cytochemical and immunological examination results and bone marrow smears of the patients were evaluated, and immunophenotyping was performed via flow cytometry. The cell extraction was performed from blood samples using standard methods and collected in 3 ml ethylene diamine tetra acetic acid (EDTA) tubes at the time of diagnosis and again after induction therapy in patients with ALL. The Real time–PCR method was used to detect POLD1 and POLD2 gene expression using a LightCycler (Roche Applied Science) in accordance with the manufacturer's protocol. RNA was isolated from the cells, and the isolated RNA samples were stored in a deep freezer at -80°C until the time of analysis. Complementary c-DNA was obtained (Transcriptor High Fidelity cDNA Synthesis Kit, Roche Diagnostics, Germany) after the extraction of RNA with a commercial kit (High pure RNA isolation kit, Roche Diagnostics, Germany). The relative expression of each gene was calculated using the formula 2(-ΔCT) and normalized to the geometric mean of expression of the two control genes.

The Ethics Committee of Cukurova University Faculty of Medicine approved the study (07.10.2016/57), which was conducted in accordance with the principles of the Declaration of Helsinki. Informed consent was obtained from the patients, control subjects, and/or legal guardians before enrollment in the study, which was conducted in accordance with local institutional regulations.

Treatment

All patients with ALL were started on the BFM-95 (BFM TR-ALL 2000)-based chemotherapy protocol. Patients were stratified into standard (SRG), moderate (MRG) and high (HRG) risk groups according the BFM protocol. Minimal residual disease assessment cannot be performed in our center.

Statistical analysis

Statistical Package for Social Sciences (version 20.0 SPSS, IL, USA) software was used for statistical analyses. Group data were defined as mean ± standard deviation (mean±SD). The Pearson correlation test was performed for correlation and Kaplan–Meier method was used for life analysis in the evaluation of the data. In addition, categorical variables (such as gender) were analyzed using the chi-square test. ANOVA and Mann–Whitney U tests were used to compare the groups. $p<0.05$ was considered statistically significant.

RESULTS

The median age of the ALL patients was 65 (7-239) months, and the median age of the control group was 62 (12-204) months. The patients were followed up for 4 days –93 months (mean 38±26 months) and treated. The survival rates of the ALL patients were 89% at 12 months and 62% in the fifth year (Figure 1). Of the total, 21 (29%) cases relapsed, and relapse was observed twice in six cases (8%). Of the relapsed ALL cases, eight (38%) were in the SRG group, 11 (52%) were in the MRG group and two (10%) were in the HRG group.

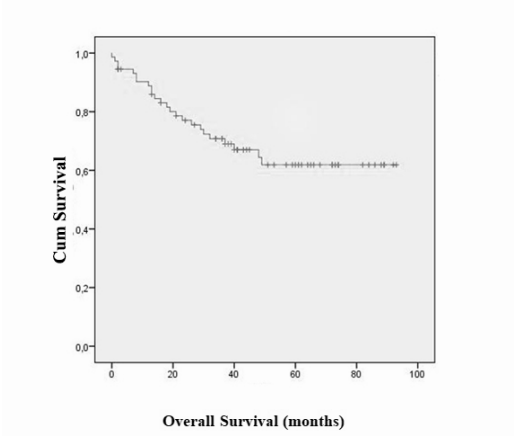

Figure 1. Overall survival in ALL patients

There was no effect of sex, age, anemia, neutropenia, thrombocytopenia, treatment risk group, organomegaly, leukopenia, degree of leukocytosis, or other clinical findings on the overall survival rate ($p>0.05$). While the 5-year survival rate was 17% in relapsed patients, it was 84% in patients without relapse, and this difference was statistically significant ($p=0.001$). Similarly, the 5-year survival rate was 21% in patients with central nervous system (CNS) involvement and 76% in patients without CNS involvement ($p=0.001$).

When the polymerase gene expression levels of ALL patients and the control group were compared, POLD1 gene expression was significantly higher in the ALL-patients at the time of diagnosis ($p=0.001$), while there was no difference in POLD2 gene expression ($p=0.246$) (Table 1).

Table 1. POLD1 and POLD2 expression in ALL and control groups

	POLD1 Mean±SD Median (min-max)	**POLD2 Mean±SD Median (min-max)**
ALL (n=73)	376.5± 685.8 108.3 (1.0-4 124.5)	511.5± 898.1 121.7 (1.0-4 153.2)
Control (n=29)	17.9± 19.8 10.2 (0.6-86.8)	125.4± 132.7 75.0 (2.6-492.7)
p	0.001	0.246

ALL: Acute lymphoblastic leukemia, POLD1: DNA Polymerase Delta 1, POLD2: DNA Polymerase Delta 2, SD: Standard deviation

The POLD1 and POLD2 gene expression levels in ALL patients according to age, gender, laboratory and clinical findings are given in Table 2. For the patients who relapsed, those with CNS involvement and those who died, the POLD1 and POLD2 gene expression levels measured at the time of diagnosis were low, but not to a statistically significant degree. The expression of the POLD1 gene in patients with thrombocytopenia was significantly higher ($p=0.023$), and the expression of the POLD2 gene was significantly higher in patients with anemia and splenomegaly ($p=0.010$ and $p=0.017$, respectively).

The levels of POLD1 and POLD2 gene expression were divided into two groups, low or high, according to the 95th percentile of the control group mean (POLD1=72.3 and POLD2=485.0), and their effect on overall survival was investigated. In patients with ALL, the 5-year survival rate was 68% in those with a high expression of POLD1 (n=41), while in patients with a low expression of POLD1 (n=32), the 5-year survival rate was 54% ($p=0.320$) (Fig. 2). In ALL cases, in patients with high expression of POLD2 (n=19), the 5-year survival rate was 68%, and in those with low expression (n=54), the 5-year survival rate was 58% ($p=0.713$) (Fig. 3).

Table 2. Demographic characteristics of patients with ALL, and comparison of their POLD1, and POLD2 gene expressions

	n	POLD1 Mean ±SD Median (min-max)	p	POLD2 Mean ±SD Median (min-max)	p
Relapse	21	318.8± 714.6 64.4 (1.0-3 165.8)	0.205	432.2± 859.8 77.7 (1.0-3 078.3)	0.170
No Relapse	52	399.8± 679.6 161.0 (1.6- ,124.4)		543.5± 919.2 169.5 (1.7-4 153.2)	
Exitus*	24	361.2± 695.3 80.7 (1.0-3 165.8)	0.432	528.2± 905.5 100.0 (1.0-3 078.3)	0.484
Survived	46	395.6± 708.7 124.7 (1.6-4 124.4)		528.7± 926.9 147.4 (1.4-4 153.2)	
CNS relapse (+)	17	354.3± 790.3 64.4 (1.0-3 165.8)	0.309	458.1± 939.2 77.7 (1.0-3 078.3)	0.230
CNS relapse (-)	56	383.3± 658.6 161.0 (1.6-4 124.4)		527.7± 893.29 169.5 (1.6-4 153.2)	
Risk group SRG	17	204.7± 357.4 70.5 (1.6-1 323.3)		350.8± 786.2 75.5 (1.4-3,125.8)	
MRG	39	479.5± 876.0 97.6 (1.0-4 124.4)	0.345	637.3± 1 079.5 121.7 (1.0-4 153.2)	0.265
HRG	17	312.1± 323.5 203.2 (18.2-1 009.9)	0.073	383.5± 399.5 203.6 (4.8-1 105.1)	0.153
Neutropenia (+)**	17	147.6± 251.6 64.4 (8.4-1 067.4)	0.173	295.9± 862.4 47.5 (1.6-3 615.5)	0.092
Neutropenia (-)	53	463.2± 776.8 155.4 (1.0-4 124.5)		601.9± 924.7 203.6 (1.0-4 153.2)	
Anemia (+)**	63	422.4± 727.7 148.0 (1.6-4 124.5)	0.102	582.6± 947.5 178.5 (1.4-4 153.2)	0.010
Anemia (-)	7	63.8± 70.7 51.9 (1.0-210.8)		32.9± 40.5 20.2 (1.0-108.3)	
Thrombocytemia (+)**	51	490.5± 788.7 181.0 (1.0-4 124.5)	0.023	675.7± 1 029.2 203.6 (1.0-4 153.2)	0.092
Thrombocytemia (-)	19	107.4± 172.6 41.07 (1.6-744.4)		130.1± 164.6 86.8 (1.8-704.3)	
Hepatomegaly (+)	35	506.1± 902.7 108.3 (1.0-4 124.5)	0.728	622.1± 997.7 174.8 (1.00-4 153.2)	0.310
Hepatomegaly (-)	38	257.2± 368.6 119.3 (1.6-1 323.3)		409.6± 795.1 110.2 (1.40-3 615.6)	
Splenomegaly (+)	31	555.7± 932.6 206.5 (6.32-4 124.5)	0.058	655.5± 980.0 293.7 (3.3-4 153.2)	0.017
Splenomegaly (-)	42	244.3± 383.9 76.9 (1.0-1 323.3)		405.2± 828.3 71.6 (1.0-3 615.6)	
Lymphadenopathy (+)	33	387.9± 639.0 176.0 (1.0-3 165.8)	0.369	464.0± 722.2 121.7 (1.0-3 078.3)	0.441
Lymphadenopathy (-)	40	367.1± 730.1 84.7 (1.6-4 124.5)		550.6± 1 028.2 121.4(1.4-4 153.1)	

ALL: Acute lymphoblastic leukemia, CNS: Central nervous system, HRG: High risk group, MRG: Moderate risk group, POLD1: DNA Polymerase Delta 1, POLD2: DNA Polymerase Delta 2, SD: Standard deviation, SRG: Standard risk group. * 3 patients lost to follow-up, ** laboratory results of 3 patients was not available at the time of diagnosis.

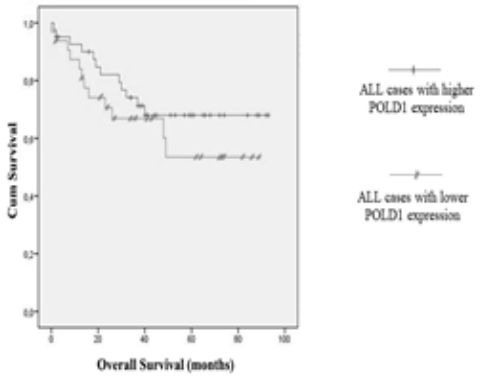

Figure 2. The impact of DNA POLD1 expression on overall survival in ALL patients.

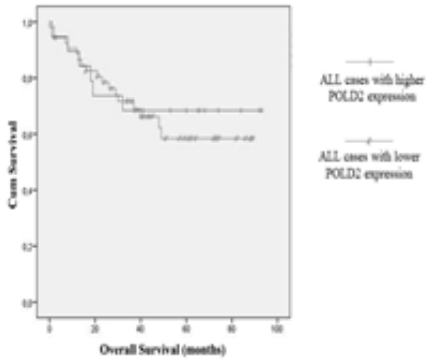

Figure 3. The impact of DNA POLD2 expression on overall survival in ALL patients.

DISCUSSION

In ALL patients, age, white blood cell count and cytogenetic values are among the most important prognostic factors[4]. Today, ALL patients are classified as low, medium and high risk to ensure the more intensive treatment of those expected to relapse and to protect those at low risk from the late side effects of treatments. That said, relapses can occur in patients who are not in the high-risk group and who do not have poor prognostic factors. The most common cause of death in childhood cancers in low-income and middle-income countries is leukemia[12]. For this reason, it is important to identify new prognostic factors to improve treatment success and survival rates in childhood acute leukemia.

The primary function of DNA replication is to continue one's bloodline through the genetic information passed down by the parents. Therefore, the replication of DNA must be complete and carried out with complete accuracy to ensure genetic stability within organisms and species. Mutations occur because of the incorrect replication, movement or repair of DNA. Today, it is known that certain diseases and cancers arise due to the effects of such mutations. The maintenance of genomic stability and prevention of carcinogenesis can be achieved through the accurate duplication of DNA. Organisms have developed mechanisms for repairing DNA damage caused by replication errors and attacks from the environment. These known mechanisms include cell cycle control proteins, various exonucleases and endonucleases, DNA single chain-specific DNA-binding proteins, and specific DNA polymerases (DNA pol delta and eta). Eukaryotic DNA polymerase delta is a conserved enzyme that plays an important role in DNA replication, DNA repair and genetic recombination, and it is thought that DNA polymerase delta synthesizes a large part of the genome by synthesizing Okazaki fragments of the lagging strand[13].

Genome sequencing has been widely used for the identification of potential biomarkers and therapeutic targets based on variations in the gene expression. In many studies, defects in the polymerase or exonuclease activity of DNA polymerase delta have been shown to increase the rate of mutation and cancer incidence in mice. Goldsby et al. reported that mice homozygous for the POLD Exo II mutation D400A developed lymphoma throughout life[6]. In another study, Goldsby et al. found that 94% of mice with homozygous POLD1D400A/D400A developed cancer and died by the age of 18 months[7]. Venkatesan et al. identified allele-specific accelerated tumorigenesis in mice with a heterozygous mutation at L604 in the polymerase active site of DNA polymerase[8]. There have been limited studies of human samples to date, although recent studies have

shown that germline mutations in the proofreading domain of POLD can lead to cancer development[9-11]. Palles et al. found that colorectal tumors, endometrial cancer and brain tumors may arise from the POLD1 S478 N variant, indicating that untreated DNA polymerase defects can contribute to carcinogenesis[9].

This study investigated the gene expression of POLD1 and POLD2 relative to the normal population in children with ALL, as well as its prognosis and relationship with other clinical presentations. A significant difference was noted in the POLD1 expression levels of the ALL-patients and the control group, while no such difference was present in POLD2 expression. This suggests that POLD1 may be more effective in DNA replication and repair in ALL patients with high clonal proliferation and mitotic rates.

Central nervous system involvement is encountered in less than 5% of B-ALL cases and in 10%–15% of T-ALL cases at diagnosis[14]. In their study of 501 patients between 2000 and 2007, Pui et al. reported that CNS relapse significantly reduced survival[15]. In this study, the effect of CNS involvement or relapse on survival in leukemia cases was statistically significant, with the 5-year survival rate of relapsed patients being 17%, compared with the 84% rate recorded in patients without relapse (p=0.001). Similarly, the 5-year survival in patients with CNS involvement was 21%, compared with 76% in those without CNS involvement, which was a statistically significant difference (p=0.001). Manley et al. reported relapse in 22% of patients diagnosed with ALL between 1992 and 2013, 9% of whom relapsed while receiving treatment, while 1.7% of patients had CNS relapses, and 85% of those experiencing a CNS relapse were in exitus[16]. In this study, relapse was recorded in 28.8% of the ALL-cases, 13.7% of cases had an isolated CNS relapse, 5.5% had an isolated systemic relapse, and 9.6% had a CNS+systemic relapse. In the patients with relapse, CNS involvement and those who died, the POLD1 and POLD2 gene expression values at the time of diagnosis were low, but not to a statistically significant degree (p>0.05).

In a study conducted by Sunamak et al. who used BFM treatment from our country, relapse was most common in MRG (61.5%), and less common in SRG (12.8%)[17]. However, in the present study, relapse was most common in patients in the MRG with 52% (11 patients), and less common in patients in HRG with 10% (2 patient). This difference may be associated with geographical and racial factors. In addition, some of the patients may have been mistakenly included in the SRG, since MRD could not be performed in our center.

When the effects of POLD1 and POLD2 expression higher or lower than the control group mean in ALL patients, it was found that patients with low levels of POLD1 and POLD2 expression than the control group had lower survival rates in the 5th year. As a result of the review of the studies in the English literature, a study was found showing the prognostic value of POLD1 in leukemia. Li et al. studied POLD1 expression in 49 newly diagnosed cases and 49 relapsed cases and found POLD1 to be significantly upregulated in relapsed ALL compared with newly diagnosed patients with ALL[18]. However, this study had some limitations. First, the results were obtained using a bioinformatics analysis. Second, the analysis of the event-free survival rate of the patients in this study could not be evaluated clearly due to the lack of clinical data. By synthesizing two-dimensional arsenene nanolayers, Wang et al. showed that with this biomedical application, arsenene was effective against acute promyelocytic leukemia cells and induced apoptosis without showing any toxicity against normal cells. They suggested that arsenene had this effect by downregulating nuclear proteins (POLD1, POLD3, and POLE) and reducing DNA replication ability, and that arsenene could be applied in the treatment of acute promyelocytic leukemia with this mechanism[19].

Liao et al. screened the core genes of Philadelphia chromosome positive/Ph-like T-cell acute lymphoblastic leukemia (Ph+/Ph-like T-ALL) using bioinformatics methods, and analyzed the core subnetworks to explore the Ph+/Ph-like T-development process. They also tried to find molecular targets that can be used in the treatment. As a result of their evaluation, they suggested that POLD1 together with RPA and POLE may be important biomarkers for the formation and development of Ph+/Ph like T-ALL[20]. The three studies mentioned above that tried to show the relationship between leukemia and POLD1 expression were bioinformatics studies, and clinical data were lacking in all these studies. Our study was a clinical study with a control group.

There were some limitations in our study, the first of which is its single-center and retrospective design. Second, we were unable to obtain sufficient RNA

from the serum samples of 50 patients with ALL, and 12 Syrian patients were excluded from the study due to poor treatment compliance. Finally, specimens were not collected from relapsed ALL patients to assess POLD1 and POLD2 expression. These factors may all limit the generalization of the study findings.

In conclusion; the POLD1 gene expression was found to be significantly higher in ALL patients at diagnosis than the control group, but there was no difference in POLD2 gene expression. In the present study we suggested that POLD1 may serve as a potential diagnostic marker and therapeutic target for the treatment of relapsed ALL. Otherwise and interestingly, the POLD1 and POLD2 expression levels measured at the time of diagnosis in patients with CNS involvement and relapse and those who died were lower than those without, and poorer survival rates were observed in those with low POLD1 and POLD2 levels. This suggests that low levels of DNA polymerase measured at the time of diagnosis may be a poor prognostic factor in ALL patients. When DNA polymerase expression measured at the time of diagnosis is detected as low, we believe that relapse can be prevented and survival rates can be increased through the application of more powerful treatment protocols or protocols formed with new and different drugs.

This study is the first clinical study in the literature to demonstrate the relationship between POLD1 and POLD2) expression and prognosis in childhood acute lymphoblastic leukemias, and the reference values of DNA polymerase delta (POLD1 and POLD2) expression in pediatric patients with ALL. Accordingly, the authors consider further comprehensive multicenter studies with a larger cohort of patients would help clarify the current findings and establish the relationship of POLD1 and POLD2 proteins with diagnosis and prognosis.

Author Contributions: Concept/Design : İB, AT; Data acquisition: AY; Data analysis and interpretation: İB, GS, SK; Drafting manuscript: AY, AO; Critical revision of manuscript: İB, SK, AT; Final approval and accountability: AY, İB, GS, SK, AÖ, AT; Technical or material support: AY, GS; Supervision: İB, SK, AT; Securing funding (if available): n/a.
Ethical Approval: Çukurova University Faculty of Medicine, Ethics Committee of Non-Interventional Clinical Research 07.1. ethical approval was obtained with the decision No. 57/3 dated 2016..
Peer-review: Externally peer-reviewed.
Conflict of Interest: The authors have declared that there is no conflict of interest.
Financial Disclosure: This work was supported by 'Cukurova University Research Projects Funding Unit' with Project number TTU-2015-5085.

REFERENCES

1. Pillai MP, Carroll WL. Acute lymphoblastic leukemia in Lanzkowsky's Manual of Peadiatric Hematol and Oncology, 7th ed. (Eds JD Fish, JM Lipton, P Lanzkowsky):413-37. Amsterdam, Elsevier Academic Press. 2022.
2. Arceci RJ, Meshinchi S. Acute Myeloid Leukemia AND Myelodysplastic Syndromes. In Pizzo and Poplack's Pediatric Oncology, 8th ed. (Eds SM Blaney, LJ Helman, PC Adamson):498-544. Philadelphia, Wolters Kluwer. 2021.
3. Siegel RL, Miller KD, Funchs, HE; Jemal A. Cancer Statistics, 2021. CA Cancer J Clin. 2021;71:7-33.
4. Rabin KR, Gramatges MM, Margolin JF, Poplack DG. Acute Lymphoblastic Leukemia. In Pizzo and Poplack's Pediatric Oncology, 8th ed. (Eds SM Blaney, LJ Helman, PC Adamson):463-97. Philadelphia, Wolters Kluwer, 2021.
5. Nelson DL, Cox MM. DNA Metabolism. In Lehninger Principles of Biochemistry, 8th ed. (Eds DL Nelson, MM Cox): 948-94. North Bay, W.H.Freeman & Co Ltd, 2021.
6. Goldsby RE, Lawrence NA, Hays LE, Olmsted EA, Chen X, Singh M et al. Defective DNA polymerase-delta proofreading causes cancer susceptibility in mice. Nat Med. 2001;7:638–9.
7. Goldsby RE, Hays LE, Chen X, Olmsted EA, Slayton WB, Spangrude GJ et al. High incidence of epithelial cancers in mice deficient for DNA polymerase delta proofreading. Proc Natl Acad Sci U S A. 2002;99:15560–5.
8. Venkatesan RN, Treuting PM, Fuller ED, Goldsby RE, Norwood TH, Gooley TA et al. Mutation at the polymerase active site of mouse DNA polymerase delta increases genomic instability and accelerates tumorigenesis. Mol Cell Biol. 2007;27:7669–82.
9. Palles C, Cazier JB, Howarth KM, Domingo E, Jones AM, Broderick P et al. Germline mutations affecting the proofreading domains of POLE and POLD1 predispose to colorectal adenomas and carcinomas. Nat Genet. 2013;45:136–44.
10. Church DN, Briggs SEW, Palles C, Domingo E, Kearsey SJ, Grimes JM et al. DNA polymerase epsilon and delta exonuclease domain mutations in endometrial cancer. Hum Mol Genet. 2013;22:2820–8.
11. Rayner E, van Gool IC, Palles C, Kearsey SE, Bosse T, Tomlinson I et al. A panoply of errors: polymerase proofreading domain mutations in cancer. Nat Rev Cancer. 2016;16:71–81.
12. Magrath I, Steliarova-Foucher E, Epelman S, Ribeiro RC, Harif M, Li CK et al. Pediatric cancer in low-income and middle-income countries. Lancet Oncol. 2013;14:104-16.
13. Larrea AA, Lujan SA, McElhinny SAN, Mieczkowski PA, Resnick MA, Gordenin DA et al. Genome-wide

model for the normal eukaryotic DNA replication fork. Proc Natl Acad Sci U S A. 2010;107:17674-9.
14. Laningham FH, Kun LE, Reddick WE, Ogg RJ, Morris EB, Pui CH. Childhood central nervous system leukemia: historical perspectives, current therapy, and acute neurological sequelae. Neuroradiology. 2007;49:873–88.
15. Pui CH, Campana D, Pei D, Bowman WP, Sandlund JT, Kaste SC et al. Treating childhood acute lymphoblastic leukemia without cranial irradiation. N Engl J Med. 2009;360:2730–41.
16. Manley S, Keenan R, Campbell H, Caswell M, Pizer B. No evidence for routine cerebrospinal fluid cytology in detecting asymptomatic central nervous system relapse in children with acute lymphoblastic leukaemia: 20 years' experience of a UK primary treatment center. Br J Haematol. 2014;164:462-4.
17. Sunamak EÇ, Özdemir N, Koka A, Yantiri L, Apak H, Celkan T. Comparison of outcomes of children with acute lymphoblastic leukemia treated with BMF protocol across 2 decades. Pediatr Hematol Oncol. 2021;38:134-46.
18. Li S, Wang C, Wang W, Liu W, Zhang G. Abnormally high expression of POLD1, MCM2, and PLK4 promotes relapse of acute lymphoblastic leukemia. Medicine. 2018;97: e10734.
19. Wang X, Hu Y, Mo J, Zhang J, Wang Z, Wei W et al. Arsenene: A potential therapeutic agent for acute promyelocytic leukaemia cells by acting on nuclear proteins. Angew Chem Int Ed Engl. 2020;59:5151-8.
20. Liao X, Zou PL, Shen YL, Guo YX, Song L, Xia JW. Second-generation sequencing analysis of Ph+ and Ph-like childhood T-cell acute lymphoblastic leukemia. Zhongguo Shi Yan Xue Ye Xue Za Zhi. 2021;29:1101-8.