

Original Article

Middle East Journal of Cancer 2013; 4(4): 139-143

Prothrombin G20210A Mutation is not a Risk Factor for Pediatric Acute Lymphoblastic Leukemia in Western Iran

Zohreh Rahimi*,***, Ziba Rahimi*, Reza Akramipour***

*Medical Biology Research Center, Kermanshah University of Medical Sciences, Kermanshah, Iran

**Department of Biochemistry, Medical School, Kermanshah University of Medical Sciences, Kermanshah, Iran

***Department of Pediatrics, Kermanshah University of Medical Sciences, Kermanshah, Iran

Abstract

Background: We conducted the present study to investigate the frequency of prothrombin G20210A mutation among acute lymphoblastic leukemia patients and healthy individuals from Western Iran and to detect the possible association between this mutation and the risk of acute lymphoblastic leukemia in our population.

Methods: The studied groups consisted of 92 children with acute lymphoblastic leukemia and 249 age- and sex-matched healthy children from Western Iran. The prothrombin G20210A mutation was identified by PCR-RFLP using the restriction enzyme of Hind III.

Results: The prevalence of prothrombin 20210 GA genotype was 6.5% in patients and 3.2% in controls ($P=0.17$). The frequency of the A allele in patients was 3.3% and in controls it was 1.6%.

Conclusion: The present study indicates the absence of any significant differences in the frequency of the prothrombin G20210A mutation between acute lymphoblastic leukemia patients and healthy individuals. The results suggest that this mutation may not be a risk factor for acute lymphoblastic leukemia in our population.

Keywords: Acute lymphoblastic leukemia, Thromboembolism, Prothrombin G20210A, Western Iran

*Corresponding Author:

Zohreh Rahimi, PhD
Professor of Clinical
Biochemistry, Medical
Biology Research Center,
Medical School, Daneshgah
Avenue, P.O.Box: 67148-
69914, Kermanshah, Iran
Tel: +98-831-4274618-21
Fax: +98-831-4276471
Email: zrahimi@kums.ac.ir,
rahimizus@yahoo.com

Introduction

Acute lymphoblastic leukemia (ALL) is the most common malignancy that comprises around 30% of all pediatric cancers.^{1,2} The five-year survival rate among Iranian children with ALL is 72.5%. Poor prognostic outcome of ALL is

associated with white blood cell counts above 50000/ml.³ ALL has been reported to be a highly prevalent cancer among pediatric patients with leukemia in Kermanshah Province of Iran.⁴ The etiology of ALL is multifactorial, involving both genetic and environmental factors.⁵

Received: April 9, 2013; Accepted: July 9, 2013

An increase in procoagulant factors or a decrease in regulatory factors results in excessive fibrin and thrombus formation.⁶ There is a wide range of thrombosis frequency (0%-36%) among ALL patients that may be due to different definitions of thrombosis, diagnostic methods for its detection, study design, and differences in treatment protocol.^{7,8}

A single nucleotide exchange (G→A) at position 20210 in the 3'-untranslated region of the prothrombin gene in patients with a family history of venous thrombosis has been described by Poort et al.⁹ The presence of this mutation is associated with an approximately 25% increase in plasma thrombin activity. The prevalence of prothrombin G20210A mutation in ALL patients has been studied.¹⁰⁻¹⁵

Previously, we have found a marked difference in the prevalence of factor V Leiden between ALL patients and healthy individuals and have indicated a trend toward increased risk of ALL (2.54-fold) in the presence of this mutation.¹⁶

In the present study the prevalence of prothrombin G20210A mutation and its possible association with ALL among pediatric ALL patients from Western Iran has been investigated.

Subjects and Methods

The sample population consisted of 92 newly diagnosed ALL children (57 males and 35 females) who referred to hematologists of two clinics at Kermanshah University of Medical Sciences. Patients had a mean age of 8.6 ± 4.4 years (1 to 16 years old). Cases were recruited from the files of patients who received hematological diagnoses at the Hematology Unit of Clinics at Kermanshah University of Medical Sciences between June 2002 and July 2011. In patients under treatment, blood samples were drawn during the course of chemotherapy. However, in previously treated patients the blood samples were taken during the follow up period. Controls comprised 249 unrelated healthy individuals that included 140 males and 109 females with a mean age of 9.6 ± 7.2 years (1 to 20 years). The ethnic background of patients and controls was Kurds. ALL patients

were treated according to the UKALL10 protocol.

Informed written consent was obtained from each individual or their parents before participation. The study was approved by the Ethics Committee of Kermanshah University of Medical Sciences and was in accordance with the Principles of the Declaration of Helsinki II.

Genotype analysis

DNA was isolated from leukocytes of EDTA-treated whole blood by using proteinase K treatment followed by phenol-chloroform extraction and ethanol precipitation as previously described.¹⁷

Prothrombin G20210A

A 345-bp fragment from exon 14 and the 3'-untranslated region of the prothrombin gene was amplified using the primers 5' TCT AGA AAC AGT TGC CTG GC 3' and 5' ATA GCA CTG GGA GCA TTG AAG C 3', as described by Poort et al.⁹ Amplification was performed at 94°C for 5 min, followed by 30 cycles at 94°C for 1 min, 55°C for 1 min, 72°C for 1 min, and a final extension period of 5 min at 72°C. A total of 10 to 15 µl of amplified PCR product was digested with 10 units of Hind III restriction enzyme and the resultant digested PCR products were subjected to electrophoresis on a 3% agarose gel. In the presence of mutated allele 20210A a fragment of 322-bp is produced. However in the presence of normal allele G the cleavage site for Hind III is lost and a 345-bp fragment remains intact.

Statistical analysis

The allelic frequencies were calculated by the chromosome counting method. We compared the genotypes and allelic frequency of prothrombin G1691A in patients with controls using the χ^2 test. Odds ratios (OR) were calculated as estimates of relative risk for disease and 95% confidence intervals (CI) obtained by SPSS logistic regression. Statistical significance was assumed at the $P < 0.05$ level. The SPSS statistical software package version 16 was used for statistical analysis.

Table 1. The frequency of prothrombin G20210A genotypes and alleles in acute lymphoblastic leukemia (ALL) patients and controls.

	Patients (n=92)	Controls (n=249)
Prothrombin genotypes		
GG	86 (93.5%)	241 (96.8%)
GA	6 (6.5%)	8 (3.2%)
AA	0 (0%)	0 (0%)
	($\chi^2=1.89$, df=1, $P=0.17$)	
Prothrombin alleles		
G	178 (96.7%)	490 (98.4%)
A	6 (3.3%)	8 (1.6%)
	(* $\chi^2=1.83$, df=1, $P=0.17$)	

*The distribution and comparisons of alleles and genotype frequencies of prothrombin in patients compared with controls were made using the χ^2 test.

Results

We studied 92 ALL patients along with 249 controls for the prothrombin G20210A mutation. The distribution of alleles and genotypes of prothrombin G20210A mutation is depicted in Table 1. The prevalence of prothrombin 20210 GA genotype was 6.5% in patients and 3.2% in controls ($P=0.17$). The frequency of the A allele in patients was 3.3%, whereas in controls it was 1.6%. The distributions of prothrombin G20210A genotypes were not statistically different compared to those in controls (Table 2). Although the presence of the prothrombin 20210GA genotype increased the risk of ALL by 2.1-fold, it was not statistically significant. We monitored patients under treatment for deep vein thrombosis (DVT) during the course of chemotherapy treatment. For those previously treated, we considered approximately a mean five-year follow up to detect the occurrence of DVT. There was no record of any incidence DVT in the files of the study patients.

Discussion

During treatment of ALL patients with asparaginase, circulating anti-thrombin is depleted that results in reduced thrombin inhibitory capacity and increased thrombin generation. The presence of thrombotic complications has been reported in leukemic patients who receive drug treatment.^{10,11,18} Most thrombotic events occur during the initial active phase of the disease (induction phase) due to a more intense treatment.⁷

The prothrombin G20210A mutation is one of the most prevalent known causes of inherited

thrombophilia.¹⁹ The prevalence of this mutation has been shown in pediatric ALL patients in German studies as 2.2%-3.5%.^{13,20} In two separate studies, the prevalence of the prothrombin G20210A mutation has been reported to be 6% and 11% among Israeli pediatric ALL patients.^{10,21} In addition, Stiakaki et al.²² have reported a high prevalence of prothrombin G20210A (14.3%) among Cretan children treated for different malignancies, including ALL.

The association between inherited thrombophilia and increased risk of venous thromboembolism (VTE) in pediatric ALL patients is controversial.¹⁰⁻¹⁵ In some studies the presence of at least one identifiable prothrombotic defect of protein C, protein S and anti-thrombin deficiency in ALL children has been found to be associated with the risk of VTE.^{11,14,15} However, the North American PARKAA study did not detect any correlation between the presence of a prothrombin 20210A mutated allele and the development of thrombosis.¹² A meta-analysis has reported a similar prevalence of genetic prothrombotic abnormalities in ALL patients and the general pediatric population.⁷

In the present study the frequency of the prothrombin 20210 GA genotype and A allele in ALL patients was non-significantly higher than controls. We did not find any report of thromboembolism in patients' files. It seemed that this mutation might not be associated with the risk of ALL in our population.

Since the role of inherited thrombophilia in childhood ALL is controversial, universal

Table 2. The odds ratio (OR) of prothrombin G20210A genotypes and alleles with respect to GG genotype or G allele, respectively in acute lymphoblastic leukemia (ALL) patients.

	Patients OR (95% CI)	Controls
Prothrombin genotypes		
GG	Reference group (n=86)	Reference group (n=241)
GA	2.1 (0.71-6.2, P=0.18, n=6)	n=8
Prothrombin alleles		
G	Reference group (n=178)	Reference group (n=490)
A	2.07 (0.71-6.1, P=0.18, n=6)	n=8

thrombophilia screening is not justified.

Conclusion

The present study indicates the absence of a significant difference in the frequency of prothrombin G20210A between ALL patients and healthy individuals and suggests this mutation may not be a risk factor for ALL in our population.

Acknowledgments

This work was financially supported by a grant from the Vice Chancellor for Research at Kermanshah University of Medical Sciences, Kermanshah, Iran.

References

- Azhar MA, Rahimi Z, Vaisi-Raygani MA, Akramipour R, Madani H, Rahimi Z, et al. Lack of association between MTHFR C677T and A1298C polymorphisms and risk of childhood acute lymphoblastic leukemia in the Kurdish population from Western Iran. *Genet Test Mol Biomarkers* 2012;16(3):198-202.
- Rahimi Z, Ahmadian Z, Akramipour R, Madani H, Mozafari H, Vaisi-Raygani A, et al. Thymidilate synthase and methionine synthase polymorphisms in children with acute lymphoblastic leukemia in Western Iran. *International J Hematol Oncol and Stem Cell Res* 2010;4:9-12.
- Karimi M, Yarmohammadi H, Sabri MR. An analysis of prognostic factors and the five-year survival rate in childhood acute lymphoblastic leukemia. *Med Sci Monit* 2002;8(12):CR792-6.
- Chanideh I. MD Thesis: The epidemiology of malignant diseases in children below 15 years old in two hospitals of Razi and Taleghani in Kermanshah Province (1995-2004) (In Persian) 2006.
- Alcasabas P, Ravindranath Y, Goyette G, Haller A, Del Rosario L, Lesaca-Medina MY, et al. Methyl-enetetrahydrofolate reductase (MTHFR) polymorphisms and the risk of acute lymphoblastic leukemia (ALL) in Filipino children. *Pediatr Blood Cancer* 2008;51(2):178-82.
- Payandeh M, Zare ME, Mansouri K, Rahimi Z, Hashemian AH, Soltanian E, et al. Protein C and S deficiency in deep vein thrombosis patients referred to Iranian blood transfusion organization, Kermanshah. *International J Hematol Oncol and Stem Cell Res* 2011;5:5-8.
- Caruso V, Iacoviello L, Di Castelnuovo A, Storti S, Mariani G, de Gaetano G, et al. Thrombotic complications in childhood acute lymphoblastic leukemia: A meta-analysis of 17 prospective studies comprising 1752 pediatric patients. *Blood* 2006; 108(7):2216-22.
- Nowak-Gottl U, Kenet G, Mitchell LG. Thrombosis in childhood acute lymphoblastic leukaemia: Epidemiology, aetiology, diagnosis, prevention and treatment. *Best Pract Res Clin Haematol* 2009; 22(1):103-14.
- Hoorn SR, Rosendaal FR, Reitsma PH, Bertina RM. A common genetic variation in the 3'-untranslated region of the prothrombin gene is associated with elevated plasma prothrombin levels and an increase in venous thrombosis. *Blood* 1996;88(10):3698-703.
- Elhasid R, Lanir N, Sharon R, Weyl Ben Arush M, Levin C, Postovsky S, et al. Prophylactic therapy with enoxaparin during L-asparaginase treatment in children with acute lymphoblastic leukemia. *Blood Coagul Fibrinolysis* 2001;12(5):367-70.
- Nowak-Gottl U, Wermes C, Junker R, Koch HG, Schobess R, Fleischhacker G, et al. Prospective evaluation of the thrombotic risk in children with acute lymphoblastic leukemia carrying the MTHFR TT 677 genotype, the prothrombin G20210A variant, and further prothrombotic risk factors. *Blood* 1999; 93(5):1595-9.
- Mitchell LG, Andrew M, Hanna K, Abshire T, Halton J, Anderson R, et al. A prospective cohort study determining the prevalence of thrombotic events in children with acute lymphoblastic leukemia and a central venous line who are treated with L-asparaginase: Results of the Prophylactic Antithrombin Replacement in Kids with Acute Lymphoblastic

- Leukemia Treated with Asparaginase (PARKAA) Study. *Cancer* 2003;97(2):508-16.
13. Nowak-Gottl U, Ahlke E, Fleischhack G, Schwabe D, Schobess R, Schumann C, et al. Thromboembolic events in children with acute lymphoblastic leukemia (BFM protocols): Prednisone versus dexamethasone administration. *Blood* 2003;101(7):2529-33.
 14. Wermes C, von Depka Prondzinski M, Lichtinghagen R, Barthels M, Welte K, Sykora KW. Clinical relevance of genetic risk factors for thrombosis in pediatric oncology patients with central venous catheters. *Eur J Pediatr* 1999;158(Suppl.3): S143-6.
 15. Wermes C, Fleischhack G, Junker R, Schobess R, Schwabe D, Sykora KW, et al. Cerebral venous sinus thrombosis in children with acute lymphoblastic leukemia carrying the MTHFR TT677 genotype and further prothrombotic risk factors. *Klin Padiatr* 1999;211(4):211-4.
 16. Rahimi Z, Rahimi Z, Akramipour R, Mozafari H, Yari K, Golpaygani MR, et al. Association of factor V Leiden mutation with pediatric acute lymphoblastic leukemia in Kermanshah Province. *International J Hematol Oncol and Stem Cell Res* 2012;6:26-31.
 17. Rahimi Z, Felehgari V, Rahimi M, Mozafari H, Yari K, Vaisi-Raygani A, et al. The frequency of factor V Leiden mutation, ACE gene polymorphism, serum ACE activity and response to ACE inhibitor and angiotensin II receptor antagonist drugs in Iranians type II diabetic patients with microalbuminuria. *Mol Biol Rep* 2011;38(3):2117-23.
 18. Payne JH, Vora AJ. Thrombosis and acute lymphoblastic leukaemia. *Br J Haematol* 2007; 138(4): 430-45.
 19. Rahimi Z, Nomani H, Mozafari H, Vaisi-Raygani, Madani H, Malek-Khosravi S, et al. Factor V G1691A, prothrombin G20210A and methylenetetrahydrofolate reductase C677T polymorphism are not associated with coronary artery disease and type 2 diabetes mellitus in Western Iran. *Blood Coagul Fibrinolysis* 2009;20(4):252-6.
 20. Nowak-Gottl U, Heinecke A, von Kries R, Nurnberger W, Nicole Munchow N, Junker R, et al. Thrombotic events revisited in children with acute lymphoblastic leukemia: Impact of concomitant Escherichia coli asparaginase/prednisone administration. *Thromb Res* 2001;103(3):165-72.
 21. Harley D, Zaidman I, Sarig G, Weyl Ben Arush M, Brenner B, Elhasid R. Prophylactic therapy with enoxaparin in children with acute lymphoblastic leukemia and inherited thrombophilia during L-asparaginase treatment. *Thromb Res* 2010;126(2):93-7.
 22. Stiakaki E, Germanakis I, Sfyridaki C, Katzikis N, Danilatou V, Kalmanti M. Prevalence of factor V Leiden and other thrombophilic traits among Cretan children with malignancy. *Pediatr Blood Cancer* 2005; 44(4):386-9.