Association of Factor V Leiden Mutation with Pediatric Acute lymphoblastic Leukemia in Kermanshah Province

Zohreh Rahimi PhD [1,2*], Ziba Rahimi BSc [1], Reza Akramipour MD [3], Hadi Mozafari MSc [1], Kheirollah Yari MSc [1], Mohammad-Reza Golpaygani MD[3], Ali Shahriari-Ahmadi MD[4]

[1] Medical Biology Research Center, Kermanshah University of Medical Sciences, Kermanshah, Iran
[2] Department of Biochemistry, Medical School, Kermanshah University of Medical Sciences, Kermanshah, Iran
[3] Department of Pediatrics, Kermanshah University of Medical Sciences, Kermanshah, Iran
[4] Department of Hematology Oncology, Medical School, Iran University of Medical Sciences, Tehran, Iran

***Corresponding author:** Zohreh Rahimi, Ph.D, Associate Professor of Biochemistry, Medical Biology Research Center, Medical School, Daneshgah Avenue, Kermanshah, Iran P.O.Box: 67148-69914
Tel: 0098-831-4274618-21
Fax: 0098-831-4276471
E-mail: zrahimi@kums.ac.ir, rahimizus@yahoo.com

Abstract

Introduction: Acute lymphoblastic leukemia (ALL) is the most common pediatric malignancy. The risk of thrombophilia increases in patients with ALL during chemotherapy. The present study aimed to investigate the frequency of factor V Leiden (FVL) mutation in children with acute lymphoblastic leukemia and its possible association with ALL.

Patients and Methods: We studied 92 patients with ALL and 249 healthy individuals from Kermanshah Province of Iran. Detection of FVL mutation was performed by PCR-RFLP using restriction enzymes of Mnl I.

Results: The frequency of FVL G1691A polymorphism was 7.8% in patients compared to 3.2% in controls (p=0.052). There was a trend towards increased risk of ALL in the presence of FVL mutation [OR=2.54, 95% CI 0.9-7.2, p=0.08].

Conclusions: Our results indicated that the frequency of both thrombophilic mutation of FVL was higher in ALL patients from Kermanshah province compared to healthy individuals and FVL mutation tended to be associated with the increased risk of ALL. Further studies needed to evaluate the association between FVL mutation and the occurrence of thromboembolism in ALL patients.

Key words: Acute lymphoblastic leukemia, Thromboembolism, Factor V Leiden, Kermanshah province

Received: 19, Apr, 2012
Accepted: 15, May, 2012

Introduction

Acute lymphoblastic leukemia (ALL) is the most common malignancy of childhood. The blockage of lymphoid cell development in any stage, leads to ALL (1, 2). In Iranian children with ALL five-year survival rate of 72.5% has been reported with an association between white blood cell counts above 50000/ml and poor prognostic outcome (3). Studying cancers in children indicated a high prevalence of leukemia (49.2%) with the highest frequency of ALL (38.2%) among children in the Kermanshah Province of Iran (4). Treatment of ALL patients with prothrombotic drug of L-asparaginase, alone or in combination with vincristine, prednisone and anthracyclines have induced antithrombin III deficiency and increased the risk of thrombotic events (5).

Factor V G1691A mutation, deficiencies of protein C, protein S, antithrombin, and elevated lipoprotein a and von Willebrand factor (VWF) concentrations are associated with venous thromboembolism in pediatric patients with ALL treated according to the BFM 90/95 protocols (60 mg/m^2 prednisone) (6).

The frequency of thrombosis in ALL patients has been reported from 0 to 36%, these large variations could be attributed to different definitions of thrombosis (symptomatic vs. asymptomatic), diagnostic methods for its detection, study design (prospective vs. retrospective), and differences in treatment protocol (7, 8).

The factor V Leiden (FVL) polymorphism is caused by a single point mutation at nucleotide1691, leading to an Arg/Gln amino acid exchange at position 506. This mutation represents one of the most important risk factors for inherited thrombophilia (9).

The frequency of FVL mutation in ALL patients has been reported in few studies (5, 6, 10-13).

Previously, we have reported a prevalence of 2.97% for FVL mutation among normal population of Western Iran (14). We demonstrated an association between FVL and deep venous thrombosis and cerebral venous and sinus thrombosis in our population (15, 16). Also, we observed an association between this mutation and sickle cell disease (17). However, we indicated that the FVL mutation was not associated with microalbuminuria and G6PD deficiency in Western Iran (18, 19).

The aim of present study was to investigate the prevalence of thrombotic risk factor of FVL and its possible association with ALL among pediatric ALL patients from Kermanshah Province of Iran.

Materials and Methods

Patients consisted of 92 children diagnosed with ALL referred to the hematologists of two clinics of Kermanshah University of Medical Sciences with the mean age of 8.64±4.43 years (1 to 16 years old) including 57 males and 35 females newly diagnosed with ALL according to French-American-British classification. Cases were recruited from the files of patients who received hematological diagnosis by the Hematology Unit of clinics of Kermanshah University of Medical Sciences in the period between June 2002 and July 2011. Two hundred and forty nine unrelated healthy individuals with the mean age of 9.6±7.2 years (1 to 20 years) including 140 males and 109 females were studied as controls. Both groups were from Kermanshah Province of Iran with Kurdish ethnic background. ALL patients treated according to UKALL10 protocol. According to this protocol during induction phase patients received E-coli L-asparaginase 6000U/m^2 at two day intervals for nine doses, daily prednisone (40 mg/ m^2) for 28 days (days 1-29), weekly vincristine (1.5 mg/m^2) for 5 weeks, daily adriamycine (45 mg/m^2) for 2 days, intrathecal methotrexate (adjusted dose for age) on days 1, 15, 29, 36, 43, 50. The patients had bone marrow aspiration 3 times on days 1, 15, 29 post treatment. In re-induction (intensification) phase patients received vincristine (1.5 mg/m^2) in the first day of therapy, daily prednisone (40 mg/ m^2) for 7 days, daily adriamycine (45 mg/m^2) for 2 days, intrathecal methotrexate on the first day of phase and on weeks 5 and 20, twice a day cytosar (100 mg/ m^2) for 5 days, etoposide (100 mg/ m^2) for 5 days and thioguanine (75 mg/m^2) for 5 days. The patients had bone marrow aspiration in the first day of therapy. In the maintenance phase which prolongs 3 to 3.5 years children received, daily 6-mercaptopurine (50 mg/ m^2), weekly methotrexate (20 mg/ m^2), prednisone (40 mg/ m^2) at 4 week intervals for 5 days and vincristine (1.5 mg/m^2) at 4 week intervals.

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.

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 (20).

Genotype Analysis

The FVL G1691A mutation was detected by the amplification of a 267-bp fragment in exon 10 of the factor V using primers 5' TGC CCA GTG CTT AAC AAG ACC A 3' and 5' TGT TAT CAC ACT GGT GCT AA 3'. Amplification was carried out for 30 cycles at 94°C for 1 min, 52°C for 1 min, 72°C for 1 min, with a final extension period of 5 min at 72°C. About 15 µl of amplified polymerase chain reaction (PCR) product was digested with 1.5 units Mnl I restriction enzyme and was subjected to electrophoresis on a 2% agarose gel. In the presence of G allele, the 267-bp fragment is digested by the Mnl I restriction enzyme to three fragments (163-bp, 67-bp and 37-bp). The G→A mutation abolishes a restriction site and produces only two fragments, of 200-bp and 67-bp (21).

Statistics

The allelic frequencies were calculated by the chromosome counting method. The genotypes and allele frequencies of FVL mutation in patients were compared to controls using 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 the statistical analysis.

Results

The characteristics of patients and healthy individuals are demonstrated in Table 1.

Table 1. Characteristics of patients and controls.

Parameters	Patients Mean±SD (n=92)	Controls Mean±SD (n=249)	P value
Age (years)	8.64±4.43	9.6±7.2	>0.05
Gender **Male** **Female**	 57 35	 140 109	>0.05
Hb (mg/dl)	11.5±2.2	11.8±2.15	>0.05
RBC ($\times10^{12}$/l)	4.3±1.4	4.6±0.7	>0.05
WBC ($\times10^{9}$/l)	4.5±2.2	7.3±2.8	<0.05

The mean white blood cells (WBC) counts in patients and controls were $4.5\pm2.2\times10^9$/l and $7.3\pm2.8\times10^9$/l ($p<0.05$), respectively. The wild type genotype (GG) of FVL was present in 83 out of 90 studied patients (92.2%), heterozygous genotype (GA) was found in 7 patients (7.8%). However, no homozygous genotype (AA) was detected in patients. In healthy individuals there were 241 subjects (96.8%) with GG genotype, 6 individuals (2.4%) with AG genotype, and 2 subjects (0.8%) had AA genotype [$\chi^2=5.84$, df=2, p=0.052] (Table 2). As indicated in Table 2 the frequency of mutant allele of 1691 A was tended to be higher (3.9%) in patients compared to that in controls (2%). The frequency of GA+AA genotype of FVL was found to be 7.8% in patients compared to 3.2% in controls [$\chi^2=3.3$, df=1, p=0.071] (Table 2).

Table 3 indicates that the mutant allele 1691A was associated with around 2-fold increased risk of ALL that did not reach to a statistical significant. Also, the presence of GA+AA genotype compared to GG genotype increased the risk of ALL by 2.54-fold [OR=2.54, 95% CI 0.9-7.2, p=0.08] (Table 3).

Table 2. The distribution of factor V Leiden genotypes and alleles in leukemia patients compared with control subjects.

Genotypes and alleles	Patients (n=92)	Controls (n=249)
FVL genotypes **GG** **GA** **AA**	 83 (92.2%) 7 (7.8%) 0	 241 (96.8%) 6 (2.4%) 2 (0.8%)
	*($\chi^2=5.84$, df=2, p=0.052)	
GA+AA	7 (7.8%)	8 (3.2%)
	*($\chi^2=3.3$, df=1, p=0.071)	
FVL alleles **G** **A**	 173 (96.1%) 7 (3.9%)	 488 (989%) 10 (2%)
	*($\chi^2=1.9$, df=1, p=0.16)	

Table 3. The odds ratio of factor V Leiden genotypes and alleles with respect to GG genotype or G allele, respectively in leukemia patients.

	Patients ORs (95% CI)	Controls
FVL genotypes GG GA+AA	 Reference group (n=83) 2.54 (0.9- 7.2, p=0.08, n=7)	 Reference group (n=241) n=8
FVL alleles G A	 Reference group (n=173) 1.98 (0.75-5.3, p=0.17, n=7)	 Reference group (n=488) n=10

Discussion

Thrombophilia is caused by inherited defects, protein deficiencies or dysfunction of hemostatic system (6). The presence of thrombotic complications has been reported in

leukemic patients receiving drug intravenously and by intramascular injection due to increased thrombin generation at diagnosis combined with reduced thrombin inhibitory capacity due to depletion of circulating anti-thrombin (AT) by asparaginase (5, 6, 22).

The mechanism of increased risk of venous thromboembolism (VTE) is associated with alterations in the haemostatic system by use of L-asparaginase alone or in combination with vincristine or prednisone during induction, early insertion of central venous catheters and inherited thrombophilia (8, 22). Most of the thrombotic events have occurred during the initial active phase of disease (induction phase) due to the more intense treatment (7).

The mutation in factor V Leiden G1691A is the most prevalent known cause of inherited thrombophilia (23). The prevalence of FVL mutation in children with ALL from two German studies was 4.4 and 2.2%, respectively (13, 24). In two separate studies from Israel frequencies of FVL were 12 and 18.5%, respectively (5, 25). In addition, Stiakaki et al. (26) have reported a high prevalence of thrombophilic mutations of FVL (19.4%) among Cretan children treated for different malignancies including ALL.

There are conflicting reports related to the association between inherited thrombophilia and increased VTE in pediatric ALL patients (5, 6, 10-13). An association between thromboembolism and the heterozygous FVL mutation in leukemic children treated according to the BFM protocols has been indicated (10). Also, a multicenter prospective study of pediatric ALL patients from Germany indicated that the presence of at least one identifiable prothrombotic defect of protein C, protein S and antithrombin (AT) deficiency in ALL children were associated with the greatest risk of VTE (6) Their study revealed that patients with multiple defects had a significantly higher risk of VTE compared with those with a single defect ($p = 0.009$). It was confirmed in similar study (14). In contrast to these studies, the North American PARKAA study failed to show any correlation between the presence of FVL mutation and the development of thrombosis (11). Caruso et al. (7) in a meta-analysis reported similar prevalence of genetic prothrombotic abnormalities in ALL patients and general pediatric population. However, pooling studies demonstrated that inherited thrombophilia increased the risk of VTE in ALL patients by approximately 8.5-fold (7).

Our study indicated that the frequency of FVL was higher in leukemic patients compared to controls and there was a trend towards increased risk of ALL in the presence of FVL mutation (2.54-fold). We did not find any report of occurrence thromboembolism in the files of patients. However, it might that the occurrence of cerebral venous and sinus thrombosis in our patients had not been diagnosed. It seems that the presence of thrombophilic mutations might not affect the risk of venous thromboembolism in our pediatric ALL patients.

Due to inconsistent reports related to the role of inherited thrombophilia in childhood ALL, universal thrombophilia screening is not justified. However, in the presence of confirmed family history of one of the higher risk prothrombotic defects, such as anti thrombin, protein C or protein S deficiency, directed screening to do replacement therapy during high risk periods of treatment has been suggested (22). Some studies have suggested prophylactic treatment with low molecular weight heparin enoxaparin or infusions of antithrombin III concentrates (27) in the preventing of thrombotic events during L-asparaginase therapy (5).

Conclusion

Our results, the first study in Iran, indicated that the frequency of FVL mutation was higher in ALL patients compared to healthy individuals and the FVL mutation tended to be associated with the increased risk of ALL in our population. Related to the role of inherited thrombophilia on the increase risk of thromboembolism further studies should be conducted to evaluate the association between FVL mutation and the occurrence of thromboembolism in large samples of ALL patients from our population.

Acknowledgments

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

References

1. Rahimi Z, Ahmadian Z, Akramipour R, Madani H, Mozafari H, Vaisi-Raygani A, Shahriari-Ahmadi A. Thymidilate synthase and methionine synthase polymorphisms in children with acute lymphoblastic leukemia in Western Iran. International J Hematol Oncol & Stem Cell Res 2010: 4: 9-12
2. Moradi A, Semnani S, Roshandel G, Mirbehbehani N, Keshtkar A, Aarabi M, et al. Incidence of childhood cancers in Golestan Province of Iran. Iran J Pediatr 2010; 20: 335-342
3. 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: CR792-796
4. 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) (Persian), 2006
5. 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: 367-370
6. Nowak-Gottl U, Wermes C, Junker R, Koch HG, Schobess R, Fleischhack 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: 1595-1599
7. 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, 2216–2222
8. 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: 103–114
9. Bertina RM, Koeleman BPC, Koster T, Rosendaal FR, Dirven RJ, de Ronde H, et al. Mutation in blood coagulation factor V associated with resistance to activated protein C. Nature 1994; 369:64–67
10. Nowak-Gottl U, Aschka I, Koch HG, Boos J, Dockhorn- Dworniczak B, Deufel T, et al. Resistance to activated protein C (APCR) in children with acute lymphoblastic leukaemia—the need for a prospective multicentre study. Blood Coagul Fibrinolysis 1995; 6:761-764
11. 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, 508–516
12. 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: 2529-2533
13. 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–S146
14. Rahimi Z, Vaisi-Raygani A, Mozafari H, Kharrazi H, Rezaei M, Nagel RL. Prevalence of factor V Leiden (G1691A) and prothrombin (G20210A) among Kurdish population from Western Iran. J Thromb Thrombolysis. 2008, 25: 280-283.
15. Rahimi Z, Mozafari H, Shariari-Ahmadi A, Alimogaddam K, Ghavamzadeh A, Aznab M, et al. Deep venous thrombosis and thrombophilic mutations in Western Iran: association with factor V Leiden. Blood Coagul Fibrinolysis. 2010; 21: 385-388
16. Rahimi Z, Mozafari H, Amiri Bigvand AH, Doulabi RM, Vaisi-Raygani A, Afshari D, et al. Cerebral venous and sinus thrombosis and thrombophilic mutations in Western Iran: association with factor V Leiden. Clin Appl Thromb Hemost. 2010; 16: 430-434
17. Rahimi Z, Vaisi-Raygani A, Nagel RL, Muniz A. Thrombophilic mutations among Southern Iranian patients with sickle cell disease: High prevalence of factor V Leiden. J Thromb Thrombolysis. 2008, 25: 288-292
18. 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: 2117-2123
19. Mozafari H, Rahimi Z, Heidarpour A, Fallahi M, Muniz A. The prevalence of factor V Leiden, prothrombin G20210A and methylenetetrahydrofolate reductase C677T among G6PD deficient individuals from Western Iran. Mol Biol Rep. 2009, 36: 2361-2364.
20. Rahimi Z, Muniz A, Parsian A. Detection of responsible mutations for beta thalassemia in the Kermanshah Province of Iran using PCR-based techniques. Mol Biol Rep. 2010; 37: 149-154
21. 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: 2117-2123
22. Payne JH, Vora AJ. Thrombosis and acute lymphoblastic leukaemia. Br J Haematol 2007; 138: 430-445
23. Rahimi Z, Nomani H, Mozafari H, Vaisi-Raygani, Madani H, Malek-Khosravi Sh, 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: 252-256

24. 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; 103:165–172
25. Harlev 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. Thrombosis Res 2010; 126: 93-97
26. Stiakaki E, Germanakis I, Sfyridaki C, Katzilakis N, Danilatou V, Kalmanti M. Prevalence of factor V Leiden and other thrombophilic traits among Cretan children with malignancy. Pediatr Blood Cancer 2005; 44: 386–389
27. Mitchell L, Hoogendoorn H, Giles AR, Vegh P, Andrew M. Increased endogenous thrombin generation in children with acute lymphoblastic leukemia: risk of thrombotic complications in L-asparginase induced antithrombin III deficiency. Blood 1994; 83: 386-391