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

Zohreh Rahimi PhD 1,2 1, Ziba Rahimi BSc 1, Reza Akramipour MD 1, Hadi Mozafari MSc 1, Kheirollah Yari MSc 1, Mohammad-Reza Golpaygani MD3, Ali Shahriari-Ahmadi MD4

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

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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/m² 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 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/m² at two day intervals for nine doses, daily prednisone (40 mg/ m²) for 28 days (days 1-29), weekly vincristine (1.5 mg/m²) for 5 weeks, daily adriamycine (45 mg/m²) 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/m²) in the first day of therapy, daily prednisone (40 mg/ m²) for 7 days, daily adriamycine (45 mg/m²) for 2 days, intrathecal methotrexate on the first day of phase and on weeks 5 and 20, twice a day cytosar (100 mg/ m²) for 5 days, etoposide (100 mg/ m²) for 5 days and thioguanine (75 mg/m²) 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/ m²), weekly methotrexate (20 mg/ m²), prednisone (40 mg/ m²) at 4 week intervals for 5 days and vincristine (1.5 mg/m²) 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 $\chi^2$ 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.

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 1691A 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 1. Characteristics of patients and controls.**

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Patients Mean±SD (n=92)</th>
<th>Controls Mean±SD (n=249)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>8.6±4.43</td>
<td>9.6±7.2</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>Gender</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>57</td>
<td>140</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>Female</td>
<td>35</td>
<td>109</td>
<td></td>
</tr>
<tr>
<td>Hb (mg/dl)</td>
<td>11.5±2.2</td>
<td>11.8±2.15</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>RBC ($\times10^12/l$)</td>
<td>4.3±1.4</td>
<td>4.6±0.7</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>WBC ($\times10^9/l$)</td>
<td>4.5±2.2</td>
<td>7.3±2.8</td>
<td>&lt;0.05</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Genotypes and alleles</th>
<th>Patients (n=92)</th>
<th>Controls (n=249)</th>
</tr>
</thead>
<tbody>
<tr>
<td>FVL genotypes</td>
<td></td>
<td></td>
</tr>
<tr>
<td>GG</td>
<td>83 (92.2%)</td>
<td>241 (96.8%)</td>
</tr>
<tr>
<td>GA</td>
<td>7 (7.8%)</td>
<td>6 (2.4%)</td>
</tr>
<tr>
<td>AA</td>
<td>0</td>
<td>2 (0.8%)</td>
</tr>
</tbody>
</table>

*($\chi^2=5.84$, df=2, $p=0.052$)

| GA+AA                 | 7 (7.8%)       | 8 (3.2%)        |

*($\chi^2=3.3$, df=1, $p=0.071$)

<table>
<thead>
<tr>
<th>FVL alleles</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>G</td>
<td>173 (96.1%)</td>
<td>488 (989%)</td>
</tr>
<tr>
<td>A</td>
<td>7 (3.9%)</td>
<td>10 (2%)</td>
</tr>
</tbody>
</table>

*($\chi^2=1.9$, 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.**

<table>
<thead>
<tr>
<th>Genotypes ORs (95% CI)</th>
<th>Controls</th>
</tr>
</thead>
<tbody>
<tr>
<td>FVL genotypes</td>
<td></td>
</tr>
<tr>
<td>GG</td>
<td>Reference group (n=83)</td>
</tr>
<tr>
<td>GA+AA</td>
<td>2.54 (0.9-7.2, p=0.08, n=7)</td>
</tr>
<tr>
<td>FVL alleles</td>
<td></td>
</tr>
<tr>
<td>G</td>
<td>Reference group (n=173)</td>
</tr>
<tr>
<td>A</td>
<td>1.98 (0.75-5.3, p=0.17, n=7)</td>
</tr>
</tbody>
</table>

**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 intramuscular 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

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