Inherited prothrombotic risk factors in children with hereditary angioedema

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Abstract
Aim: Hereditary angioedema is characterized with recurrent mucocutaneous angioedema, abdominal pain, edema of larynx and extremities. Dermal vascular thrombosis and systemic coagulation may occur in patients with hereditary angioedema due to inhibition of activated factor XII, thrombin and plasmin. Aim of this study was to screen patients with HAE for prothrombotic genetic risk factors before treatment.

Material and Methods: Ten patients with hereditary angioedema who were followed up at our clinic were included in our study. The type and frequency of attack, use of prophylaxis and family history of hereditary angioedema were questioned and prothrombotic risk factors were studied.

Results: Among the 10 included patients, five of them were male (50%) and five were female (50%). Four patients had abdominal edema (40%), four patients had edema of hands, feet and face (40%). One patient (10%) had heterozygous factor V G1691A mutation, another one had also heterozygous protrombin G20210A mutation. The heterozygous methylene tetrahydrofolate reductase (MTHFR) mutation were identified in seven patients (70%) and homozygous MTHFR mutation were found two patients (20%).

Conclusion: In patients with hereditary angioedema, evaluation of prothrombotic risk factors was crucial to estimate attack frequency-severity and treatment related thrombosis risk.

Keywords: Hereditary angioedema; prothrombotic risk factors; C1 inhibitor; thrombosis.

INTRODUCTION
Hereditary angioedema (HAE) is a rare and recurrent, life-threatening type of angioedema. It has autosomal dominant inheritance and the estimated incidence is 1/10000 to 1/150000, with equal occurrence in both genders. Typical symptoms of the disorder include recurrent mucocutaneous angioedema, abdominal pain and asphyxia due to laryngeal edema (1,2). Erythema marginatum can be observed during prodromal period (1,3). Angioedema primarily involves the extremities, oropharynx and visceral organs. Two phenotypic variants have been described. Type I is characterized by a quantitative decrease in C1 esterase inhibitor (C1-INH), which results in diminished functional activity (C1-INH-HAE type I), and type II is characterized by normal or high levels of C1-INH, which is dysfunctional (4,5).

In C1 inhibitor deficiency, complement, kinin-bradykinin, coagulation and fibrinolytic systems are activated beyond control. As a result, increased vascular permeability, submucosal and subcutaneous angioedema develops. In addition to edema, C1-INH deficiency may cause vascular thrombosis and systemic coagulation as well (6). Based on predisposition for thrombosis in patients with HAE and the potential effects of C1 inhibitors used in treatment or danazol in prophylaxis of HAE on increased thrombosis risk, aim of this study was to screen patients with HAE for prothrombotic genetic risk factors before treatment.

MATERIAL and METHODS
The study was launched upon 2017/122 numbered
approval of Ethics Committee. The study included 10 patients with HAE who were under follow-up in Department of Pediatric Immunology and Allergy. The patients diagnosis was made according to the recently published international consensus algorithm for the diagnosis, therapy and management of HAE (7).

Following Ethics Committee approval, written consent for participation in the study was obtained from patients and their legal guardians on a volunteer basis.

Detailed patient history, age, gender, presence of blood relation between parents, C4 and C1-INH levels as diagnosis were recorded. C1-INH, and C4 levels were measured by nephelometric method using a Siemens BN2 device produced in Germany in 2013. Additional information on attack frequency, attack form, history of receiving prophylaxis between attacks and family history of hereditary angioedema were collected. Patients were screened for genetic mutations of prothrombotic risk factors including Factor V Leiden- Prothrombin G20210A-Methylene Tetrahydrofolate Reductase (MTHFR) and Plasminogen Activator Inhibitor (PAI). 2 ml peripheral bloods were taken with EDTA tube and genomic DNA isolation was performed by Roche Manga Pure LC DNA isolation kit. DNA concentration sad justed as it was 10ng/µl.

Factor II (prothrombin) (G20210A), factor V (G1691A), MTHFR (C677T and A1298C), factor XIII (V34L), PAI-1 (4G/5G) mutations were studied with multiplex PCR method with GML company’s CVD certificated SNP panel kit (Switzerland). Each patient’s 10µl PCR product was run on the (ABI 3500) (Applied Biosystems) sequencing device and results were obtained by fragment analysis in Gene Mapper_5 Soft Ware program.

Table 1. Demographic characteristics of the patients

<table>
<thead>
<tr>
<th>Patients</th>
<th>Age (Months)</th>
<th>Gender</th>
<th>C4 Level (N:16-38 mg/dl)</th>
<th>C1-INH Level (N: 210-345 mg/L)</th>
<th>Attack freq/w</th>
<th>Attack form</th>
<th>Family history</th>
<th>Prophylaxis</th>
<th>Factor V mutation</th>
<th>Prothrombin G20210A mutation</th>
<th>MTHFR mutation</th>
<th>PAI mutation</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>135</td>
<td>M</td>
<td>3.58</td>
<td>42.1</td>
<td>1/months</td>
<td>Abdomen</td>
<td>Sibling</td>
<td>(-)</td>
<td>N</td>
<td>N</td>
<td>Het</td>
<td>Het</td>
</tr>
<tr>
<td>2</td>
<td>75</td>
<td>M</td>
<td>3.34</td>
<td>48.4</td>
<td>1/2-3 months</td>
<td>Abdomen</td>
<td>Sibling</td>
<td>(-)</td>
<td>N</td>
<td>N</td>
<td>Het</td>
<td>Het</td>
</tr>
<tr>
<td>3</td>
<td>205</td>
<td>F</td>
<td>2.15</td>
<td>29.9</td>
<td>1/month</td>
<td>Abdomen</td>
<td>Sibling</td>
<td>(-)</td>
<td>N</td>
<td>N</td>
<td>Het</td>
<td>Het</td>
</tr>
<tr>
<td>4</td>
<td>129</td>
<td>M</td>
<td>5.42</td>
<td>43.3</td>
<td>None</td>
<td>-</td>
<td>Sibling</td>
<td>(-)</td>
<td>N</td>
<td>N</td>
<td>Het</td>
<td>N</td>
</tr>
<tr>
<td>5</td>
<td>207</td>
<td>M</td>
<td>5.42</td>
<td>45.3</td>
<td>None</td>
<td>-</td>
<td>Sibling</td>
<td>(-)</td>
<td>N</td>
<td>N</td>
<td>Het</td>
<td>Het</td>
</tr>
<tr>
<td>6</td>
<td>84</td>
<td>F</td>
<td>2.94</td>
<td>41.7</td>
<td>1/week</td>
<td>Hand-feet-face</td>
<td>None</td>
<td>(-)</td>
<td>N</td>
<td>N</td>
<td>Het</td>
<td>N</td>
</tr>
<tr>
<td>7</td>
<td>170</td>
<td>F</td>
<td>7.0</td>
<td>90</td>
<td>1/week</td>
<td>Abdomen</td>
<td>Mother/Aunt</td>
<td>(-)</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>Hom</td>
</tr>
<tr>
<td>8</td>
<td>148</td>
<td>F</td>
<td>4.85</td>
<td>36.0</td>
<td>1/1-2 weeks</td>
<td>Hand-feet-face</td>
<td>Mother</td>
<td>(-)</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>Hom</td>
</tr>
<tr>
<td>9</td>
<td>210</td>
<td>F</td>
<td>5.88</td>
<td>79.9</td>
<td>1/2 weeks</td>
<td>Hand-feet-face</td>
<td>Father</td>
<td>(-)</td>
<td>N</td>
<td>N</td>
<td>Het</td>
<td>N</td>
</tr>
<tr>
<td>10</td>
<td>156</td>
<td>M</td>
<td>6.55</td>
<td>44.4</td>
<td>1/month</td>
<td>Abdomen</td>
<td>Mother/Aunt</td>
<td>(-)</td>
<td>Het</td>
<td>Het</td>
<td>Het</td>
<td>N</td>
</tr>
</tbody>
</table>


** Statistical Analysis

Statistical Analysis

SPSS (SPSS for Windows, Version 17.0, SPSS Inc, U.S.A) program was used for statistical analysis. Variables of qualitative data were given as number and percentage while data of quantitative variables were given as median (±SD).

RESULTS

Ten patients- 5 males (50%) and 5 females (50%) were enrolled in the study. Mean age was 151.90 ± 48.21 months (range: 75-210 months). Three of the patients (2 male, 1 female) and another two (2 male) were siblings. None of the parents had blood relation. At the time of diagnosis, mean C4 level was 4.71±1.62 mg/dl and mean C1 esterase inhibitor level was 50.10± 19.22 mg/dl. Attack frequency was once monthly in 3 (30%), once in 1-2 weeks in 4 (40%) and once in 2-3 months in 1 (10%) of the patients. Two (20%) patients had no attack history.

Clinical presentations of the attacks were abdominal pain in 4 (40%) and swelling of hands-feet and face in 4 (40%) of the patients. Family history of angioedema was present in siblings of 5 patients (50%), mother and aunt of 3 (30%) patients, father of 1 (10%) patient, 1(10%) patient had no family history. None of the patients in the study was receiving prophylaxis. Prothrombotic risk factors were evaluated in 5 patients findings were as follows: factor V Leiden mutation, heterozygote in 1 patient (10%), Protrombin G20210A mutation, heterozygote in 1 patient (10%), MTHFR mutation, heterozygote in 7 patients (70%), homozygote in 2 patients (20%). PAI mutation was also detected as heterozygote in 4 patients (40%), homozygote in 1 patient (10%) (Table1).
DISCUSSION

HAE is a rare condition characterized by C1-INH deficiency that presents with attacks in children. Hereditary and acquired phenotypic variants have been described. Idiopathic histaminergic acquired angioedema, idiopathic nonhistaminergic acquired angioedema, acquired angioedema related to angiotensin-converting enzyme inhibitor and acquired angioedema with C1-INH deficiency are subtypes of acquired HAE; HAE with C1-INH deficiency, HAE with factor XII mutation and HAE of unknown origin are subtypes of hereditary HAE (4). Although there are several studies conducted on HAE in adults, studies in children are limited due to small number of cases.

HAE is usually diagnosed in the first decade of life. In the study by Nanda et al. mean age of the pediatric patient group was 158.4 months (8). In the study by Read et al. 111 HAE patients from 28 centers were included; mean age was 84 months with a range of 0-17 years (9). Mean age of our study group was 151.9 ±48.21 months and this was consistent with the literature.

Based on autosomal dominant inheritance of the disease, HAE is often present in family history. Family history of HAE was found in 90% of our study group. Family history was positive in 86% and 84% of the different studies (8-10).

HAE is a clinical condition that usually presents with attacks. The studies reported attacks were commonly localized in the abdominal region our findings were consistent with these results (8,11). However Kesim et al. have reported attacks commonly originated from the extremities (12). The relatively rare laryngeal or scrotal involvement was not detected in our study group. Similar to the reported case groups in literature, 2 of our patients were diagnosed based on family history but were asymptomatic.

Patients with HAE may experience HAE attacks due to exposure to various factors. Trauma, stress, infections are the most common factors known to trigger attacks. However, unknown factors also exist. In the study by Nygren et al. majority of abdominal attacks were triggered by psychological factors while cutaneous attacks were mostly triggered by trauma or sports activities (11). Psarros et al. concluded %94.7 of the attacks were due to unknown factors (13). In our study, factors causing the majority of attacks were not identified, although a history of trauma was the most common factor that could be identified. Similarly, in the study by Kesim et al. the triggering factor for attacks could not be detected (12). In our study population, history of trauma was the most common factor that could be identified.

The treatment of HAE requires planning in three phases; treatment of attacks, short-term prophylaxis and long-term prophylaxis. Treatment options include C1-INH replacement in acute conditions, danazol and anti-fibrinolytic agents for short- and long-term prophylaxis (6,14,15). In our study group, C1-INH concentrate was used to treat acute attacks; none of the patients have received short- or long-term prophylaxis. Any complications, including thrombosis, was not observed in patients treated with C1-INH.

C1-INH have an essential role in molecular stages of complement activation, inflammation and activation of contact phase in coagulation. In patients with hereditary angioedema, its role on the coagulation system, particularly during attacks, have been investigated. Factor VII activation have been observed in patients with HAE (15). Although coagulation system was activated in patients with HAE, increased prevalence of thromboembolism was rarely observed due to mechanisms against thrombosis, such as fibrinolysis (15,16). In our study population, no clinical condition suggesting thrombosis was observed. On the other hand, HAE were not reported in different studies including children with venous or arterial thrombosis (16,19).

C1 inhibitor deficiency does not cause only edema, it may cause dermal vascular thrombosis and coagulation (20). Additionally, decreased levels of PAI1-PAI2 would impair plasmin activation that allows factor XII activation and thus break the prekallikrein-bradykinin cascade which would increase thrombosis predisposition and HAE risk (21-23).

Recently, studies were conducted in patients with HAE comparing plasma enzyme levels (prothrombin, D dimer, plasminogen etc.) during attacks and attack-free periods. Very few studies that investigate genetic prothrombotic risk factors in HAE patients are available in literature. Factor XII mutations have been frequently reported in patients with HAE, although clinical manifestations suggesting thrombosis were not detected. Our study population was not screened for Factor XII mutation. However, based on other genetic prothrombotic risk factors and an adult patient with heterozygote Factor V Leiden mutation accompanied by purpura fulminans was reported (21,24).

Considering genetic prothrombotic risk factors and clinical features, our patient who had homozygous PAI mutation had relatively frequent attacks. We did not find any association between MTHFR polymorphisms and HAE attacks. On the other hand, MTHFR polymorphisms without hyperhomocysteinemia do not have any effect on thrombosis (25).

The limitations of this study are relatively small number of the included children with HAE, and not including all of the reported prothrombotic risk factor.

CONCLUSION

Patients with HAE should be screened for genetic risk factors before hormone replacement therapy and pregnancy or initiation of agents that are androgenic and
increase tendency for thrombosis, due to predisposition for coagulation. Thrombosis should be considered in clinical conditions that may occur during follow-up or treatment of patients with HAE.

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