Novel SERPING1 gene mutations and clinical experience of type 1 hereditary angioedema from North India

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Abstract

Background: There is paucity of literature on long term follow-up of patients with Hereditary angioedema (HAE) from developing countries. Objective: This study was carried out to analyse the clinical manifestations, laboratory features and genetic profile of 32 patients (21 male and 11 female) from 23 families diagnosed with HAE between January 1996 and December 2019. Methods: Data were retrieved from medical records of the Paediatric Immunodeficiency Clinic, Post Graduate Institute of Medical Education and Research, Chandigarh, India. Results: Median age at onset of symptoms was 6.25 years (range 1–25 years) and median age at diagnosis was 12 years (range 2-43 years). Serum complement C4 level was decreased in all patients. All patients had low C1- esterase inhibitor (C1-INH) quantitative level (type 1 HAE). SERPING1 gene sequencing could be carried out in 20 families. Of these, 11 were identified to have a pathogenic disease-causing variant in the SERPING1 gene. While 2 of these families had a previously reported mutation, remaining 9 families had novel pathogenic variants in SERPING1 gene. Because of non-availability of C1-INH therapy in India, all patients were given long term prophylaxis (attenuated androgens or tranexamic acid or a combination of the 2). Life-threatening episodes of laryngeal oedema were managed with fresh frozen plasma infusions. Only one disease related mortality was reported in the entire cohort. Conclusions: We report largest single centre cohort of patients with HAE from India. Attenuated androgens, fibrinolytic agents and fresh frozen plasma may be effectively used for management of HAE in resource limited settings.

Impact statement:

We report the largest single centre cohort of type 1 hereditary angioedema (HAE) from India. Most patients had novel mutation in*SERPING1* gene. Clinical profile of patients in the present series was found to be similar to several previously reported series. Because of lack of availability of C1-INH therapy in India, patients were managed using attenuated androgens, tranexamic acid and fresh frozen plasma. Only one disease related mortality was reported in the entire cohort. Results of this study suggest that use of TA, stanozolol and FFP may still be an effective treatment option for patients with type 1 HAE in resource constrained settings.

Introduction:

Hereditary angioedema (HAE) is an uncommon primary immunodeficiency, clinically characterized by recurrent, non-pruritic oedema involving the face, extremities, genitalia and upper airways.[1]Occasionally this disease may manifest with pain abdomen due to oedema involving gastrointestinal tract or an acute and life-threatening airway obstruction due to laryngeal oedema.[2,3] The disease often starts in childhood, worsens at puberty and may persist throughout life.[2,4–6] Detection of low serum levels of C1-esterase inhibitor (C1-INH) (quantitative and functional) along with low serum C4 are suggestive of a diagnosis of HAE.[5–7]

HAE is a rare genetic disorder most often caused by mutations in Serpin family G member 1 (*SERPING1)* gene that leads to deficiency of C1-INH protein.[8] The disease is inherited in an autosomal dominant manner.

However, spontaneous occurrences have also been reported in up to a quarter of patients[2] Epidemiologic studies suggest that the prevalence varies from 1:10,000 to 1:150,000.[9–12] HAE has been reported infrequently from developing countries.[13–21] There are no long-term follow-up studies and no data on genetics of HAE from India. In this study we report our experience with HAE over last 2 decades. To the best of our knowledge, this would be one of the longest follow-up studies on paediatric HAE.

Patients and methods:

This study was carried out in the Allergy-Immunology Unit, Advanced Paediatrics Centre, Postgraduate Institute of Medical Education and Research, Chandigarh, North India. Our institute is a federally funded, not-for-profit tertiary care referral hospital for North-West India. The study included all patients diagnosed to have HAE between January 1996 and December 2019. Data were retrieved from medical records and entered in a predesigned proforma. The study was approved by Institute Ethics Committee and an informed consent was obtained from patients or their parents. Serum complements C4 was measured by end-point nephelometry using a semi-automated nephelometer, MININeph from The Binding Site, Birmingham, UK. Functional C1-INH activity was assessed by MicroVue C1 inhibitor Plus enzyme immunoassay kit from Quidel, USA. Our laboratory is regularly participating in an external quality assurance scheme i.e. UK National Quality Assurance Scheme (UK NQAS) for special proteins since 2010. Quantitative C1-INH was estimated using radial immunodiffusion method and by semi-automated nephelometry. Patients were diagnosed to have HAE if they had characteristic clinical manifestations of disease with or without a suggestive family history with low C4 and either a low quantitative C1-INH (type 1 HAE) or low functional activity of C1-INH (type 2 HAE). Patients with suspected HAE but with normal quantitative or functional C1INH levels were not included in this analysis. Family members of patients with HAE for whom complete clinical details and laboratory data were not available were also excluded from this analysis.

Because of non-availability of recombinant or plasma derived C1-INH therapy in India, stanozolol 2-4 mg/day), danazol (100-600 mg/day) or tranexamic acid [TA] (30-50 mg/kg/day) was used for long-term prophylaxis. For short term prophylaxis during planned surgeries, stanozolol (2-4 mg/day) and fresh frozen plasma, FFP (10 ml/kg) were used. Acute episodes of life-threatening laryngeal oedema were managed using FFP (10 ml/kg).

Sanger sequencing for *SERPING1* gene:

We started doing Sanger sequencing of the *SERPING1* gene in our cohort of patients with HAE since 2018. For Sanger sequencing, 200 µl of EDTA blood was used. Genomic DNA was extracted using QIAamp DNA extraction kit as per the manufacturers' protocol (Qiagen, Hilden Germany). All exons of *SERPING1* gene were amplified using oligonucleotide primers as mentioned in Table 1 and Figure 1. These oligonucleotide primers were designed to cover exon/intron junctions of all exons. Each exon was amplified using Polymerase Chain Reaction (PCR). The PCR product was checked on 1.5% of agarose gel electrophoresis followed by purification and this purified product was used for Sanger Sequencing using the ABI Big Dye terminator kit and Agilent 2100 Bioanalyzer System. The sequencing data were analyzed using Finch TV and Codon code aligner software. *In silico* prediction analysis was used for all novel mutations detected in the*SERPING1* gene. The pathogenic nature of these variants was inferred using 3 free, online bioinformatics tools for prediction of functional effects of amino acid substitution in proteins viz. Provean, PolyPhen-2 and FATTHM.

Results:

In this study, we included 32 patients (21 male and 11 female) from 23 families who were diagnosed to have HAE. Median age at onset of symptoms was 6.25 years (range 1–25 years) and median age at diagnosis was 12 years (range 2-43 years) with median delay in diagnosis of 6.5 years (range 0-28 years). Clinical manifestations of patients in this study are detailed in Table 2. All patients had swelling over face (eyelids and/or lips) (Supplemental Figure 1). Recurrent episodes of erythema marginatum preceding a flare of disease were noted in 4 patients. Although abdominal symptoms were noted in almost 1/3rdof patients, only 1 patient presented with acute surgical abdomen and underwent exploratory laparotomy. No patient in this

study had central nervous system complaints. Stress, exercise and blunt trauma were the only identifiable triggers for flare of symptoms. However, most patients reported no definite trigger for their episodes. All patients had low serum complement C4 levels and low serum C1-INH levels. C1INH activity was also low in all patients in who it was tested. Thus, all patients in the present study had type 1 HAE.

Most common differential diagnosis at presentation was allergic angioedema. Patients who were diagnosed to have allergic angioedema had itching or redness over the swelling and had history of response to antihistaminic drugs and/or corticosteroids that were prescribed elsewhere. All these features were absent in patients who were diagnosed to have HAE. However, 1 patient presented with recurrent episodes of periorbital puffiness and fever. A clinical possibility of HAE was considered initially as C1-INH levels were low. However, because of recurrent episodes of fever accompanying the periorbital puffiness and elevated inflammatory parameters (erythrocyte sedimentation rate and C-reactive protein), a clinical possibility of autoinflammatory syndrome was also considered. Next generation sequencing revealed a pathogenic variant in the *TNFRSF1A* gene that encodes for Tumour Necrosis Factor receptor (TNFR1) protein.

SERPING1 gene sequencing could be carried out in 29 patients from 20 families till the time of this analysis. Of these, 17 patients from 11 families were found to have a pathogenic variant in the*SERPING1* gene, while no pathogenic variant was detected in 12 patients from 9 families. Most mutations in the SERPING1 gene in our cohort were located in exon 7 and exon 8 (in 4 and 3 families respectively). Missense mutations were most common and seen in 5/11 families while non-sense, frameshift and splice site mutations were seen in 2/11 families each (Figure 2 and Figure 3). Nine out of these 11 families had a novel mutation while in remaining 2 families a previously reported mutation was identified. In addition, 20 benign polymorphisms in *SERPING1* gene were observed in 14 patients from 13 families (Supplementary Table 1). Intron 6 polymorphism (c.1029+20A>G) was most common (in 6 patients) followed by exon 8 polymorphism (c.1438 G>A; p.Val480 Met) and intron 3 polymorphism (c.251-106 T>G) [in 4 patients each].

Most patients who were diagnosed prior to 2015 were initiated on stanozolol (2 mg per day) or danazol (200 mg/day). However, over last 4 years, patients were initiated on either a combination of stanozolol and TA or TA alone. Depending on the response, stanozolol was either discontinued after 6 months to 1 year or continued at a dose of 0.5-2 mg per day. Stanozolol had to be initiated in patients who failed to respond to TA alone (Table 2). Four patients who were initially given stanozolol and TA, stopped taking treatment after 25 years of age because they had minimal attacks thereafter.

Short term prophylaxis was used in 2 patients. First patient was a 6-year-old girl who was diagnosed to have type 1 HAE in 2015. She was given short term prophylaxis in 2018 as she had to undergo radical excision of mandibular cyst. She was managed by increasing the dose of stanozolol and by giving FFP infusions [10 ml/kg/dose]. Second patient was a 29-year-old female who was diagnosed to have HAE in 2001. She conceived in 2018 and remained asymptomatic during pregnancy despite any prophylaxis. She was planned for an elective cesarean section in view of non-progress of labor. She was managed with FFP (2 units) before and during cesarean section and stanozolol 2 mg/day (initiated on the day of cesarean section). Both patients tolerated the procedure well.

On demand therapy was used for acute episodes of laryngeal edema only. FFP (10 ml/kg, maximum 2 units) infusion was used for 15 such episodes. All patients showed prompt response and resolution of symptoms with single dose of FFP.

Four patients reported virilization (menstrual irregularities, hirsutism and hoarseness of voice) after taking stanozolol. One patient also developed hypertension and excess weight gain. None of the patients treated with attenuated androgens showed any evidence of hepatic or haematological abnormalities. Other than one patient who had 2 spontaneous abortions during first trimester while she was being continued on TA prophylaxis, no side effects related to TA therapy were reported in this study.

One patient in our cohort died due to the disease. She was taking stanozolol and TA as long-term prophylaxis. She developed an acute episode of laryngeal oedema and collapsed before her parents could access FFP infusion. Another patient in this study died due to road traffic accident. In addition, at least 3 families

could recall the death of one of their family members who died due to an episode of laryngeal oedema in the past.

Mean follow-up period in this study is 57 months (+ 81.5, range 4-288). The total follow-up period of the entire cohort is 1824 patient-months.

Discussion

HAE is an uncommon autosomal dominant disorder characterized by recurrent episodes of subcutaneous oedema, reduced complement C4 and low antigenic and/or functional levels of C1-INH. HAE is a potentially life-threatening medical condition. Depending on the C1-INH antigenic and functional levels, HAE has been classified into 3 different types.[7] In majority (80-85%) there is a reduction of both antigen and functional levels of C1-INH protein (type I HAE) and in 15-20% patients C1-INH antigen levels are normal or elevated but it is dysfunctional (type II HAE). A rare form of hereditary angioedema (HAE nlC1-INH) is characterized by normal levels and function of C1-INH protein. HAE nlC1-INH is most commonly caused by mutation of coagulation factor XII (*F12*) gene. Recent advances in the genetic studies have identified some new genes that are associated with HAE nlC1-INH. These include mutations in *angiopoietin 1* gene (HAE-ANGPT1),*plasminogen* gene (HAE-PLG) and *Kininogen-1*gene.[8,22–25] Acquired angioedema (AAE-C1-INH) has mostly been reported in association with drugs, autoimmune diseases and B-cell lymphoproliferative disorders.[26] Patients with AAE have older age at onset (4^{th} or 5^{th} decades of life). These patients have low levels or abnormal function of C1-INH and low levels of C1q. The present study, however, focussed on patients with type I HAE.

Published literature on HAE from developing countries is very limited.[13–19,27–29] To the best of our knowledge, no long-term follow-up studies are available from India, especially in children. Moreover, there are limited data on genetic profile of patients with HAE from developing countries including India. Studies conducted in China[27] and Japan[30]reported that the incidence of HAE in Asian countries is less as compared to studies from Latin American and Western countries such as Brazil[31], Hungary[32], Denmark[33] and Germany.[34] However, precise epidemiologic figures are not available.[35]Table 3 shows an overview of published studies on type 1 and 2 HAE including few paediatric studies.[12,27,30–34,36–46]

Diagnosis of HAE can be easily made from the characteristic oedema and is supported by a positive family history. Low serum C4 level is a cost-effective method of screening for HAE.[2,3] In our cohort, the characteristic swelling was present in all patients and low C4 levels were detected in all. It has, however, been reported that C4 levels may occasionally be normal in HAE.[2,9,36]Assessment of serum C1-INH level (antigenic and functional) helps in establishing an accurate diagnosis. All patients in the present study had low C1-INH antigen levels.

Median age at onset of symptoms was 6.25 years in the present study which is comparable with several other previously published studies (Table 3). Median age at the time of diagnosis was 12 years with median delay in diagnosis of 6.5 years. This is largely because of lack of awareness amongst primary care physician and most patients were initially managed elsewhere as allergic angioedema.

No definite triggers were discernible in most patients in the cohort except few patients who identified blunt trauma, psychosocial stress and exercise as potential triggers. Frequency of attacks were discernibly less during pregnancy in 2 patients in our study. One of these 2 patients was continued on TA prophylaxis and she had an uneventful pregnancy. The other patient experienced 2 first trimester abortions while she was on TA prophylaxis even though she had no episodes of angioedema during both pregnancies. Pregnancy has variable effect on the clinical course of HAE.[47–49] Attenuated androgens are considered contraindicated during pregnancy and plasma derived C1-INH and TA are considered safe. Mental stress has also been reported to be an important trigger in these patients.[7,50]

There can be substantial inter-individual variation in the onset of symptoms of HAE, duration of symptoms, frequency of attacks and severity of symptoms. These differences can exist within the members of the same family as was the case in several of our patients. In the Hungarian cohort, the clinical attacks were found to be

more severe in children who had earlier age of disease onset.[32] Recurrent abdominal pain may be seen in 30-100% of patients with HAE.[27,31,51] It may be the initial presenting symptom in 40-80% of children.[16,52] In the presented study, 1/3rd of all patients reported having gastrointestinal symptoms. Patients with predominant abdominal symptoms may inadvertently be subjected to exploratory laparotomy.[30] In one study, 3 patients presented with oedematous abdominal attacks as initial symptoms.[32] One patient in the present cohort had a history of exploratory laparotomy for acute surgical abdomen, several years before he was diagnosed to have HAE.

Erythema marginatum could be an early manifestation of HAE and may precede a disease flare. It may easily be confused with urticaria and is more commonly reported in children and with type 1 and 2 HAE.[7,53] Erythema marginatum was seen in 3 patient in the present series.

In children, the only recommended drugs for management of acute episode of HAE are C1-INH concentrate.[7] Solvent detergent –treated plasma (SDP) or fresh frozen plasma (FFP) may be considered when C1-INH therapy is not available.[7] As C1-INH concentrate is not readily available commercially in our country, we had to administer FFP in our patients with laryngeal oedema and patients showed prompt resolution of symptoms. FFP may be considered as on demand therapy in countries where C1-INH therapy is not easily available.[21]

Patients and their parents were explained about need of long-term prophylaxis and available treatment options. All patients opted for long term prophylaxis as effective on demand treatment is not available in India and access to FFP in emergency situations may not be always be possible. TA has favourable side effect profile when compared to attenuated androgens. However, studies carried out in the past have reported safety of attenuated androgens even in prepubertal children.[7,50] In India, we do not have access to plasma derived C1-INH therapy. Therefore, we considered attenuated androgens (stanozolol) as the agent of choice for long term prophylaxis of HAE. Most patients in our cohort showed reduction in frequency and severity of HAE attacks with stanozolol prophylaxis. However, few patients continued to have disease flare and even severe airway obstruction requiring plasma therapy while they were taking stanozolol prophylaxis. It has been observed that attenuated androgens are more effective than TA.[7] However, TA was considered an upfront therapy in several patients in the present study considering their side effect profile and these patients showed good response to therapy.

Patients with HAE are prone to develop disease flare and occasionally life-threatening laryngeal oedema during surgical procedures especially dental surgeries.[7] Plasma derived C1-INH therapy is recommended to be used as short-term prophylaxis during these procedures. Use of attenuated androgens and FFP have also been suggested if C1-INH is not available.[54,55] We successfully used stanozolol and FFP for short term prophylaxis in 2 patients in the present series.

Genetic profile of patients with HAE has been studied for the first time in India. We found mutations in the *SERPING1* gene in 11 out of 20 families who were screened. Nine of these 11 families had a novel pathogenic variant in the *SERPING1* gene. Missense mutations in exon 7 and 8 were the most common mutations in our cohort. Mutation spectrum in *SERPING1* gene is quite heterogeneous and more than 450 mutations spread over the entire gene have been reported so far.[33,56–59] Missense mutations have been reported to be the most common mutations in *SERPING1* gene.[8,60–62] while some populations have reported that nonsense or frameshift mutations are most common.[33] Missense mutations in exon 8 have also been reported to be the most common mutation in certain populations.[60,61,63] Few authors have also reported a genotype-phenotype correlation and have shown that non-sense, frameshift and deletion mutations are associated with a more severe disease phenotype.[56,64] However, several other authors have reported no genotype-phenotype correlation and have suggested that polymorphisms in bradykinin receptor or contact system proteins may be responsible for this phenotypic heterogeneity.[33,60] In the present study, we observed no genotype-phenotype correlation. Family members with same mutations were noted to have wide variation in age of symptom onset and disease severity suggesting a variable penetrance.[8]

We did Sanger sequencing for all 8 exons of SERPING1 gene, however, 9 out of 20 families were not found

to have any pathogenic variant. All these families had characteristic clinical manifestations of HAE with low C4 and low C1-INH. All except 2 of these patients also had suggestive family history. We could have missed mutations in the promoter region and deep-intronic regions of the *SERPING1* gene in these patients. Recently a deep intronic novel pseudoexon activating mutation in the intron 6 of *SERPING1* gene has been reported in a family with HAE.[65] Therefore, some of the patients with HAE in this study may have this deep intronic mutation or a variant in the promoter region of the gene.

Conclusion:

This is the first study on long-term follow-up of HAE from India. The major strength of the study is its duration of follow-up (1824 patient-months) which is one of the longest reported so far in children and genetic profile of HAE in India has been studied for the first time. In resource constraint settings where C1-INH concentrate is not available, FFP may be an effective option for acute management. Though recent guidelines for management of HAE in children recommend use of C1-INH for long term prophylaxis, our experience suggests that in developing countries and resource limited settings, attenuated androgens and fibrinolytic agents may also have a role in management. In the present situation, when several options are available and more effective options are being explored for long term prophylaxis and for acute management of HAE, all attempts must be made to avail these medications for patients. Use of TA, attenuated androgens and FFP may not be advocated for patients with HAE in developed countries who have access to modern treatments. However, despite best of the efforts, the modern treatments for HAE may not be available in several developing countries as is also the case in India. Our experience suggests that use of TA, stanozolol and FFP may still be an effective treatment option for patients with HAE in these countries.

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Figure legends:

Figure 1: Optimization of the PCR conditions for the amplification of exons of *SERPING1* gene: Lane 1-7: PCR products of different exons (1, 2, 3, 4, 5-6, 7, 8) of *SERPING1* gene: Lane 8: 100 bp DNA ladder.

Figure 2: Distribution of mutations on *SERPING1* gene in the present study

Figure 3: Pedigree charts of 11 families wherein a mutation was detected in SERPING1 gene along with their sequence chromatograms from each family

Supplemental Figure 1: Facial and periorbital swelling in a 3-year-old girl during an episode of hereditary angioedema and (A) and normal face during asymptomatic period (B)

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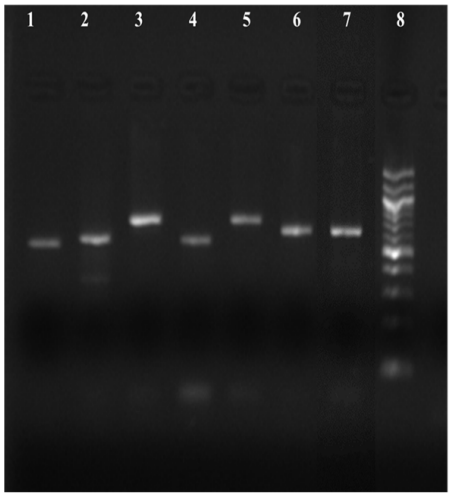

Lane no	*SERPING1* exon (no)	Product size (bp)
1	*SERPING1* Exon 1	533
2	*SERPING1* Exon 2	555
3	*SERPING1* Exon 3	709
4	*SERPING1* Exon 4	548
5	*SERPING1* Exon 5,6	720
6	*SERPING1* Exon 7	639
7	*SERPING1* Exon 8	602
8	100 bp DNA ladder	

Figure 1