Cascade Screening of Hereditary Angioedema in Pakistan

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ABSTRACT

Objective: To establish cascade family screening of newly diagnosed hereditary angioedema (HAE) patients.

Study Design: Cross-sectional, observational study.

Place and Duration of the Study: Department of Immunology, The Armed Forces Institute of Pathology / CMH / NUMS, Rawalpindi, Pakistan, from September 2021 to June 2024.

Methodology: Eighty-nine parents, siblings, and children of 10 diagnosed patients of HAE were screened. Thirty-two family members were screened by using C1 esterase and complement C4 levels, whereas 57 patients were not available or willing for blood samples, and a questionnaire designed for HAE was recorded from patients / index cases. Baseline characteristics of HAE-Index and HAE-Screened patients were analysed using inferential statistics (independent t-test, Chi-square / Fisher's exact, and Mann-Whitney U test), selected based on data distribution (assessed by the Shapiro-Wilk's test).

Results: A total of 10 cases were followed for screening in families. Upon cascade screening of 89 individuals, 16 confirmed and 24 probable cases of HAE among family members (symptomatic and asymptomatic) were identified. Out of these 40 positive patients, 26 were male and 14 were female. Three patients among these screened individuals died secondary to laryngeal oedema. Out of ten index cases, two did not have any family history of hereditary angioedema, hence suspected for *de novo* mutation in the *SERPING-1* gene.

Conclusion: Cascade screening helps in facilitating early diagnosis of asymptomatic or mildly symptomatic family members of HAE patients. This will provide better guidance for avoiding potential triggers, appropriate prophylaxis, and better management of HAE patients. Young patients of HAE need genetic counselling for a 50% risk of HAE in offspring and are recommended for prenatal diagnosis.

Key Words: *C1 esterase, C4, Hereditary angioedema, Laryngeal oedema, Screening*.

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INTRODUCTION

Hereditary angioedema (HAE) is a serious, life-threatening health problem for patients and their families. HAE is primarily an autosomal dominant disorder, but also results from *de novo* mutations in 25% of cases.[1] HAE is caused by a mutation in the *SERPING1* gene (OMIM#606860) located on chromosome number 11.[2] There are no differences in its incidence based on race or gender, and it ranges from 1: 10,000 to 1: 50,000.[3] This potential life-threatening disease is clinically distinguished by intermittent episodes of cutaneous angioedema (non-pruritic and asymmetric without urticaria and submucosal swelling with severe abdominal pain), which are the most common features, but these patients can also present with genital swelling, laryngeal oedema, and joint swelling.[4]

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Laryngeal oedema can lead to asphyxiation, and this is the primary cause of mortality in patients with HAE.[5] Numerous factors, such as stress, trauma, infections, surgical and dental procedures, and estrogen hormones, are known to cause HAE episodes.[6]

HAE is categorised into three types, Type I HAE is caused by reduced production or lack of C1 esterase inhibitor (INH). Type II HAE is characterised by normal or raised C1-INH levels with a functional defect, and Type III HAE is associated with normal levels and function of C1-INH, but the defect lies in other regulators of the kinin system, such as the factor XII gene, plasminogen gene, *angiopoietin* gene, *kininogen-1*, *myoferlin*, *HS3OST*, and *HAE* of an unknown mutation, which can only be diagnosed with genetic testing.[7] Angioedema is also linked to several other conditions, such as connective tissue diseases, infections, leukaemia, lymphoma, and medications, including ACE inhibitors and estrogen, and needs to be differentiated.[8] Screening the families of patients with HAE is crucial for early detection, genetic counselling, and appropriate management.[9] Screening involves a comprehensive assessment of family history, clinical evaluation, and genetic testing. Genetic counselling should be offered to affected families to discuss inheritance patterns, recurrence risks, and the availability of prophylactic and acute treatment options.[10] Overall, screening

the families of HAE patients facilitates early diagnosis, appropriate management, and preventive measures to mitigate the burden of this potentially life-threatening condition. HAE patients are treated with three different strategies, including on-demand treatment, short-term prophylaxis, and long-term prophylaxis.[11] In many developing countries such as Pakistan, no C1-INH product is available for acute attacks, so, they rely on FFPs at a dose of 20 ml/kg for acute attacks of HAE. The use of androgens is considered as a cost-effective and accessible long-term prophylaxis; however, their use requires caution due to side effects and contraindications in pregnancy.[12] HAE patients, if planning any minor or major surgery, must be provided with C1-INH/FFPs as prophylactic treatment.[13]

The aim of this study was to determine the utility of cascade screening in HAE patients. The diagnostic facilities for C4 and C1 esterase levels are available at only a few laboratories, whereas molecular diagnosis is still not available in Pakistan. No specific treatment, including C1 INH concentrate treatment, is available in Pakistan. Furthermore, there is also a need to establish an HAE registry at the country level.

METHODOLOGY

This cross-sectional, observational study was conducted at the Department of Immunology, The Armed Forces Institute of Pathology / CMH / NUMS, Rawalpindi, Pakistan, from September 2021 to June 2024. Initially, 10 patients were diagnosed as index cases ascribed to HAE-index. Families were offered screening of all first-degree relatives in a three-generation pedigree, including children, siblings, parents, uncles, aunts, and grandparents, who were included in the study. A total of 32 suspected symptomatic and asymptomatic family members were screened with C4 (normal range: 0.2 to 0.5 g/L) and C1 esterase levels (normal range: 0.22 to 0.38 g/L) on the binding site SPA plus automated protein analyzer (UK). Fifty-seven family members of index cases, who were not available for blood samples, were screened for probable HAE with a questionnaire designed for HAE. Categorical (nominal) variables are presented as numbers and percentages, while continuous variables are reported as means ± standard deviations (SD) or as medians with interquartile ranges (25^{th}-75^{th} IQR), where appropriate. Inferential statistics such as the independent t-test (parametric test), and non-parametric tests, including the chi-square test or Fisher's exact test, and the Mann-Whitney U test, were applied according to the data distribution, which was evaluated by using the Shapiro-Wilk's test. A p-value below 0.05 was regarded as indicating statistical significance.

RESULTS

A total of 89 first-degree relatives of 10 index patients were identified for HAE screening, and 40 were labelled with HAE, resulting in a diagnostic yield of 45%. However, 40% (n = 16) of whom were tested deficient for C1 esterase, and 60% (n = 24) were deemed probably deficient based on family history and clinical symptoms as per the designed questionnaire. Thirty-two (36%) family members were screened by using C1 esterase and complement C4 levels, whereas 57 patients were not available or willing for blood samples, and a questionnaire designed for HAE was recorded from patients / index cases. The overall willingness to participate in cascade family screening (CFS) was therefore 64%. Out of the 10 index cases, two did not have any family history of HAE, hence suspected for *de novo* mutation in the *SERPING-1* gene. Baseline characteristics of patients are summarised in Table I and a summary of index cases screening is presented in Table II. A family tree of only one family is also drawn (Figure 1).

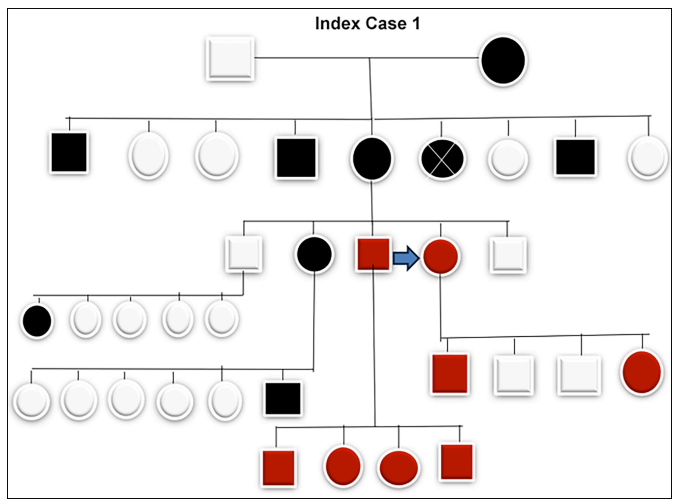

Figure 1: A representative family tree with affected indivisuals.
Family history of index case 1 suggested many family members with similar symptoms. A total of eight family members had confirmed diagnosis (red) of HAE, and about nine family members had probable diagnosis (black) of HAE in four generations of index case 1. One female patient with a black circle and white cross was died with laryngeal oedema.

Table I: Baseline characteristics of index and screened patients.

Characteristics	All (n = 50)	HAE-index (n = 10)	HAE-screened (n = 40)	p-value
	Median (IQR) or Mean ± SD			
Age (year)	25 (28)	24.5 (9.75)	25 (33.50)	0.837*
Age of onset (year)	11.27 ± 5.95	13.4 ± 7.21	10.7 ± 5.55	0.208**
Age of diagnosis (year)	25 (28)	24.5 (17.25)	23.5 (38.0)	0.666*
Delay in diagnosis (year)	7 (26.3)	6.5 (11.50)	7.5 (32.35)	0.634*
Gender, n (%)				
Male	33 (66%)	7 (70%)	26 (65%)	0.765***
Female	17 (34%)	3 (30%)	14 (35%)	

**Mann-Whitney U test. **Independent samples t-test ***Fisher's exact test (the p-value appears significant as CFS was carried out for HAE-index patients who were previously tested and diagnosed). HAE: Hereditary angioedema, IQR: Interquartile range, SD: Standard deviation.*

Table II: Summary of index cases screening of HAE in the Pakistani population.

Family No.	Gender	Age of diagnosis	Onset of symptoms at age	C1 esterase levels g/l	C4 levels g/l	Family history	Death from laryngeal oedema in family	Confirm cases in family screening	Probable cases in family screening	Total patients
Family 1	Male	36	15	0.09	0.07	Yes	1	7	9	17
Family 2	Female	20	12	0.05	0.09	Yes	1	1	3	5
Family 3	Male	30	14	0.07	0.10	Yes	0	1	3	5
Family 4	Male	22	12	0.07	0.06	Yes	0	1	4	6
Family 5	Male	15	6	0.04	0.08	No	0	0	0	1
Family 6	Male	37	2	0.02	0.04	Yes	1	1	1	3
Family 7	Female	28	25	0.14	0.11	Yes	0	3	1	5
Family 8	Female	24	7	0.06	0.21	No	0	0	0	1
Family 9	Male	25	21	0.05	0.06	Yes	0	2	2	5
Family 10	Male	30	14	0.09	0.07	Yes	0	0	1	2
Total										50

DISCUSSION

HAE is a rare autosomal dominant condition with recurrent episodes of swelling of the skin, gastrointestinal system, and upper airways. As it is not very common, its diagnosis can be difficult, especially in settings with resource-limited countries such as Pakistan. This study highlights the value of cascade family screening in discovering undiagnosed HAE cases among relatives of known patients. Cascade screening is essential for identifying relatives who may carry the disease-causing gene but are asymptomatic or only mildly symptomatic. As a result, prompt intervention such as preventative therapy initiation, appropriate acute attack management, and genetic counselling is made possible by early diagnosis using cascade screening.

A non-interventional survey of patients with HAE conducted by Banerji *et al*. in 2017 in the United States of America showed that 78.4% of patients with HAE Type I had a positive family history for HAE,[14] thereby substantiating the rationale of cascade screening in low-income settings. Another study conducted by Aabom *et al*. in 2009 at the Danish HAE Comprehensive Care Centre, Odense University Hospital, on health- related quality of life in the Danish population showed that 70% of patients with HAE had a positive family history,[15] whereas this study showed a positive family history of HAE in 80% of patients.

A study conducted by Henao *et al*. in 2016 in the USA concluded that C4 was traditionally considered a cost-effective screening test to rule out HAE, but about 10% of cases show C4 levels within the normal range.[16] Another case report published by Karim *et al*. in 2004 also suggests that HAE can exist with normal C4 levels.[17] A study conducted by Jindal *et al*. concluded that low C4 level is only sensitive in about 80-85% of HAE cases.[18] This study also showed two cases of HAE with normal C4 levels, so C4 screening alone is not recommended to rule out HAE in all cases.

A study conducted by Minafra *et al*. in 2020-2021 in Brazil analysed the data from previous studies on HAE and concluded that a total of 3,292 patients were diagnosed with HAE, and 411 (12.5%) were died because of asphyxia caused by laryngeal oedema.[19] Data from the present study showed 3 (7.5%) deaths from laryngeal oedema out of 50 diagnosed patients of HAE. Low deaths from laryngeal oedema in the present study may be because of the smaller data available in Pakistan.

A study was conducted at the Queen Mary Hospital, a tertiary referral hospital in Hong Kong, from 2019 to 2022, where 63 volunteer relatives of 12 families were screened, and 29 (46%) new cases were diagnosed on the basis of deficient C1 esterase and low C4 levels, and about 52% of newly diagnosed cases were symptomatic.[20] In this study, a total of 32 available relatives were screened with C1 esterase and C4 levels and 16 (50%) cases were confirmed on these blood investigations. Out of these new 16 confirmed cases, the majority of patients had mild-to-moderate symptoms, whereas only two patients were asymptomatic.

This study highlighted the problems at the national level that need to be addressed, including strategies for awareness of HAE in general medical practitioners because of its rarity and resemblance to allergy and urticaria. It is necessary to enhance diagnostic facilities at tertiary care centres in Pakistan, as most tertiary care centres do not have C1 esterase levels facilities. It is the need of the time to establish molecular diagnosis of HAE at the country level, especially for HAE Type II and Type III, because these types are usually missed by routine investigations for HAE. Genetic counselling of the family and young adult patients is very crucial, and prenatal diagnosis is also helpful for the next generation. Limitations of this study include that it is a single-centre study from the Northern region of Pakistan, unavailability of C1 esterase levels at most tertiary care centres, transport of samples at 4°C from long distances and molecular diagnosis for Type II, and HAE with normal C1 INH levels and function.

CONCLUSION

This study highlighted the importance of cascade family screening in patients with HAE, as many family members initially can go unnoticed because of the asymptomatic phase or mild symptoms. This will provide guidance for newly diagnosed cases to avoid potential triggers, use of

appropriate prophylaxis, better management, genetic counselling of patients and parents, and awareness for prenatal diagnosis of HAE. All children with HAE must receive all compulsory vaccines along with the influenza vaccine annually.

ETHICAL APPROVAL:
The Ethical Review Committee of the Armed Forces Institute of Pathology (AFIP) Rawalpindi, Pakistan, provided ethical approval letter prior to the initiation of the research work.

COMPETING INTEREST:
The authors declared no conflict of interest.

PATIENTS' CONSENT:
Informed consents were taken from all patients included in the study, and detailed history was recorded.

AUTHORS' CONTRIBUTION:
MH: Concept, design, and drafting of the work.
MOR: Critical revision of the manuscript.
MA: Interpretation of the data and critical revision of the manuscript for the important intellectual content.
MAH: Data collection, acquisition, and data analysis.
All authors approved the final version of the manuscript to be published.

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