# HEREDITARY ANGIONEUROTIC EDEMA: CLINICAL AND LABORATORY FINDINGS IN 58 SUBJECTS

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Hereditary angioneurotic edema (HANE) is an autosomal dominant disorder in which underlying quantitative or functional defects of complement C1 inhibitor (C1INH) and serum complement components (e.g., C4) are observed [1, 11, 28, 32, 37]. Clinically, HANE presents as non-pitting edema of the extremities, face, trunk, airway or abdominal viscera, apparently arising either spontaneously or following trauma [5, 18, 19, 22].

In the past few years we have diagnosed hereditary C1INH defects in 58 patients, thus bringing to over 700 the cases reported in the international scientific literature. The relative weight of our case-study warrants an in-depth analysis of clinical and laboratory findings with special focus on problems related to the diagnosis of the disorder.

## MATERIALS AND METHODS

### *Patients*

Fifty-eight patients belonging to 21 families were diagnosed as having HANE based on history, symptoms and laboratory testing (i.e., reduced values in immunochemical and/or functional assays). At the time of diagnosis, the 24 male and 34 female patients ranged in age from 4 to 70 years.

### *Complement assays and laboratory data*

*Buffers* - Standard procedures were followed in the preparation of 0.015 M Veronal buffer, pH 7.5, containing 0.1% gelatin, $1.5 \times 10^{-4}$ M $Ca^{++}$ and $5 \times 10^{-4}$ M $Mg^{++}$, and Veronal buffer, pH 7.5, containing 0.01 M ethylenediamine-tetraacetate (EDTA) and 0.1% gelatin. Veronal buffer, pH 7.5, containing $2 \times 10^{-2}$ M $Mg^{++}$ and $8 \times 10^{-3}$ M ethyleneglycol-tetraacetate (EGTA), was prepared as previously described [9, 21, 33].

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*Hemolysis tests* - Total hemolytic complement (CH50) activity was determined according to MAYER[27]. CH50 was also run after incubation of the serum in low ionic strength buffer[15].

*Alternative pathway activity (APA)* - APA was evaluated using the method of PLATTS-MILLS and ISHIZAKA[31] or according to a modified version (2sAPA) of the method previously described[29,30].

*Immunochemical methods* - Serum values of C3, C4, immunochemical C1INH, IgG, IgA and IgM were determined by means of single radial immunodiffusion assays according to MANCINI et al.[26], using specific antisera. The antisera employed were purchased from the Behring Institute.

*C1INH functional tests*[1] - C1INH was assessed functionally employing the method described by LACHMANN et al.[25], or by means of the heterolysis of $C_2H_5CO$-Lys(e-Cbo)-Gly-Arg-pNa (Immuno AG, Wien; Nyegaard & Co., Oslo).

Statistical analysis of the data was done, when applicable, using Student's *t*-test for paired or unpaired data ($p<0.05$).

## RESULTS

Characteristics of the HANE patients are reported in tab. 1. The incidence of affected family members in different generations was 52.1%. The male/female ratio was 0.705 (24/34 patients). The mean age at the time of diagnosis was 31.9 ± 18.9 years, ranging from 4 to 70 years. The incidence of cutaneous, laryngeal or abdominal attacks in the symptomatic patients was 94.2% (49/52), 48% (25/52) and 88.4% (46/52), respectively. Stress could cause attacks in 88.4%, and trauma in 78.8% of symptomatic patients. Stress and trauma were often associated in the pathogenesis of the attacks. Certain patients were unable to ascribe some episodes to discernible causes, either mental or physical.

Laboratory findings in HANE patients are reported in tab. 2. A functional C1INH defect (type II) was encountered in 15.5% of our patients (9/58), whereas an immunochemical defect in the enzyme molecule (type I) was revealed in 84.5% of our patients (49/58). Functional C1INH, C4 levels and CH50 titres were significantly reduced in both type I and type II HANE as compared to controls. Immunochemical C1INH was significantly reduced in type I and augmented in type II HANE. APA titres and C3, IgG, IgA and IgM levels were normal in both type I and type II HANE.

## DISCUSSION

When evaluating the affected family members in different generations who furnished a complete clinical and laboratory picture, the incidence of 52.1% found can be considered in good keeping with the disorder's typical autosomal dominant mode of transmission. Hence, according to our case-study, HANE can be expected to develop in 52.1% of children resulting from the marriage between affected and unaffected individuals. The male/female ratio (0.705, 24/34) confirms the non-sex-linked nature of HANE heredity.

| ♂/♀ ratio | mean age at diagnosis (range) | edema (symptomatic patients) | | | | | | causes of the attacks (symptomatic patients) | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | cutaneous | | laryngeal | | abdominal | | stress | | trauma | | unknown | |
| | | n° | (%) | n° | (%) | n° | (%) | n° | (%) | n° | (%) | n° | (%) |
| 0.705 | 31.9 ± 18.9 (4-70) | 49/52 | (94.2) | 25/52 | (48) | 46/52 | (88.4) | 46/52 | (88.4) | 41/52 | (78.8) | 19/52 | (36.5) |

Tab. 1 - Characteristics of the HANE patients.

These observations concur with what has previously been reported in the literature[22]. In the case of an exclusively functional deficiency (15.5% of our patients, a percentage in good keeping with that reported by ROSEN et al.[35]), the physicochemical properties of the C1INH molecule are abnormal and vary among individuals. According to CARBONARA et al.[4] and DE MARCHI et al.[8], the alterations are the result of a series of point mutations capable of modifying the active site on the enzyme.

Electrophoretic mobility of C1INH in these patients varies widely and the enzyme is sometimes found complexed to albumin. Other studies of molecular biology involved the primary structure of C1INH, cDNA cloning and localization of the gene to chromosome 11[2,7]. In order to account for the quantitative deficiency (84.5% of our patients) being transmissible in an autosomal domi-

| | type I | type II | controls | p values | | |
|---|---|---|---|---|---|---|
| | | | | type I *vs* controls | type II *vs* controls | type I *vs* type II |
| C1INH (RID) (mg/dl) | 5.3 ± 3.2 (0-11) | 39.8 ± 10.3 (25-60) | 28 ± 10 (15-35) | <0.001 | <0.001 | <0.001 |
| functional C1INH (%) | 17 ± 6 (10-23) | 19.7 ± 7.9 (10-29) | 100 ± 30 (70-130) | <0.001 | <0.001 | ns |
| C4 (RID) (mg/dl) | 8.2 ± 5.9 (0-28.6) | 5.5 ± 5.6 (0-14) | 34 ± 12 (25-45) | <0.001 | <0.001 | ns |
| CH50 (U/ml) | 64.7 ± 47.1 (0-130) | 74.3 ± 53.2 (0-120) | 145 ± 17 (110-180) | <0.001 | <0.001 | ns |
| APA (U/ml) | 34.3 ± 10.6 (20-43) | 32.8 ± 10.9 (0-43) | 39 ± 8 (30-40) | ns | ns | ns |
| C3 (RID) (mg/dl) | 146.9 ± 30.2 (95-205) | 124.2 ± 40.4 (30-160) | 120 ± 40 (70-170) | ns | ns | ns |
| IgG (mg/dl) | 1,079 ± 254 (727-1,783) | 1,252 ± 377 (586-1,696) | 1,151 ± 281 (564-1,765) | ns | ns | ns |
| IgA (mg/dl) | 210 ± 87 (98-538) | 285 ± 135 (79-500) | 265 ± 115 (85-385) | ns | ns | ns |
| IgM (mg/dl) | 190 ± 109 (82-600) | 169 ± 72 (82-296) | 180 ± 95 (53-375) | ns | ns | ns |

RID = radial immunodiffusion; ns = not significant.

Tab. 2 - Laboratory findings in HANE patients. Values are expressed as mean ± SD; ranges are in brackets.

nant pattern, it is necessary to bear in mind that unlike other enzymes, C1INH becomes irreversibly bound to its substrate and is therefore consumed during complement activation reaction [10, 12]. Under normal conditions, continuous production of the inhibitor maintains serum levels within normal limits. As soon as synthesis is in any way reduced, there is a dramatic fall in serum C1INH levels as a direct result of its continual consumption. STOPPA-LYONNET et al. [36] recently proposed a pathogenetic mechanism common to both forms of HANE, by postulating that some structural properties of the C1INH gene make it prone to undergo occasional structural rearrangements. Although most of the alterations will result in a biosynthetic deficit of the protein (type I HANE) some of them, depending on the kind and extent of the molecular lesion, will allow the expression of a functionally impaired C1INH (type II HANE).

Considering solely HANE's vertical mode of transmission, in heterozygotes one would expect C1INH values near 50% of normal. However, we have observed enzyme levels reduced to 18.9% of normal, suggesting an increased C1INH catabolism in patients with an immunochemical defect. Conversely, the 9 patients who had a functional defect presented an average immunochemical value of C1INH somewhat higher than normal. The enzyme in this case is functionally inactive, as demonstrated by results of the heterolysis of N-acetyl-L-tyrosine-ethyl-ester or $C_2H_5CO$-Lys(e-Cbo)-Gly-Arg-pNa. No longer properly inhibited, the patient's complement system is consumed. Serum C4 levels were reduced as compared to controls, and the complement consumption was more marked in type II than in type I HANE. The chronic depletion of complement components seems to be preponderant in the early phases of activation along the classical pathway, whereas C3 is spared, with serum values approaching normal. Further confirmation of complement component depletion is given by CH50, in which average values fall far below the normal range. Moreover, in two seriously affected patients, one having type I and the other type II HANE, CH50 was undeterminable.

The tendency towards complement depletion in HANE is corroborated by the substantial reduction in CH50 after serum incubation in low ionic strength buffer. All the cases studied in this manner resulted in undetectable residual CH50, contrary to the mild reduction seen with normal sera. This technique may prove useful as a diagnostic tool in mass screening programs. In seriously affected patients, however, the method could not be used, inasmuch as they had undetectable CH50 levels *a priori*. In the light of what has been reported in this study, several observations can be made:

- a functional C1INH assay is the method of choice in the diagnosis of HANE: it is capable of revealing the disorder in 100% of cases;
- an immunochemical assay of C1INH will detect only type I HANE;
- an immunochemical assay of C4 is useful when C1INH functional tests are not available: this complement component is consistently reduced in both types of HANE;
- CH50 testing is of little diagnostic value since total hemolytic complement activity is reduced in a variety of other congenital or acquired pathologies involving the complement system;
- the CH50 assay after incubation in low ionic strength buffer may be utilized in mass screening programs for qualitative evaluation. However,

there is the drawback of the test not being applicable in cases of frank hypocomplementemia.

While the classical pathway of complement activation is chronically being depleted in most cases, the alternative pathway is not equally affected by the lack of C1INH. In fact, values obtained in patients were close to those from controls. Moreover, immunoglobulin levels were found to be within normal limits on the whole.

A positive family history of angioedema was encountered in 100% of our patients. This is not surprising, considering the heritable nature of the disorder. Allergic phenomena were typically absent from almost all patients' histories. This stresses the fact that the pathogenesis of HANE hinges on complement and/or complement-related multienzyme systems in the plasma and is not an IgE-mediated mechanism [13, 14, 19].

The conspicuous absence of allergy from the patients' histories may actually be attributable to patients' inaccuracy in remembering and relating of past medical disturbances, given the overwhelming preponderance of angioedema symptoms which dominate their accounts [16].

The age of onset of HANE was below 15 years in 33 out of 52 (64%) symptomatic patients studied. In 6 patients the diagnosis was made based on laboratory tests performed between the ages of 4 and 17 years and before any overt manifestation of the disease. Among symptomatic patients, the average age of onset was 13.4 years, ranging from 7 to 45 years.

The factors involved in bringing on an attack are manifold, but can be reduced to two principal areas: trauma (even of modest entity) and stress (mental and/or physical). However, certain patients were unable to ascribe some episodes to discernible causes, either mental or physical.

From a clinical point of view, trauma and stress often have an additive effect. Physical trauma can initiate an angioedema attack on its own, or act as an additional stress aggravating a preexisting anxiety state. Moreover, once prodromic symptoms appear heralding the onset of a crisis, the resulting anxiety is sufficiently stressful to precipitate a full-blown attack. In this case, the patient senses the on-coming edema, becomes agitated, realizes the gravity of the situation and often embarks on a desperate search for medications which rarely provide relief.

Stress alone is capable of provoking an episode of angioedema in patients who are particularly emotional or sensitive. The role of trauma in the etiopathogenesis of HANE is linked to the closely interacting relationships existing between complement and multienzyme systems in the plasma, particularly the coagulation cascade [3, 6, 17, 23, 24, 34]. The mechanism(s) of action involved in the case of mental stress causing a crisis is unclear [22].

Symptoms in the prodromic stage are mostly subjective. The patient may sense a kind of 'tension' at the site of an imminent attack, beginning a few minutes to several hours prior to the appearance of edema. On occasion, edema is preceded by a transient macular or serpentine erythema. Symptoms and signs of the full-blown attack are primarily determined by the characteristic edema. In the event of skin involvement, affected areas appear pale, are painless and non-pruritic, and on rare occasions present with a rash. The parts of the body most often involved are the face, particularly the lips and periorbital soft tissues, hands, feet and scrotum.

Of all possible mucosal sites, the most life-threatening situation is represented by laryngeal edema which, in the absence of i.v. injection of C1INH concentrate, often requires emergency tracheostomy. Warning signs of glottal involvement are changes in voice timbre or dysphagia[19]. Severe dyspnea and signs of acute respiratory distress are also characteristic during an attack. Mortality figures for laryngeal angioedema, based on information provided by the patients and regarding 34 of their relatives affected with HANE, reflect the gravity of the event: left untreated, it accounted for death in 2 men and 5 women (20.5%). Of the women, 4 (3 mothers and one daughter) died during childbirth. An explanation for the particular danger posed by childbirth is likely to be found in the decreased plasma concentrations of C1INH associated with pregnancy, even in healthy women. The precarious equilibrium existent in a woman with HANE is easily upset when the stress of labor and delivery supervenes. It is sufficient to bring on an acute episode.

On the other hand, the incidence of abdominal symptomatology was great, with patients reporting frequent bouts of colic, nausea, vomiting and diarrhea. These cases must necessarily be differentiated from acute abdominal crises of surgical interest and vice versa.

Although the frequency with which abdominal, cutaneous, laryngeal or other episodes occurred was variable, 19 patients reported 1 to 4 attacks per month, each lasting for about 60h. These patients were therefore symptomatic for 30 to 120 days out of the year.

*Associated pathology*: 2 of our patients were affected by associated lesions. One with HANE and lupus rash, the other with terminal uremia secondary to chronic membranoproliferative glomerulonephritis. This case was C3 nephritic factor (C3NeF)-positive, treated by maintenance hemodialysis and extensively illustrated in a previous report from our group[20].

SUMMARY

An in-depth analysis of clinical and laboratory findings in 58 patients affected by hereditary angioneurotic edema (HANE) is reported with special focus on problems related to the diagnosis of the disorder. The functional C1 inhibitor (C1INH) assay is the method of choice in the diagnosis of HANE, as it is capable of revealing the disorder with 100% accuracy. The immunochemical assay of C1INH detected HANE in 84.5% of the cases, i.e., immunochemical deficiency of C1INH (type I HANE). C4 was markedly reduced in both type I and type II HANE; thus, C4 levels can be particularly useful when C1INH functional tests are not available. CH50 testing is of little diagnostic value since total hemolytic complement activity is reduced in a variety of other congenital or acquired pathologies involving the complement system. The CH50 assay after incubation in low ionic strength buffer may be utilized in mass screening programs for qualitative evaluation. However, the test has the drawback of not being applicable in cases of frank hypocomplementemia. While a depletion of the complement classical pathway was detected in most cases, no alteration in the complement alternative pathway was recorded, nor there was any reduction in immunoglobulin levels. Family history was positive in 100% of the cases. Attacks were almost always brought on by stress and/or trauma, though the causes were sometimes unknown. Edema could be cutaneous (non-pitting and non-pruritic) in 94.2%, laryngeal (often life-threatening) in 48% and abdominal (almost always painful) in 88.4% of patients. Associated pathologies were found in 2 patients, i.e., lupus rash and C3NeF-positive chronic membranoproliferative glomerulonephritis, respectively.

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