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C1 Inhibitor and Diagnosis of Hereditary Angioedema in Newborns

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ABSTRACT. Symptoms of hereditary angioedema may present during the child's first years. Attacks may be a particular threat to the narrower airway of the child. An early diagnosis is most valuable because effective C1 inhibitor (C1 INH) concentrate is available. We present a reference area for the antigenic and functional determination of C1 INH by using uncontaminated umbilical cord blood from 80 normal newborns collected by puncturing vessels in the newly delivered placenta. We examined two full-term babies (1 and 2) from mothers with hereditary angioedema type I the same way. The concentration of C1 INH antigen was determined by radial immunodiffusion. The C1 INH functional assay was based on the addition of a known quantity of C1s, which enzymatically splits a chromogenic substrate. The test was performed in the presence of methylamine and heparin in a kinetic microtiter plate assay. Citrated plasma was used in both assays. The data obtained in the 80 cord blood samples (2.5–97.5 percentile) were 0.11–0.22 g/L for C1 INH antigen (adults, 0.15–0.33 g/L) and 47.2–85.9% for C1 INH function (percentage of adults). In cord blood, baby 1 had an antigenic value of 0.12 g/L (7.5 percentile) and C1 INH function of 61.8% (42 percentile). The corresponding values for baby 2 in cord blood were less than 0.05 g/L (0.106 g/L < 2.5 percentile) and 34.3% (12.9% < 2.5 percentile). Baby 2 had markedly lower C4 values yet much higher C4 activation products than baby 1. At 4 mo, baby 1 had an antigenic C1 INH value of 0.24 g/L. At 6 mo, baby 2 had an antigenic value of 0.13 g/L, which is considerably low for the age. At 19 mo of age this child had abdominal pain, distension, and massive amounts of watery diarrhea. C1 INH concentrate (500 U) was administered, and 4 wk of symptoms resolved within 6 h. This work supports the assumption that the diagnosis of hereditary angioedema can be made at delivery by assessing C1 INH antigen and function. (*Pediatr Res* 35: 184–187, 1994)

Abbreviations

C1 INH, C1 inhibitor
HAE, hereditary angioedema
AU, artibrary units

HAE is a rare disease inherited as an autosomal dominant trait. It is caused by lack of functional C1 INH, a pivotal protein in the regulation of the kallikrein-kinin system and early part of the complement cascade. HAE exists in two forms: type 1 has reduced amounts of C1 INH protein, and type II has the antigenic concentration of C1 INH that is normal but mostly consists of a dysfunctional protein.

Patients with HAE may experience various degrees of colicky vomiting and s.c. swelling in nearly every part of the body, even brain edema (1). Sometimes annular or serpentine-like rash heralds attacks. Submucous edema in the throat may proceed to fatal laryngeal edema, even in infancy (2).

The edema persists for about 2 to 5 d and is often experienced after a history of emotional upset, infections, or traumas (1). Tooth extraction has led to several serious attacks of laryngeal edema and death (3). The disease therefore requires special attention during events commonly encountered in childhood, such as tonsillectomy, intubation of the larynx, or laryngitis. The edema is caused by extravasation of fluid through wrinkled and leaky endothelial cells in the postcapillary venules, most probably the result of excess bradykinin formation (4) and/or a peptide liberated from C2 (5, 6). Conventional antiallergic therapy in the form of antihistamines, corticosteroids, or epinephrine is insufficient. Edema affecting the throat should be treated by i.v. injection of C1 INH concentrate (1), which the proteins should always have with them.

A relatively large family in northern Norway has HAE. Most of the affected children have had their first typical symptoms at 3–4 y of age. In one case, however, a mother did notice different phonation, edema of the mouth and tongue, and enlargement of the uvula in her 6-mo-old baby. This condition arose during a period of primary teeth eruption. An early diagnosis of HAE can therefore be of vital importance, and thus we examined the possibility of making the diagnosis at the time of delivery. For practical and ethical purposes we decided to use umbilical cord blood to establish a reference area for C1 INH antigen and function.

Some studies have assessed antigen C1 INH concentrations in umbilical cord blood (7–9). The antigen concentration of C1 INH in newborns is about two thirds the concentration in adults (adults, 0.15–0.33 g/L). Most complement values, C1 INH included, increase considerably during the child's first few days, rapidly surpassing adults (10). To our knowledge no reference values for the common functional assay of C1 INH in newborns exist. Functional assays detect even the rarer form of HAE (type II). The aim of the present study was to establish a range for the function of C1 INH in newborns and to see whether a diagnosis could be made in two babies born to mothers with HAE.

MATERIALS AND METHODS

Samples were taken from 100 consecutive newborns during spring 1991. Eighty of these infants who fulfilled the following criteria were selected for analysis: *1*) born at term, *i.e.* ± 14 d

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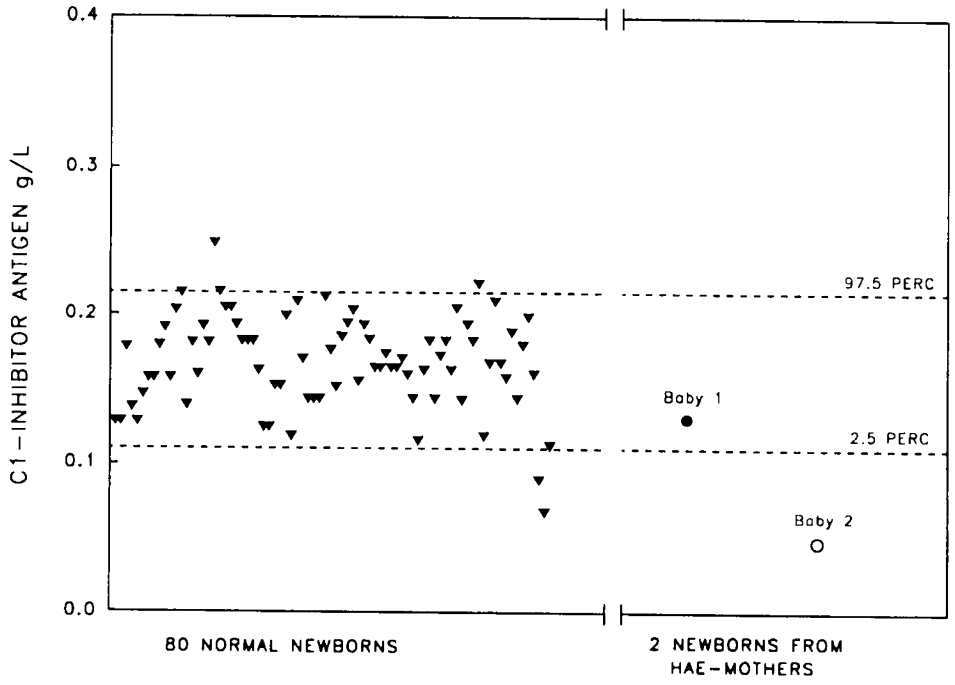

Fig. 1. Concentration of C1 INH antigen in umbilical cord blood from 80 normal newborns and two babies born to mothers with HAE type I. *Dotted lines* represent upper and lower reference values as defined by 97.5 and 2.5 percentiles, respectively.

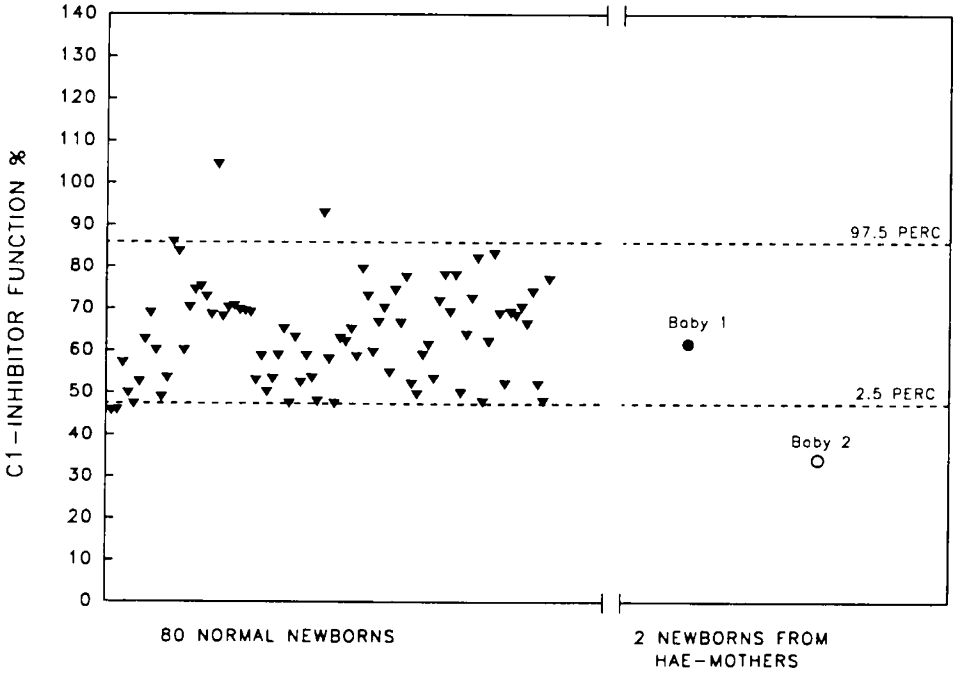

Fig. 2. Functional activity of C1 INH in umbilical cord blood from 80 normal newborns and two babies born to mothers with HAE type I. Values are in percentages of those in normal adults. *Dotted lines* represent upper and lower reference values as defined by 97.5 and 2.5 percentiles, respectively.

(term was estimated by ultrasonography in gestational wk 17 or by use of Naegele's rule); *2*) birth weight > 2500 g; *3*) Apgar score at 5 min > 8; *4*) no signs of maternal infection; and *5*) no maternal medication except for a short period when four mothers were using antibiotics, oxazepam, or terbutaline. Two babies born to mothers with HAE type I were included. They fulfilled the above mentioned criteria. Vessels in the umbilical cord were punctured with an 18-gauge needle near the basis of the fetal part of placenta shortly after delivery of placenta. Approximately 15 mL of blood was drawn into a 20-mL plastic syringe (Terumo, Leuven, Belgium) and distributed into siliconized Vacutainer tubes (Vacutainer, Becton Dickinson, France) containing 0.125 M sodium citrate. Plasma was obtained after centrifugation at 800 × *g* for 10 min and frozen in aliquots at −70°C in Nunc Cryo Tubes (Nunc, Roskilde, Denmark). The regional ethical committee approved the study.

C1 INH antigen concentration in citrated plasma was determined with radial immunodiffusion using the Nor-Partigen kit kindly provided by Behringwerke AG (Marburg, Germany). The diameter after radial immunodiffusion of the sample was correlated to a standard curve in a semilogarithmic plot.

Functional C1 INH activity in citrated plasma was assayed in microplates by modification of a procedure described by Munkvad *et al.* (11) and by using reagents in a commercial kit kindly

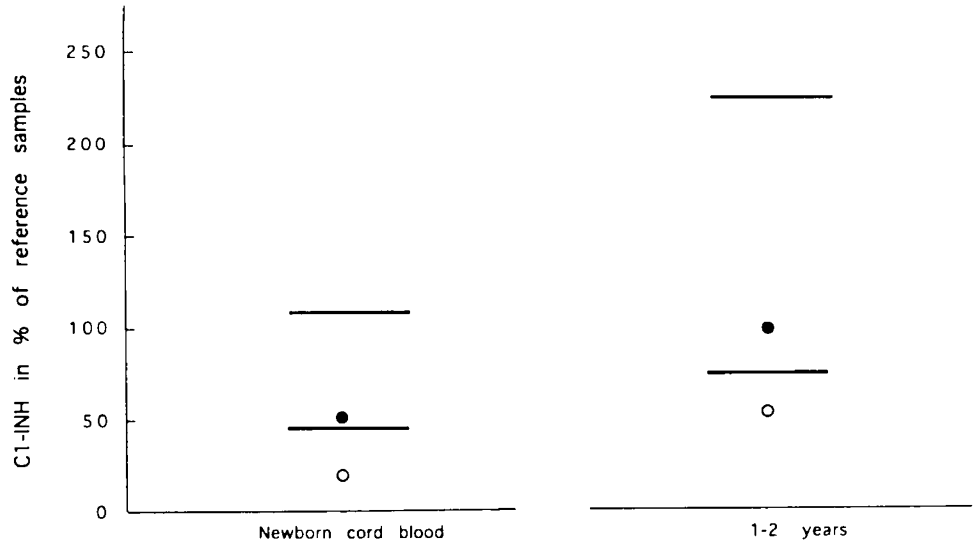

Fig. 3. Concentration of C1 INH antigen in cord blood from two babies born to mothers with HAE type I. *Left*, Baby 1, *filled circle*; baby 2, *open circle*. *Horizontal bars* depict ± 2SD in our reference material based on cord blood from 80 newborns. *Right*, the same babies' values at 4 and 6 mo are inserted into the reference area presented by Roach *et al.* (10) for the age period 1–2 y.

provided by Behringwerke AG. Briefly, plasma was diluted three times in an imidazole buffer with the addition of 0.12 M methylamine hydrochloride and 3000 IU/L heparin (final concentrations). Ten microliters of diluted plasma and 100 μL of C1s solution were incubated at room temperature for 1 min in microplate wells. Sixty microliters of chromogenic substrate (MeOC-Lys(Cbo)-Gly-Arg-pNA) were added to a final concentration of 0.5 mM to assay the excess C1s not inhibited by plasma. The increase in absorbance at 410 nm was recorded by repeated measurements in a microplate reader, and the rate of the reaction during the 5 min immediately after substrate addition was calculated. The functional concentrations of C1 INH in the tests were calculated according to a reference curve based on dilutions of normal citrated pool plasma.

Quantification of C4. C4 antigen was measured by two different methods. The first method involved nephelometric quantification by the Behring nephelometer with their routine C4 reagents. The amount is given in grams per liter. The second method involved enzyme immunoassay with goat antiserum to C4 (Quidel, San Diego, CA) as capture antibody and a murine MAb to C4c (Quidel) as detection antibody. The amount is given in percent of a normal standard (100%).

Quantification of C4 activation products. An MAb against C4c, also expressed in C4b and iC4b, was used as capture antibody in an enzyme immunoassay. The antibody was a kind gift from C. E. Hack and has been characterized elsewhere together with the method (12). The amount is given in AU per milliliter according to a standard made by activation of a serum pool with heat-aggregated immunoglobulins and defined to contain 1000 AU/mL.

RESULTS

Figure 1 shows the distribution of C1 INH antigen values from 80 normal newborns and two babies born to affected mothers. Median C1 INH concentration was 0.17 g/L, and the 2.5–97.5 percentile reference range was 0.11–0.22 g/L. Baby 1 had 0.12 g/L (7.5 percentile), whereas baby 2 had less than 0.05 g/L (0.106 g/L < 2.5 percentile).

Figure 2 shows the corresponding C1 INH functional values. The values refer to percentage of a pool of healthy adult blood donors. Median value was 64.5%, and the 2.5–97.5 percentile reference range was 47.2–85.9%. Baby 1 had a C1 INH value of 61.8% (42 percentile), and baby 2 had a C1 INH value of 34.3% (12.9% < 2.5 percentile) based on five independent measurements.

No significant correlation was observed between the C1 INH values and weight or between C1 INH and time from birth to collection of blood (correlation coefficients: 0.03 to 0.17, Spearman's rank correlation). Thus, this material showed no deterioration of C1 INH as an effect of time elapsed (range, 2–59 min). The correlation coefficient between the functional and antigenic C1 INH values was 0.69 ($p < 0.0001$).

The two babies from affected mothers were reexamined for C1 INH antigen concentrations after 4 mo (baby 1) and 6 mo (baby 2). The concentrations rose markedly during this period from 0.12 g/L to 0.24 g/L in baby 1 and from less than 0.05 g/L to 0.13 g/L in baby 2, a definite subnormal concentration for the age (Fig. 3).

The concentration of C4 in cord blood from baby 1 was 0.15 g/L and 76%, respectively, in the two different methods used. The corresponding values in baby 2 were 0.06 g/L and 19%. C4 activation products (C4b/ib/c) were 29 AU/mL in baby 1 and 86 AU/mL in baby 2. Thus, the ratio between C4b/ib/c and C4 was 0.39 for baby 1 and 4.34 for baby 2.

DISCUSSION

The normal procedure for collecting umbilical cord blood by holding the opened tubes under a dripping cord permits contamination of urine, amnion fluid, maternal blood, feces, Wharton's jelly, or talcum from gloves. Such contaminants are probable candidates for complement or contact activation by which C1 INH might be consumed. This situation made us establish the method of puncturing the fetal vessels in the newly born placenta for collection of blood. A possible disadvantage of this method is the longer period of time from when the baby is born to when the blood sample is drawn (median, 14 min), which might lead to a decline in C1 INH values. However, this material showed no such deterioration.

The two mothers with HAE both received 1000 U of C1 INH concentrate intravenously 2–4 h before delivery. The 105-kD protein is unlikely to cross the placenta, and to our knowledge no active transport has been reported for C1 INH across the placental barrier. The substitution of the mothers are therefore not expected to interfere with the interpretation of the data.

Our results are in accordance with others in that complement values for term infants are generally in the range of 60 to 100% of adult normals (13). The reference area obtained for functional

C1 INH is almost identical to the antigenic concentration presented by Johnson *et al.* (8), who referred to percentage of adult values, and Ferlazzo *et al.* (7), who compared them with their adult values.

Our analyses were performed in citrated plasma (1 mL sodium citrate/9 mL blood). It is generally assumed and in accordance with observation in our laboratory that all the isotonic sodium citrate additive stays extracellularly and dilutes plasma only. This observation is important because newborns exhibit the exceptionally high hematocrit value of 0.6 (14), and the citrate would thus contribute to a relatively higher dilution in this plasma as compared with adults (hematocrit value, 0.42). Conceivably, it is possible that the amount of protein in 5 mL of citrated plasma from newborns corresponds to approximately 4 mL of serum or EDTA plasma. Protein concentration in serum would accordingly be 25% higher than in citrated plasma or, conversely, citrated plasma concentrations 80% of serum concentrations. This aspect should be kept in mind when comparing different reference values.

We present a reference area for C1 INH antigen and function. The method of umbilical cord blood sampling allows uncontaminated collection and yields generous amounts of blood for supplementary analyses. One of the two babies we examined who were born to mothers with HAE had low C1 INH and C4 values, the C4 mainly consisting of degradation products. At 19 mo of age this child had abdominal pain, distension, and massive watery diarrhea. He was admitted to a hospital once during this period, where no cause was found. The symptoms persisted for 4 wk until 500 U of C1 INH concentrate was administered i.v., after which the symptoms disappeared within 6 h. He has remained well since that time. On the basis of clinical and biochemical data, we consider this child to be affected. This work supports the assumption that the diagnosis of HAE can be made at delivery by assessing C1 INH antigen and function.

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