The effect of hormonal changes on the clinical manifestations of hereditary angioedema caused by C1-inhibitor deficiency

PhD thesis

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1. INTRODUCTION

Hereditary angioedema caused by the deficiency of the C1 inhibitor (C1-INH-HAE) is a rare disorder of autosomal dominant inheritance. It is characterized by recurrent, acute episodes of subcutaneous and/or submucosal edema formation, resulting from the release of bradykinin. A number of genetic mutations leading to functional C1-INH deficiency have been identified. To date, no correlation could be demonstrated between genotype and the clinical phenotype of the disorder. By contrast, the functional activity of C1-INH (C1-INH$_f$) correlates with disease severity.

The location, frequency, and severity, as well as the time of their initial onset and the duration of individual episodes may vary greatly. Therefore, it exhibits substantial intra- and inter-individual variations even within the same family. Edematous attacks can be induced – among others – by mechanical trauma, psychological stress, infections, surgical and diagnostic procedures performed in the head-neck region, certain medicinal products (e.g. ACE inhibitors). Fluctuations in the sex hormone balance of the body in puberty and during menstrual periods, or the use of estrogen-containing oral contraceptives, hormone replacement therapy, and pregnancy are additional, possible triggering factors. The time of onset, the severity, and the location of these episodes are not predictable. Prophylaxis with antifibrinolytic agents and attenuated androgens may reduce the number of edematous attacks. The exact mode of the action of attenuated androgens is not known; their use is empirical. These agents enhance the hepatic of plasma proteins, along with the expression of mRNA for C1-INH in peripheral blood mononuclear cells (PBMC). Androgens have been shown to reduce thyroxin-binding globulin (TBG) levels, and this is followed by an increase in basal metabolism. Changes of free thyroid hormone levels within the normal range have been observed in relation to this effect.

The clinical symptoms of C1-INH-HAE may cause a possibly life-threatening condition. Moreover, the prophylactic agents in current use are contraindicated or not recommended during pregnancy and breastfeeding.

As a result, patient management is often a great challenge for medical professionals. According to the international therapeutic guidelines, the only option for the management of pregnant women with C1-INH-HAE is to administer plasma-derived (pd)C1-inhibitor concentrate. Nevertheless, only limited data have been published – mainly as case reports – on this treatment modality. A search of the literature revealed just a single conference abstract on a prospective follow-up study of 22 pregnant women with C1-INH-HAE. The US FDA allocated pdC1-INH concentrate to pregnancy category 'C'.

Endocrine imbalance other than that of the sex hormones (such as overt and subclinical thyroid disease) may also be responsible for the worsening of angioedema symptoms. Thyroid hormones stimulate the synthesis of many plasma proteins (including complement factors) by controlling cellular metabolism. Only a few publications have contributed data on the relationship between hereditary angioedema and thyroid function. These papers are focused on the occurrence of immunoregulatory disorders – among others thyroiditis – in C1-INH-HAE. In autoimmune diseases, the increased formation of immune complexes may lead to enhanced consumption of C1-INH, and may thereby worsen the symptoms of C1-INH-HAE. According to clinical experience, treatment with thyreostatic agents, or supplementation with thyroid hormones is accompanied by a decrease in the number of edematous episodes. No reports have been published on any possible relationship between endocrine profile and complement levels, as well as the number of angioedematous episodes in C1-INH-HAE patients with normal thyroid function. Similarly, the effect of long-term prophylaxis with attenuated anabolic steroids on thyroid function has not yet been reported in C1-INH-HAE.

2. OBJECTIVES

Clinical experience is limited on the evolution of angioedematous episodes, as well as on the course and on the influencing factors of the pregnancies and postpartum periods in women with C1-INH-HAE. No data is available on the relationship among thyroid hormones and the complement levels characteristic of C1-INH-HAE, and the impact of the former on angioedematous symptoms. Thus, our study aimed to answer the following questions:

1. Is the percentage of premature births and gynecological complications different between pregnant women with C1-INH-HAE, and the female Hungarian population?

2. Is there any change in the clinical course, frequency, location, and severity of edematous attacks during the three trimesters of pregnancy and in the postpartum period? If yes, then to what extent are these influenced by known triggering factors, or by the presence of a foetus with inherited C1-INH deficiency?

3. Is pdC1-INH concentrate effective and safe for acute therapy and for prophylaxis in female patients with C1-INH-HAE?

4. Is our group of C1-INH-HAE patients with normal thyroid function different from the healthy, Hungarian general population?

5. Is there any correlation among the complement and thyroid parameters, as well as the clinical symptoms of C1-INH-HAE patients?

6. Are there any differences between thyroid function in C1-INH-HAE patients treated/not treated with danazol?

7. Are the indices suggestive of the activation of the fibrinolytic system correlated with the parameters of thyroid function in patients with C1-INH-HAE?

All these questions focus on exploring the role of hormonal influence – which is important in the pathomechanism of C1-INH-HAE and its relationship with clinical manifestations, as well as at the identification of new possible triggering factors.

3. METHODS

The group of pregnant women with C1-INH-HAE, the implementation of the study, and considerations for the processing of data

We have reviewed the data of the 72 female patients registered with the Hungarian Angioedema Center. Our retrospective study analyzed the course of 118 pregnancies of 41 female patients in the period between 1979 and 2009.

Data collection was performed with questionnaires, from sources including the National C1-INH-HAE Register, medical records, hospital discharge notes, and patient diaries. Data extraction focused on the frequency, location, and severity of edematous episodes; on factors with possible influence on pregnancy, its course, or on the period of breastfeeding; as well as on the therapy administered for angioedema attacks before or during pregnancy or breastfeeding. We also surveyed the location and severity of edematous episodes that had occurred during the 2-year period before pregnancy, during pregnancy, and in the postpartum period. We rated the attacks also by trimesters, using the following scoring system: 0 = symptom-free trimester; 1 = mild cutaneous or abdominal attacks; 2 = severe cutaneous, moderate-to-severe abdominal, or laryngeal edema. The study was conducted with the approval of the Regional Research Ethics Committee; informed consent had been obtained from all subjects.

The evaluation of thyroid hormone and complement parameters; patient data

Our retrospective, case-controlled study analyzed the data accumulated by the National Angioedema Center in 2011. According to the family screening, the evaluation of clinical symptoms and of complement parameters (C1q, C4, C1-INH antigenic and functional level), all the 146 patients registered with the Center had hereditary C1-INH deficiency. The time of onset, location, and severity of the attacks, as well as the administered therapy were ascertained by reviewing the entries of patient diaries, and the medical information recorded at the follow-up visits. On these occasions, we determined (in symptom-free subjects) thyroid hormone levels (thyrotropin [TSH], free thyroxine [fT4], free triiodothyronine [fT3], anti-thyroid antibodies (anti-thyroglobulin [a-TG], and anti-thyroid peroxidase [a-TPO]), and complement parameters (C4, antigenic and functional C1-INH levels). Additionally, fibrinogen, D-dimer, an prothrombin fragment 1+2 (F1+2) levels were determined in a limited number of subjects.

In the group of healthy controls, we measured the levels of thyroid hormones (TSH, fT4, and fT3), anti-thyroid antibodies (a-TG, a-TPO). Patients younger 16 years of age (n=16), and those with any overt or subclinical thyroid disease (n=13) were excluded from the study. Only individuals with a normal thyroid profile, hereditary C1-INH deficient patients (with parameters in the normal reference range for age and clinical status), and healthy controls were enrolled.

We examined 117 euthyroid, C1-INH deficient individuals (107 had type I, 10 had type II C1-INH HAE), 52 (44.4%) of them were males and 65 (55.6%) were females; their mean age was 39.7 years (range: 16 to 81 years). Subgroups were defined by danazol treatment.

The sex- and age-matched control group consisted of 150 healthy, euthyroid individuals: 70 (46.7%) males, 80 (53.3%) females with a mean age of 38.5 years (range: 16 to 73 years). The controls were free of known disease, they were not receiving medicinal products, and their diagnostic evaluation did not reveal clinical or laboratory findings suggestive of C1-INH-HAE. The study was approved by the Institutional Review Board of Semmelweis University.

Laboratory methods

The serum concentrations of antigenic C1-INH and of C4 were determined with a method based on radial immunodiffusion. Functional C1-INH levels were measured with ELISA, using a C1-Inhibitor Enzyme Immunoassay kit

(Quidel, San Diego, CA, USA). A Liaison chemiluminiscence immunoassay analyzer (DiaSorin SpA, Saluggia, Italy) was applied to quantify serum TSH, fT4, fT3, anti-TPO, and anti-thyroglobulin levels. Fibrinogen level was determined with the Clauss method. Commercial kits were applied to measure F1+2 concentration (Enzygnost) and D-dimer level (Innovance D-dimer kit), according to the manufacturers' instructions.

Statistical analysis

Statistical analyses were performed with the SPSS software package (version 20, SPSS Inc., Chicago, Illinois, USA). As the variables followed non-normal distribution, non-parametric tests were applied. The chi-square test and Fisher's exact test were used to assess the relationship of two variables within the sample. Friedman's two-way analysis of variance by ranks was performed for dependent samples. Two or more independent subgroups were compared with the Mann–Whitney, or the Kruskal-Wallis tests, whereas the Spearman's correlation coefficient was applied to calculate correlations. All statistical analyses were two-sided, and $p < 0.05$ indicated a statistically significant difference.

4. RESULTS

4.1. The characteristics of the pregnancies in study subjects

The outcomes of the 118 (including 2 twin) pregnancies of the study subjects with C1-INH-HAE were as follows: 84 neonates (40 boys and 44 girls) were born and 36 abortions (9 spontaneous and 27 artificial) occurred (***Figure 1***). The percentage of spontaneous abortions among female patients with C1-INH-HAE was similar to that seen in the Hungarian general population (7.6% *vs*. 25%).
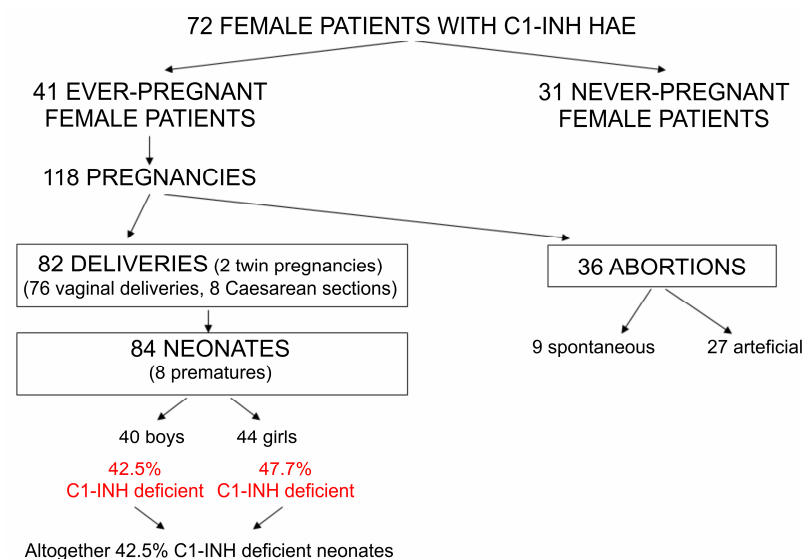

Figure 1: The demographic properties of female patients with C1-INH-HAE and their neonates.

4.2. Full-term pregnancies *(n=82/118)*

At the time of delivery, the median age of these 82 women was 25 years (range: 18 to 42 years), and they gave birth to 2 neonates on average. Stillbirths did not occur. There were 8 premature births (9.7%) – this corresponds to the population average (8 to 12%). Half of the premature neonates were C1-INH deficient. Delivery usually occurred on the 38th to 39th weeks of gestation; 76 subjects (90%) delivered by the vaginal route, and 8 (10%) had Caesarean section. The latter procedure was not preformed more frequently on our pregnant subjects with C1-INH-HAE than in the Hungarian general population (10% *vs*. 25%).

4.3. The frequency and location of edematous attacks

The disease symptoms worsened in 48% of patients with C1-INH-HAE, whereas pregnancy was associated with the attenuation of attacks in 33% of cases. In 19% of the pregnancies, the ensuing physiological changes had no effect on attack properties, and – compared with the preceding 2-year

period – the mean number of episodes remained unchanged during the year of the pregnancy. In total, 44% of full term pregnancies were symptom-free (*Figure 2/a*). In the remaining 56% (n=46), the 26 women, who gave birth to 48 babies reported angioedematous attacks that had occurred in various locations.
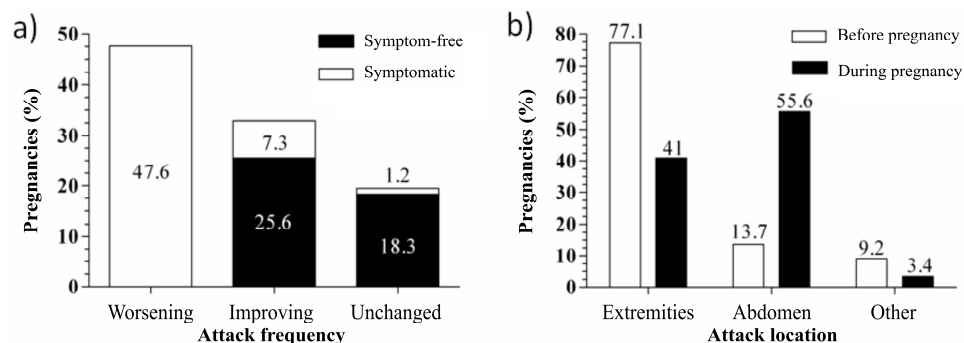

Figure 2: The impact of pregnancy on attack frequency *(a)*. The distribution by location of angioedema episodes occurring before and during pregnancy *(b)*.

During the period before the pregnancy, angioedema occurred mainly (77%) on the extremities. Abdominal attacks predominated (57%) during pregnancy, either as abdominal only episodes (37%), or combined occurrences of abdominal and extremital edema (19%). In the case of the latter, the location exhibiting the most severe symptoms were taken into account, as these required intervention for relief (*Figure 2/b*).

A comparison of the locations of the edematous episodes occurring before and during pregnancy in the same female subjects revealed that the frequency of abdominal attacks was significantly higher during pregnancy (χ^2 test, $p<0.0001$).

4.4. The time of the onset and the characteristics of edematous episodes

Angioedematous episodes occurred in the first, second, and third trimesters in 40, 31, and 31 (49%, 37%, and 37% of) pregnancies, respectively. Considering the severity and the location of the attacks occurring in each pregnancy, the first trimester was the most critical period. However, the absolute number of angioedema symptoms was the greatest in the third trimester (**Table 1**). In female patients with C1-INH-HAE, an attack frequency of 17 episodes per year was typical during pregnancy, whereas mean annual attack frequency was as low as 4 episodes per year during the period before the pregnancy. Thus, pregnancy was accompanied by a significant, 4.2-fold increase in attack frequency (Mann–Whitney test, $p=0.0392$).

Seventy-eight per-cent (n=21/27) of our multiparous subjects reported that their pregnancies were similar with regard to the frequency and severity of the attacks experienced. This was confirmed by the symptom scores recorded during our survey. In 7 subjects, an edematous episode occurred during 10 terms. In 6 of these cases, mild, subcutaneous edema formation was limited to the extremities, and only one patient suffered genital edema, which did not interfere with uneventful vaginal delivery. Laryngeal edema developed in one subject, whereas two others experienced simultaneous abdominal and extremital edema-formation. In the postpartum period, a transient increase in attack frequency was observed in a smaller proportion (n=6) of our subjects, mainly in the form of abdominal attacks. In these 7 patients, 5 extremital, and 20 abdominal edematous attack occurred within a period as short as 4 months only.

Table 1: The rating of the trimesters by the occurrence of C1-INH-HAE attacks.

	1st trimester 0 to 12 weeks of gestation	2nd trimester 13 to 24 weeks of gestation	3rd trimester 25 to 40 weeks of gestation	Whole term of pregnancy	Postpartum period (4 months)
Pregnancies with attacks	40	30	31	10	6
Mean attack severity (SD)	0.585 (0.666)	0.402 (0.564)	0.561 (0.787)	0.304 (0.628)	0.260 (0.681)
Mean attack number (SD)	3.78 (7.07)	2.29 (5.40)	6.59 (12.39)	0.12 (0.33)	0.28 (0.45)

A statistically significant fluctuation was observed in the numbers and severity of the attacks occurring during pregnancy (Friedman's test, $p=0.0022$, and $p=0.0092$, respectively).

4.5. Time of the onset of the initial angioedema attack, and changes in the number of edematous episodes during pregnancy

The number of edematous attacks was significantly (Mann–Whitney test, $p<0.0001$) greater during pregnancy in subjects who had suffered their first episode in their lives before the age of 8 years than in those whose disease manifested itself at a later age (**Table 2**).

The Kruskal–Wallis test showed a strong, significant difference between subjects with the first onset of angioedema attacks at 0 to 8, or at 9 to 18 years of age. This difference was still significant (although it was below the borderline of statistical significance) between the age groups of 0 to 8 and over 18 years of age, in favor of the former group. As major physiological changes in sex hormones begin after the age of 8 years, we have chosen this age as the cut-off point.

Table 2: The number of angioedema episodes occurring during pregnancy in the subgroups defined by the time of the onset of the initial attack.

Mean symptom scores (±SD) by age at the initial onset of symptoms (n of pregnancies)	1ST TRIMESTER	2ND TRIMESTER	3RD TRIMESTER	TOTAL SYMPTOM SCORE mean (±SD)	p-value (Kruskal-Wallis test)	p-value subgroups 0-8 vs. 9-18 years of age	p-value subgroups 0-8 vs. >18 years of age
0 to 8 years (28 pregnancies[a])	**7.46** (10.218)	**5.61** (8.13)	**15.61** (16.63)	**28.71** (27.956)			
9 to 18 years (27 pregnancies)	**1.48** (2.901)	**0.48** (1.477)	**2.41** (6.71)	**4.37** (7.596)	0.0006	<0.001	<0.05
>18 years (23 pregnancies)	**2.65** (4.292)	**0.78** (1.565)	**1.65** (3.737)	**5.09** (8.028)			
78+4[b] pregnancies in total							

[a] Including 2 twin pregnancies.
[b] These two subjects (with 2 pregnancies each) are still symptom-free (they never had an attack).

4.6. The effect of triggering factors during pregnancy

Angioedema symptoms occurred significantly less frequently during the third trimester in patients in whom menstrual periods had been identified as a triggering factor of edematous episodes. Those subjects by contrast, who pinpointed mechanical trauma as a triggering factor, had significantly more episodes during all three trimesters than those not susceptible to this possible trigger. The correlation was the strongest in the third trimester ($p<0.0001$).

4.7. The influence of the fetus on the angioedematous symptoms of the mother

The transgenerational transmittance of C1-INH-HAE was 45% (n=38/84), which corresponds to the rate one would expect based on Mendelian inheritance. According to our observation, the presence of a C1-INH deficient fetus was associated with a significant increase in the number of edematous episodes during the third trimester ($p=0.039$, **Table 3**). However, we did not find any correlation between the gender of the fetus as well as the severity and the frequency of maternal angioedema symptoms during the pregnancy.

Table 3: The influence of a C1-INH deficient fetus on angioedema symptoms experienced by the mother during pregnancy.

Trimester	Mean attack number (±SD)		P-value (Mann–Whitney U test)
	Mothers pregnant with a non-C1-INH-deficient fetus (n=46)	Mothers pregnant with a C1-INH deficient fetus (n=38)	
1st	3.54 (8.07)	5.03 (7.77)	0.076
2nd	1.35 (2.35)	3.47 (7.43)	0.166
3rd	5.07 (11.55)	9.08 (13.86)	**0.039**

All values are means (±SD); n = the number of full-term pregnancies

4.8. The efficacy and safety of human plasma-derived C1-INH concentrate

When available, we used pdC1-INH concentrate exclusively to relieve angioedema occurring during pregnancy. In total, 30 female patients received altogether 91 (500-IU) vials of human pdC1-INH concentrate as acute therapy or prophylaxis, during their 118 pregnancies (**Table 4**).

Table 4: The utilization of pdhC1-INH during pregnancy, delivery, and in the postpartum period (each vial of pdC1-INH concentrate contains 500 IU).

	FULL TERM PREGNANCY	PREGNANCY TERMINATED BY ABORTION	DELIVERY	POSTPARTUM & BREASTFEEDING PERIOD
N° of vials administered:				
RELIEF OF ACUTE EPISODES	36	8	0	12
SHORT-TERM PROPHYLAXIS	0	6	9	0
LONG-TERM PROPHYLAXIS	20	0	0	0
ALL INSTANCES [vials]	56	14	9	12
91 vials altogether				

The relief of acute symptoms started within 15-60 minutes of dosing. When used for prophylaxis, pdC1-INH prevented angioedema in every instance. Worsening of the symptoms or the recurrence of episodes did not occur. We administered long-term prophylaxis to 2 subjects, in whom severe edematous attacks occurred just about every other day, after they had discontinued prophylactic danazol upon becoming pregnant. None of the mothers treated with pdC1-INH gave birth to a premature neonate. Spontaneous abortions were not related to the use of pdC1-INH. All of our female patients treated with pdC1-INH delivered a healthy (aside from inherited C1-INH deficiency) neonate. Viral transmission (of HBV, HCV, or HIV) did not occur.

4.9. Parameters of thyroid function in C1-INH-HAE patients and in healthy controls

Table 5 summarizes the parameters of thyroid function in the 117 ptients with hereditary C1-INH deficiency, and in healthy controls.

TSH and anti-TG levels were not different between the patients and the controls. In C1-INH deficient subjects, the levels of fT4 and of fT3 were significantly lower, whereas anti-TPO levels were significantly higher (although still within the reference rage) than in the controls.

Table 5: Thyroid hormone levels of C1-INH-HAE patients and of healthy controls (IQR: interquartile range)

Parameters of thyroid function (reference range)	Median (IQR)		P-value (Mann-Whitney U-test)
	CONTROL group (n=150)	C1-INH-HAE patients (n=117)	
TSH (0.3-3.6 mU/L)	1.6 (1.0 – 2.2)	1.5 (1.0 – 2.2)	0.72
fT4 (10.9-21.9 pmol/L)	15.2 (13.3 – 17.1)	14 (12.5 – 16.0)	**0.002**
fT3 (3.4-6.5 pmol/L)	5.1 (4.5 – 5.7)	4.6 (4.2 – 5.0)	**<0.0001**
a-TG (5-100 IU/mL)	6 (2.0 – 13.5)	5 (5.0 – 5.3)	0.22
a-TPO (1-16 IU/mL)	1.5 (0.3 – 4.2)	2.5 (1.5 – 5.2)	**<0.0001**

(Only subjects with thyroid hormone levels normal for age and clinical status were analyzed.)

4.10. The relationship between thyroid and complement parameters in patients with C1-INH-HAE

The possible relationship between thyroid and complement parameters were studied in parallel blood samples from 115 C1-INH-HAE patients. The C1-INH$_f$ levels of 2 subjects were unavailable.

We studied the proportions of patients with C1-INH$_f$ levels below or above the median (30%) among subjects with a normal fT4 level below or above the median. The percentage of patients with decreased C1-INH$_f$ activity was greater among subjects with low-normal than among those with high-normal fT4 levels (37/58 ill. 24/57; p=0.020; df: 1; Pearson's χ^2 value: 5.429). With low-normal fT4 levels, the risk of a decreased C1-INH$_f$ level was 2.4 times of that seen in patients with high-normal fT4 (OR [95% CI]: 2.42 [1.14-5.13]). The comparison of C1-INHa and free thyroid hormone levels did not reveal any correlation (p=0.968), and we found only a marginal relationship between C4 and fT4 levels (p=0.054; df: 1; Pearson's χ^2 value: 3.726).

4.11. Thyroid parameters of C1-INH-HAE patients by the numbers of angioedema episodes

The median number of angioedema episodes occurring in our 117 C1-INH-HAE patients in a year was 4. Therefore, we divided the study population

into two subsets with an attack frequency of ≤4, or >4 per year and then, analyzed their thyroid parameters.

Table 6: Thyroid hormone levels of C1-INH-HAE patients experiencing ≤4 or >4 angioedema episodes per year.

Thyroid parameters (reference range)	Median (IQR)		P-value (Mann-Whitney U-test)
	Subjects with ≤4 attacks/year (n=63)	Subjects with >4 attacks/year (n=54)	
TSH (0.3-3.6 mU/L)	1.5 (1.0-2.3)	1.4 (0.9-2.0)	0.28
fT4 (10.9-21.9 pmol/L)	**15.2** (12.8-16.5)	**13.3** (12.1-15.0)	**0.01**
fT3 (3.4-6.5 pmol/L)	4.8 (4.2-5.1)	4.5 (4.2-4.9)	0.08
a-TG (5-100 IU/mL)	5 (5.0-5.0)	5 (5.0-5.5)	0.20
a-TPO (1-16 IU/mL)	2.4 (1.6-5.7)	2.6 (1.5-5.1)	0.54

As shown in **Table 6**, fT4 levels were lower in the subset with a greater (>4) than in that with a lower (≤4) per-year attack number. Using the contingency table method and the χ^2 test, we analyzed the proportions of patients with a normal fT4 level below or over the median in these two subsets. The proportion of patients with a low-normal fT4 level was greater in the subset with >4 per-year attack number (34/54 *vs*. 26/63; p=0.02; df: 1; Pearson's χ^2 value: 5.477). A similar comparison with fT3 values also showed a significant difference (36/54 *vs*. 27/62; p=0.01; df: 1; Pearson's χ^2 value: 6.216). However, no significant correlation could be demonstrated for TSH levels.

4.12. Thyroid hormone levels of C1-INH-HAE patients treated vs. not treated with danazol

We also analyzed the study population of 117 C1-INH-HAE patients according to treatment with danazol (n=54, patients on long-term prophylaxis), or non-treatment with danazol (n=63) (**Table 7**).

Table 7: Thyroid hormone levels of C1-INH-HAE patients treated/not treated with danazol.

Thyroid parameters (reference range)	Median (IQR)		P-value (Mann-Whitney U-test)
	C1-INH-HAE patients not treated with danazol (n=63)	C1-INH-HAE patients treated with danazol (n=54)	
TSH (0.3-3.6 mU/L)	1.3 (0.9-2.2)	1.5 (1.1-2.2)	0.36
fT4 (10.9-21.9 pmol/L)	**13.4** (12.2-15.3)	**15** (12.7-16.8)	**0.02**
fT3 (3.4-6.5 pmol/L)	**4.5** (4.0-4.9)	**4.8** (4.3-5.2)	**0.01**
a-TG (5-100 IU/mL)	5 (5.0-5.1)	5 (5.0-5.4)	0.61
a-TPO (1-16 IU/mL)	2.3 (1.5-4.5)	2.8 (1.6-6.2)	0.60

The fT4 and fT3 levels of patients receiving long-term danazol prophylaxis were significantly higher than in those not treated with danazol (Mann-Whitney U-test; p=0.02 and p=0.01, respectively). This difference was significant also between the danazol-taking/not-taking subgroups of healthy controls (p<0.001; *Figure 3*).

On the other hand, the fT4 and fT3 levels of patients treated with danazol and of the healthy controls were not significantly different (p=0.41 and p=0.08, respectively) (*Figure 3*). By contrast, the anti-TPO levels of patients treated/not treated with danazol were significantly higher than of the healthy controls (p=0.001 for both comparisons). We did not find any other significant differences between the two subsets with regard to other thyroid parameters.
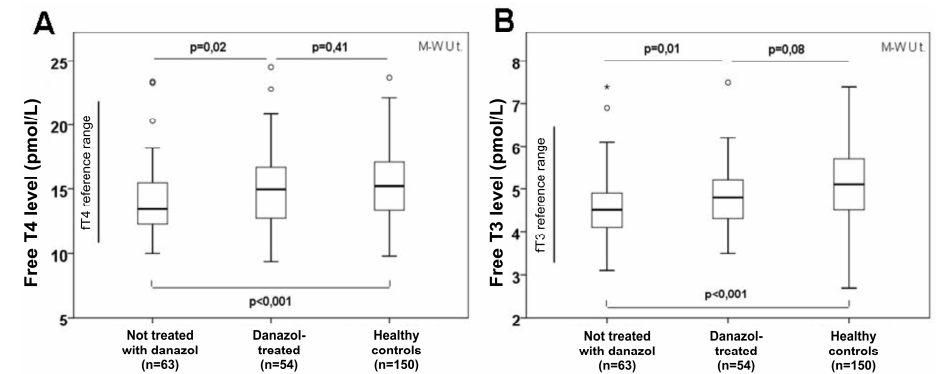

Figure 3: The fT4 (Figure 3/A) and fT3 levels (Figure 3/B) in patients treated/not treated with danazol. (M-W U-t = Mann–Whitney U-test).

4.13. Relationships among thyroid parameters, D-dimer, F1+2, and fibrinogen levels in C1-INH-HAE patients

The levels of fibrin degradation products (D-dimer, F1+2), and fibrinogen levels were determined in blood samples drawn during symptom-free periods of the current year in 31 out of the 117 C1-INH-HAE patients. Spearman's correlation showed a significant, negative correlation between fT4 and D-dimer levels (p=0.02; R:-0.406). We studied the distribution of patients in the low/high-normal (below/above-median) fT4 subsets according to the 0.5 µg/mL cut-off point between the normal and the abnormal range of D-dimer level (median: 0.6 µg/mL). We found that the proportion of patients with low-normal fT4 level was greater in the subset with high D-dimer levels, and *vice versa* (n=14/16 vs. 2/11; p=0.009; Fisher's exact test).

Grouping the patients according to their F1+2, fibrin degradation product, and fT4 levels did not reveal any significant difference. Nevertheless, it appeared that lower fT4 levels tend to be associated with higher F1+2 levels and conversely, higher fT4 levels appeared to belong to lower F1+2 levels. A correlation between fibrinogen and free thyroid hormone levels could not be demonstrated, probably because of the small sample size.

5. CONCLUSIONS

1. The rates of premature birth and of Caesarean section among female patients with C1-INH-HAE correspond to the Hungarian average.

2. In the majority of patients, both the frequency and the location of acute edema formation change. Pregnancy may aggravate, or alleviate angioedema attacks, or may leave these unchanged. The number and severity of edematous episodes occurring during pregnancy exhibit significant fluctuations.

   a) The first trimester is the most difficult for the patients. The number of the attacks peaks during the third trimester. In the majority of cases, pregnancy follows a similar course as far as the symptomatology is concerned.

   b) Abdominal attacks occur more frequently during pregnancy. Edematous attacks are uncommon at full term. In a proportion of the patients, the postpartum period is again dominated by abdominal episodes.

   c) Patients with an early initial onset of the disease endure edematous attacks in greater numbers during pregnancy. Patients who have found that the menstrual periods are possible triggering factors may experience angioedema symptoms significantly less often during pregnancy. By contrast, patients who have identified mechanical trauma as a triggering factor go through significantly more instances of edematous attacks during all three trimesters.

   d) The number of edematous episodes increases significantly during the third trimester in female C1-INH-HAE patients pregnant with a C1-INH deficient fetus.

3. The US FDA allocated pdC1-INH concentrate to pregnancy category 'C'. In our knowledge, this is the largest study so far in support of the safety of pdC1-INH concentrate. We have administered this preparation with success, both as an acute treatment and for prophylaxis. Currently, pdC1-INH concentration is the treatment of choice is pregnancy, as it is harmless both for the mother and for the fetus.

4. The levels of free thyroid hormones was significantly lower, whereas that of anti-TPO vas significantly higher in our C1-INH-HAE patients than in the healthy controls.

5. Below-median C1-INH activity is more common among patients with low-normal fT4 levels. Moreover, fT4 level is lower in patients who experience a greater number of angioedema episodes per year.

6. In patients on long-term danazol prophylaxis, the levels of free thyroid hormones were significantly higher than in those not receiving danazol.

The difference in fT4 and fT3 levels is similarly significant between patients not taking danazol and healthy controls. On the other hand, free thyroid hormone levels of danazol-treated patients and of the healthy controls were not significantly different. This observation might offer a new alternative for research into the mode of action of danazol.

7. A correlation was demonstrated between the indices of the activity of the fibrinolytic system and the parameters of thyroid function. We found a significant, negative correlation between fT4 and D-dimer levels. The proportion of patients with low-normal fT4 levels is greater in the subgroup with high D-dimer levels, and *vice versa*.

Therefore, patients with low-normal free thyroid hormone levels experience more angioedema episodes. This is possibly related – among others – to the activation of the fibrinolytic system, as suggested by elevated D-dimer levels.

We can conclude that in C1-INH-HAE patients, physiological, hormonal changes influence the frequency of angioedema episodes. Our finding suggest that the endocrine system has an outstanding role in the pathophysiology of C1-INH-HAE.

6. LIST OF PUBLICATIONS

Thesis-related publications in English:

1) Czaller I, Csuka D, Zotter Z, Veszeli N, Takács E, Imreh É, Varga L, Farkas H. (2016) Thyroid hormones and complement parameters in hereditary angioedema with C1-inhibitor deficiency.
Ann Allergy Asthma Immunol, 117(2): 175-179.   IF: 3,475* (2015)

2) Czaller I, Visy B, Csuka D, Füst G, Tóth F, Farkas H. (2010) The natural history of hereditary angioedema and the impact of treatment with human C1-inhibitor concentrate during pregnancy: a long-term survey.
Eur J Obstet Gynecol Reprod Biol, 152(1): 44-49.   IF: 1,764 (2010)

3) Farkas H, Csuka D, Gács J, Czaller I, Zotter Z, Füst G, Varga L, Gergely P. (2011) Lack of increased prevalence of immunoregulatory disorders in hereditary angioedema due to C1-inhibitor deficiency.
Clin Immunol, 141(1): 58-66.   IF: 4,046 (2011)

4) Zotter Z, Csuka D, Szabó E, Czaller I, Nébenführer Z, Temesszentandrási G, Füst G, Varga L, Farkas H. (2014) The influence of trigger factors on hereditary angioedema due to C1-inhibitor deficiency. Orphanet J Rare Dis, 9: 44.   IF: 3,358 (2014)

Other English-language publications not related to the subject of the thesis:

1) Bohács A, Bikov A, Ivancsó I, Czaller I, Böcskei R, Müller V, Rigó J Jr, Losonczy G, Tamási L. (2016) Relationship of Circulating C5a and Complement Factor H Levels With Disease Control in Pregnant Women With Asthma. Respir Care, 61(4): 502-509.   IF: 1,922* (2015)

2) Farkas H, Csuka D, Zotter Z, Szabó E, Czaller I, Varga L, Fejes J, Füst G, Harmat G. (2013) Treatment of attacks with plasma-derived C1-inhibitor concentrate in pediatric hereditary angioedema patients.
J Allergy Clin Immunol, 131(3): 909-911.   IF: 11,248 (2013)

3) Csuka D, Kelemen Z, Czaller I, Molnár K, Füst G, Varga L, Rajczy K, Szabó Z, Miklós K, Bors A, Farkas H. (2011) Association of celiac disease and hereditary angioedema due to C1-inhibitor deficiency. Screening patients with hereditary angioedema for celiac disease: is it worth the effort?
Eur J Gastroenterol Hepatol, 23(3): 238-244.   IF: 1,757 (2011)

4) Czaller I, Molnár K, Csuka D, Varga L, Farkas H. (2011) Successful outcome using C1-inhibitor concentrate in acute pancreatitis caused by hereditary angioedema.
Gastroenterol Nurs, 34(1): 60-63.   IF: 0,705 (2011)

5) Kelemen Z, Visy B, Csuka D, Czaller I, Füst G, Farkas H. (2010) Abdominal symptoms of hereditary angioedema and early weaning.
Eur J Clin Nutr, 64(9): 1025-1027.   IF: 2,561 (2010)

6) Farkas H, Czaller I, Csuka D, Vas A, Valentin S, Varga L, Széplaki G, Jakab L, Füst G, Prohászka Z, Harmat G, Visy B, Karádi I. (2010) The effect of long-term danazol prophylaxis on liver function in hereditary angioedema - a longitudinal study.
Eur J Clin Pharmacol, 66(4): 419-426.   IF: 3,032 (2010)

7) Kristóf K, Madách K, Czaller I, Bajtay Z, Erdei A. (2009) Mathematical analysis of clinical data reveals a homunculus of bacterial mimotopes protecting from autoimmunity via oral tolerance in human.
Mol Immunol. 46(8-9): 1673-1678.   IF: 3,202 (2009)

Papers not related to the subject of the thesis and published in Hungarian professional journals:

1) Bíró A, Dombai B, Oroszi D, Eszes N, Czaller I, Tamási L. (2016) Asztma okozta rizikó terhességben. *[in Hungarian]*
Med. Thor, 69(1): 35-39.

2) Czaller I, Csomor J, Demeter J, Losonczy Gy, Gálffy G. (2014) Szolid tumornak indult, non-Hodgkin lymphoma pulmonalis manifesztációja lett.*[in Hungarian]*
Med. Thor, 67(5): 340-344.

3) Gálffy G, Tamási L, Szondy K, Czaller I, Losonczy Gy, Müller V. (2012) Tüdődaganat kemoterápiája mellett észlelt korai és késői hányinger – hányás gyakorisága különböző antiemetikum-kezelések mellett. *[in Hungarian]*
Med. Thor, 65(5): 358-364.

4) Gálffy G, Czaller I. (2012) Sikeres célzott kezelés időskori, nem-kissejtes tüdőcarcinoma esetén. *[in Hungarian]*
Med. Thor, 65(4): 273-274.