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Changes of coagulation parameters during erythema marginatum in patients with hereditary angioedema

Kinga Viktória Kőhalmi[a,b], Blanka Mező[c], Nóra Veszeli[c], Szabolcs Benedek[d], Adrienne Fehér[e], Ágnes Holdonner[a], Milos Jesenak[f], Lilian Varga[a], Henriette Farkas[a,*]

[a] Hungarian Angioedema Reference Center, 3rd Department of Internal Medicine, Semmelweis University, Budapest H-1125, Hungary
[b] Hospital of Hospitaller Brothers of St. John of God, Department of Rheumatology, Budapest H-1023, Hungary
[c] MTA-SE Research Group of Immunology and Hematology, Hungarian Academy of Sciences and Semmelweis University, Budapest H-1125, Hungary
[d] 3rd Department of Internal Medicine, Semmelweis University, Budapest H-1125, Hungary
[e] Department of Laboratory Medicine, Semmelweis University, Budapest H-1085, Hungary
[f] Department of Pediatrics, Martin University Hospital, Martin, Slovakia

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ABSTRACT

Background: Hereditary angioedema (HAE) with C1-inhibitor deficiency (C1-INH-HAE) is characterized by recurrent episodes of subcutaneous/submucosal edema, which may be preceded by erythema marginatum (EM) as a prodromal symptom. Our aim was to analyze the changes occurring in the parameters of the coagulation system during the development of EM and HAE attacks.

Materials and methods: Eight C1-INH-HAE patients (1 male, 7 females, median age: 41.7 years) were studied. Blood samples were obtained from all patients (during symptom-free periods, EM, and HAE attacks), as well as from 20 sex- and age-matched healthy controls. Prothrombin time (PT), activated partial thromboplastin time (aPTT), fibrinogen, D-dimer, Factor V, Factor VII, Factor X, Factor XI, and Factor XII levels were measured.

Results: D-dimer levels were significantly lower, whereas aPTT was significantly prolonged in healthy controls vs. the values measured during the symptom-free period ($p = 0.0497$; $p = 0.0043$), in the presence of EM ($p = 0.002$; $p = 0.0002$), or during HAE attacks ($p < 0.0001$; $p = 0.0002$). We observed the following differences between samples taken during HAE attacks vs. in symptom-free periods: D-dimer levels were significantly elevated ($p = 0.0391$), while aPTT was significantly shorter during HAE attacks ($p = 0.0159$). D-dimer levels were significantly higher during EM than in symptom-free periods ($p = 0.0078$). Comparing the samples drawn during EM or during HAE attacks, there were no significant differences in the study parameters.

Conclusions: D-dimer levels were elevated during EM and this suggests that EM may be part of the HAE attack. Nevertheless, further research into the complement and kinin-kallikrein systems is needed in more patients for a better understanding of the pathomechanism of EM.

1. Introduction

Hereditary angioedema is a rare, autosomal dominant disorder characterized by recurrent subcutaneous and/or submucosal edema formation. The disease is caused by a mutation in the gene encoding C1-inhibitor protein (i.e. in the *SERPING1* gene). In C1-INH-HAE, coagulation, fibrinolytic, complement, and kinin systems – all regulated by the C1-INH – may undergo activation, which is followed by the production of bradykinin.

The process starts on negatively charged surfaces and can lead to the activation of Factor XII. Activated Factor XII mediates the conversion of prekallikrein to kallikrein, and the latter cleaves high-molecular-weight kininogen to bradykinin. Furthermore, activated Factor XII activates also Factor XI [1]. The accumulation of prekallikrein and of activated Factor XI increases the quantity of active Factor XII through a positive feedback mechanism, and this further enhances the activation of the entire system [2]. The activation of the coagulation system may be triggered both by tissue factor and by activated Factor XII. The end

Abbreviations: aPTT, activated partial thromboplastin time; C1-INH, C1-inhibitor; C1-INH-HAE, hereditary angioedema with C1-inhibitor deficiency; EM, erythema marginatum; HAE, hereditary angioedema; PT, prothrombin time

* Corresponding author at: Hungarian Angioedema Center, 3rd Department of Internal Medicine, Semmelweis University, Kútvölgyi Street 4, H-1125 Budapest, Hungary.
E-mail address: farkas.henriette@med.semmelweis-univ.hu (H. Farkas).

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product of blood coagulation is fibrin. The latter is cleaved to D-dimers by plasmin, which is regulated – among others – by the C1-inhibitor [3]. In view of the foregoing, studying coagulation in C1-INH-HAE is necessary.

In 82.5% of C1-INH-HAE patients, HAE attacks are preceded by prodromal symptoms [4]. These might include malaise, fatigue, sadness, restlessness, irritability, skin rash, nausea, abdominal pain, and stomach upset – all these are subjective symptoms. The only objective phenomenon is erythema marginatum (EM) [4–7]. According to American, Danish, and Hungarian surveys, EM occurs in 42 to 58% of C1-INH-HAE patients [8–10]. Patients experience EM as an isolated occurrence, a prodromal symptom – alternatively, this cutaneous manifestation may accompany angioedema attacks [11].

The pathophysiology of EM is unclear. However, according to a single report by STARR *et al,* in a skin biopsy specimen obtained from a C1-INH-HAE patient with EM, cross-reactivity was found in stromal tissue with an antibody that recognizes bradykinin. However, this could also have resulted from the accumulation of (high-/low-molecular-weight) kininogen. This is much more probable, given the very short half-life of bradykinin [12].

In the literature, a single report was published on the monitoring of complement parameters during EM [13]. No other biomarkers have been measured with the purpose of monitoring the pathophysiological changes that occur during EM in C1-INH-HAE patients. Therefore, our aim was to evaluate the coagulation parameters during EM in patients with C1-INH-HAE.

2. Materials and methods

In our prospective study, 8 out of 176 C1-INH-HAE patients followed-up in the Hungarian Angioedema Reference Center (Budapest, Hungary) (1 male, 7 females, median age: 41.7 years, 25% percentile: 25.7 years, 75% percentile: 50.41 years) were studied (Table 1). These patients experienced EM on multiple occasions during their lifetime. Additionally, 20 sex- and age-matched healthy controls (4 males, 16 females; median age: 39.03 years, 25% percentile 35.39 years, 75% percentile 44.84 years) were enrolled in the study.

Sodium citrate-anticoagulated blood samples were obtained between 2017 and 2019 from all patients (during symptom-free periods, EM, and HAE attacks), as well as from the healthy controls. According to the standard procedure adopted by the Hungarian Angioedema Reference Center, samples from the symptom-free period were those obtained from patients who had been symptom-free for at least 72 h before blood sampling and had not received acute treatment for a HAE attack in the preceding 7 days, as well as who did not harbor an infection. The following coagulation parameters were measured in each sample: D-dimer, activated partial thromboplastin time (aPTT), prothrombin time (PT), fibrinogen, Factor V, Factor VII, Factor X, Factor XI, and Factor XII activity.

D-dimer levels (mg/L) were measured with the Innovance D-dimer kit based on a particle-enhanced immunoturbidimetric assay (Siemens Healthcare Diagnostics, Marburg, Germany). Prothrombin time and activated thromboplastin time were determined in plasma, using standard laboratory methods. Fibrinogen level (g/L) was measured in the samples from the patients and from the healthy controls with a modified Clauss method. Excess thrombin was added to diluted citrate samples. The time required to convert fibrinogen to fibrin correlates with the concentration of fibrinogen. Coagulometry and commercially available Siemens Healthcare kits (Marburg, Germany) were applied to measure Factor V, Factor VII, Factor X, Factor XI, and Factor XII levels (%).

Table 1
Age and gender of the eight C1-INH-HAE patients enrolled in the study.

C1-INH-HAE patients	Age (years)	Gender (1 = male; 2 = female)
Patient #1	51.33	1
Patient #2	35.76	2
Patient #3	58.52	2
Patient #4	41.34	2
Patient #5	47.66	2
Patient #6	22.35	2
Patient #7	20.3	2
Patient #8	42.02	2

The reference ranges were as follows: PT assay: 8.0–11.0 sec, aPTT assay: 28.0–40.0 sec, Factor V: 62–152%, Factor VII: 61–199%, Factor X: 70–171%, Factor XI: 67–196%, Factor XII: 35–200%, fibrinogen: 1.5–4.0 g/L.

The coefficient of variation for the Factor XI and Factor XII assays was 2–14%. We determined the following coefficients of variation for the instrument used: Factor V activity – normal control: 5.61%; Factor V activity – pathological control: 6.32%; Factor VII activity – normal control: 4.85%; Factor X activity normal control: 4.37%; Factor X activity pathological control: 4.28%; D-dimer – normal control: 5.08%; d-dimer – pathological control: 3.02%; aPTT – normal control: 3.7%; PT – normal control: 1.33%; PT – pathological control: 2.35%; fibrinogen – normal control: 47%; fibrinogen pathological control: 5.13%.

Anticoagulation could have interfered with some of the blood tests (e.g. D-dimer level); however, anticoagulant therapy was defined as a criterion for exclusion from recruitment to the study.

All patients gave informed consent. The study was approved by the Research Ethics Committee of Semmelweis University (Budapest), and it was implemented in conformity with the declaration of Helsinki.

2.1. Statistical analysis

We performed the statistical analyses with the GraphPad Prism software, version 8.0.1 (GraphPad Software, San Diego, California, USA). The samples of the healthy controls, as well as those obtained from the patients in symptom-free periods, during HAE attacks, and during EM were compared with the paired *t*-test and with the Mann-Whitney test. The levels of the coagulation parameters in samples obtained in symptom-free periods, during HAE attacks, and during EM were evaluated with the paired t-test (Wilcoxon test) in all cases. A p value of < 0.05 was considered statistically significant in all analyses.

3. Results

The D-dimer levels of healthy controls were significantly lower than those of the patients in blood samples obtained in symptom-free periods ($p = 0.0497$), during EM ($p = 0.002$), and during HAE attacks ($p < 0.0001$). By contrast, aPTT was significantly prolonged in samples from healthy controls than in those drawn from patients in symptom-free periods ($p = 0.0043$), during EM ($p = 0.0002$), or during HAE attacks ($p = 0.0002$). Factor XI levels were significantly lower in healthy controls ($p = 0.0187$) than in the patients during EM ($p = 0.0424$) and during HAE attacks ($p = 0.0410$) (Fig. 1).

Considering fibrinogen, prothrombin time, Factor V, Factor VII, Factor X, and Factor XII, the levels measured in the patients' blood samples obtained in symptom-free periods, during EM, or during HAE attacks did not differ significantly from those of the healthy controls.

Comparing blood samples drawn during HAE attacks with blood samples taken during symptom-free periods, we found that D-dimer levels were significantly elevated [1.9 (0.80–4.80) mg/L vs. 0.59 (0.41–1.50) mg/L; $p = 0.0391$; median (25–75th percentile)], whereas aPTT was significantly shortened during HAE attacks [23.55 (22.10–26.35) sec vs. 24.85 (23.08–27.00) sec; $p = 0.0159$]. Furthermore, D-dimer levels were significantly higher during EM than in the symptom-free period [3.50 (0.62–12.02) mg/L vs. 0.59 (0.41–1.50) mg/L; $p = 0.0078$]. In all three sample types, median D-dimer levels were the highest in those drawn during EM. No significant differences were found between D-dimer levels measured in samples

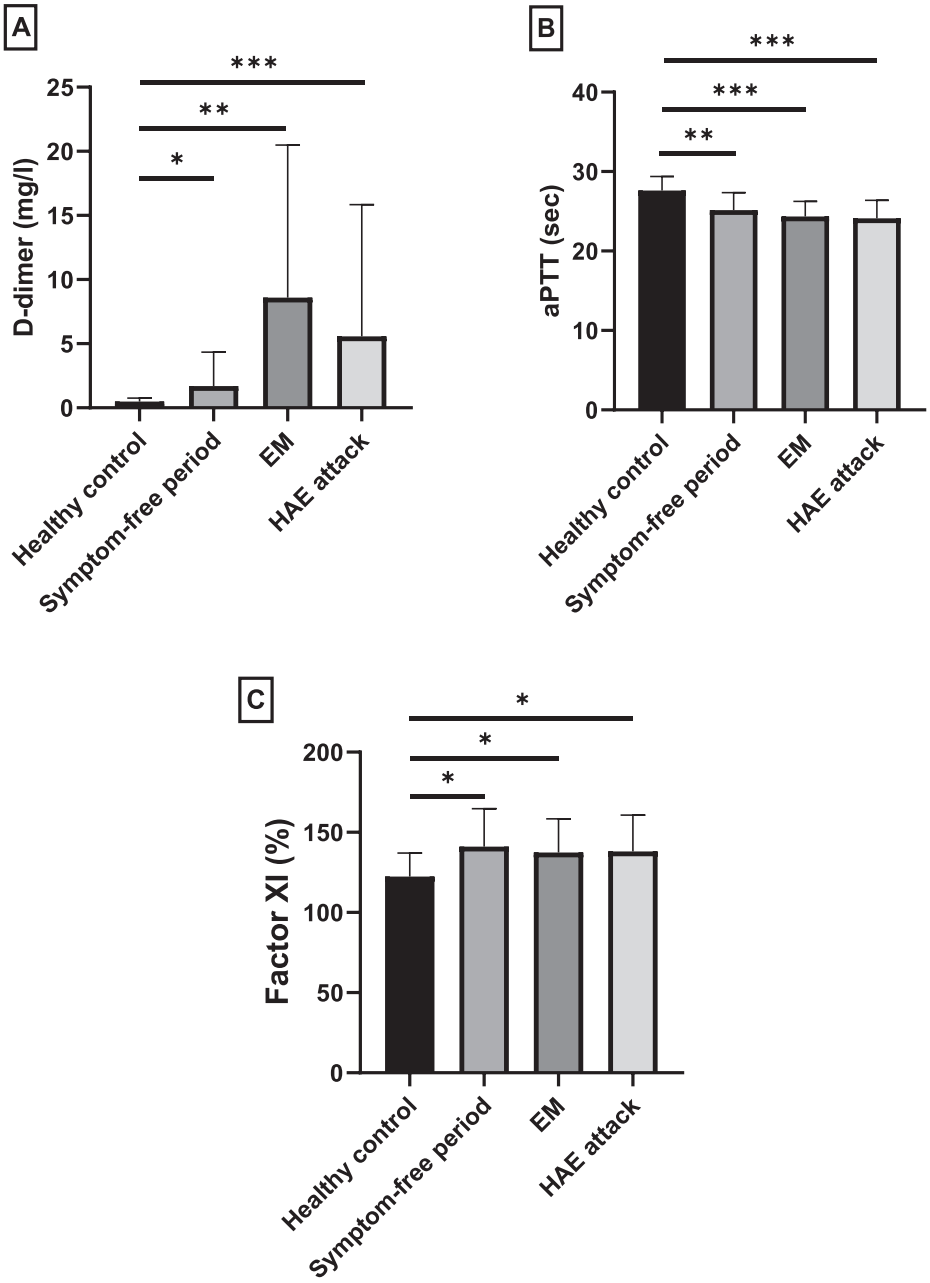

Fig. 1. D-dimer levels (A), activated partial thromboplastin time (B), and Factor XI levels (C) in 20 healthy controls, compared with those measured in samples obtained from 8 C1-INH-HAE patients in symptom-free periods, during HAE attacks, and during EM.

obtained during EM vs. during HAE attacks ($p = 0.8438$) or between aPTT measured during symptom-free periods vs. during EM ($p = 0.1215$), or during EM vs. during HAE attacks ($p = 0.8227$) (Fig. 2).

With regard to prothrombin time (PT), fibrinogen, Factor V, Factor VII, Factor X, Factor XI, and Factor XII levels, we did not find significant differences among the samples obtained in symptom-free periods, during EM, or during HAE attacks from the eight C1-INH-HAE patients studied (Fig. 3).

4. Discussion

This is the first study to report the measurement of coagulation parameters during erythema marginatum, the sole objective prodromal symptom of C1-INH-HAE. It confirmed differences in coagulation biomarkers determined in blood samples obtained from C1-INH-HAE patients during symptom-free periods, EM, and HAE attacks, as well as in samples from healthy controls. In previous studies into the coagulation parameters of C1-INH-HAE patients, comparisons were made only between values measured in samples obtained in symptom-free periods and during HAE attacks, as well as these were only occasionally compared with the values of healthy controls.

Findings by Cugno et al., and Csuka & Veszeli et al. clearly showed that compared with healthy controls, the D-dimer concentrations of patients are higher as early as in the symptom-free period, and further elevation occurs in samples obtained during HAE attacks [14,15]. Reshef et al. similarly found elevated D-dimer levels in blood samples drawn during HAE attacks compared with those from symptom-free periods; however, these authors did not study healthy controls [16]. As far as the D-dimer is concerned, our results are in agreement with these earlier findings – and include a new and remarkable observation. In particular, a significant elevation can be ascertained in comparison

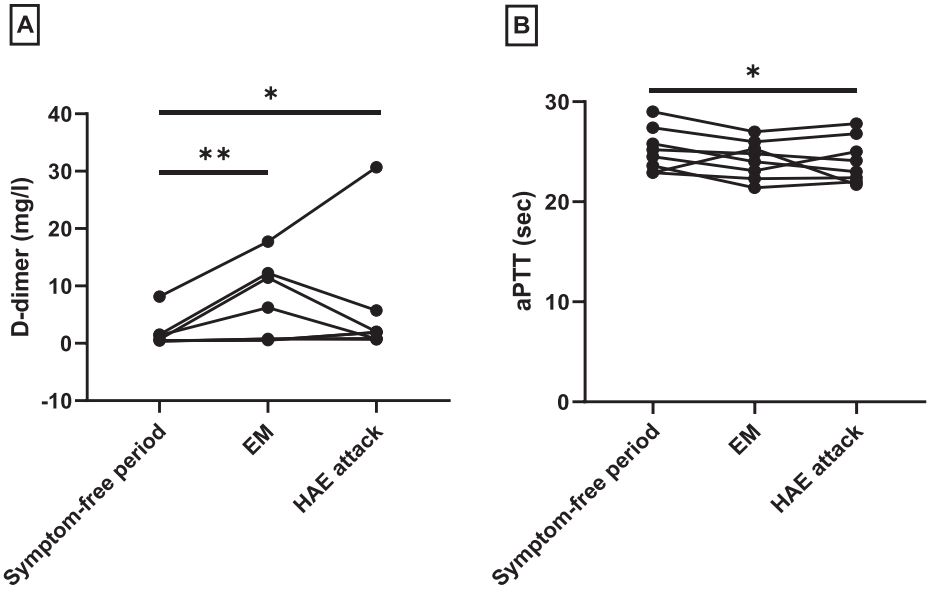

Fig. 2. D-dimer levels (A) and activated partial thromboplastin time (B) in samples obtained during symptom-free periods, during HAE attacks, and during EM blood from 8 patients with C1-INH-HAE.

with patient samples obtained in symptom-free periods as well as with those from healthy controls. Moreover, the highest D-dimer levels were measured during EM. Our observation is the first to shed light on the fact that the development of erythema marginatum may be regarded as the initial phase of an HAE attack because this is the period when the elevation of the D-dimer level begins.

According to a study by BORK et al. conducted earlier in 146 patients with different types of angioedema, aPTT was significantly shortened in C1-INH-HAE patients than in the 26 healthy controls included [17]. In addition to the similarity of our findings with those of Bork et al. regarding the differences compared with the values found in healthy controls and with those measured in symptom-free periods, our study supplemented the observations of that study with the measurement of aPTT in samples obtained during EM and during HAE attacks. We found that compared with symptom-free periods, aPTT shortened significantly during HAE attacks; however, we could not detect any significant differences when comparing the values measured in symptom-free periods with those determined during EM, or between the latter and those measured during HAE attacks. However, it should be noted that aPTT was shorter in all three sample types obtained from C1-INH-HAE patients than in the samples from healthy controls.

In our knowledge, the prothrombin time of C1-INH-HAE patients was measured, so far, in just a single study, which found shortened PT during HAE attacks vs. symptom-free periods. We could not confirm these results and did not find any difference in any respect compared with samples obtained during EM [14].

According to two studies by Cugno et al., as well as to the study by Csuka & Veszeli et al., Factor XII activity was elevated during HAE attacks, compared with symptom-free periods [14,18,19]. Healthy controls were included in the study by Cugno et al., as well as in the Hungarian study [14,19]. Similar to our findings, Cugno et al. did not find any difference in Factor XII activity between samples from healthy controls and those drawn from patients during symptom-free periods. By contrast, Csuka & Veszeli et al. showed the elevation of Factor XII activity compared with healthy controls as early as during the symptom-free period and this activity increased further – in contrast with our findings – during HAE attacks [14,18,19]. Our failure to detect any elevation of Factor XII level in samples drawn during symptom-free periods – in comparison with those obtained during HAE attacks or during EM – might be attributed to the small sample size.

With regard to Factor XI, these results confirm the previous observation of our research team that compared with healthy controls, a significant elevation of the level of this coagulation factor can be observed as early as in the symptom-free period [14], although this has not been confirmed by a previous study [20]. Our research goes beyond these studies by showing that compared with those of the controls, Factor XI levels are elevated in patients even during EM, as well as during HAE attacks.

In our knowledge, Factor V levels have never been measured before in C1-INH-HAE patients. However, based on our results, this coagulation factor is unlikely to play a role either in the occurrence of EM or in the development of HAE attacks, as we did not find a significant correlation among any of our sample types with regard to Factor V level.

NIELSEN et al. determined Factor VII levels during symptom-free periods in 21 C1-INH-HAE patients and in their 23 normal siblings. They found that although the values measured were in the normal reference range, they were nearly twice as high as were those of the healthy siblings of the patients. Our study did not confirm this finding; however, we supplemented measurements with the determination of Factor VII level in samples obtained during HAE attacks and during EM, but we could not find any difference between these [21].

In conclusion, according to our investigations, pathophysiological changes begin during EM – as we found elevated D-dimer levels as early as upon the occurrence of this prodromal symptom. Notwithstanding this, further research into the complement and kinin-kallikrein systems in a larger patient population is necessary for a better understanding of the complex pathomechanism of EM. If we succeed in elucidating the molecular changes occurring during EM, we might deepen our knowledge of the pathophysiology of HAE. This might provide an objective basis for new, individualized therapeutic strategies such as administering acute treatment for HAE as early as during EM.

Declaration of funding

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CRediT authorship contribution statement

Kinga Viktória Kőhalmi: Conceptualization, Data curation, Formal analysis, Methodology, Project administration, Resources, Software, Supervision, Validation, Visualization, Writing - original draft, Writing -

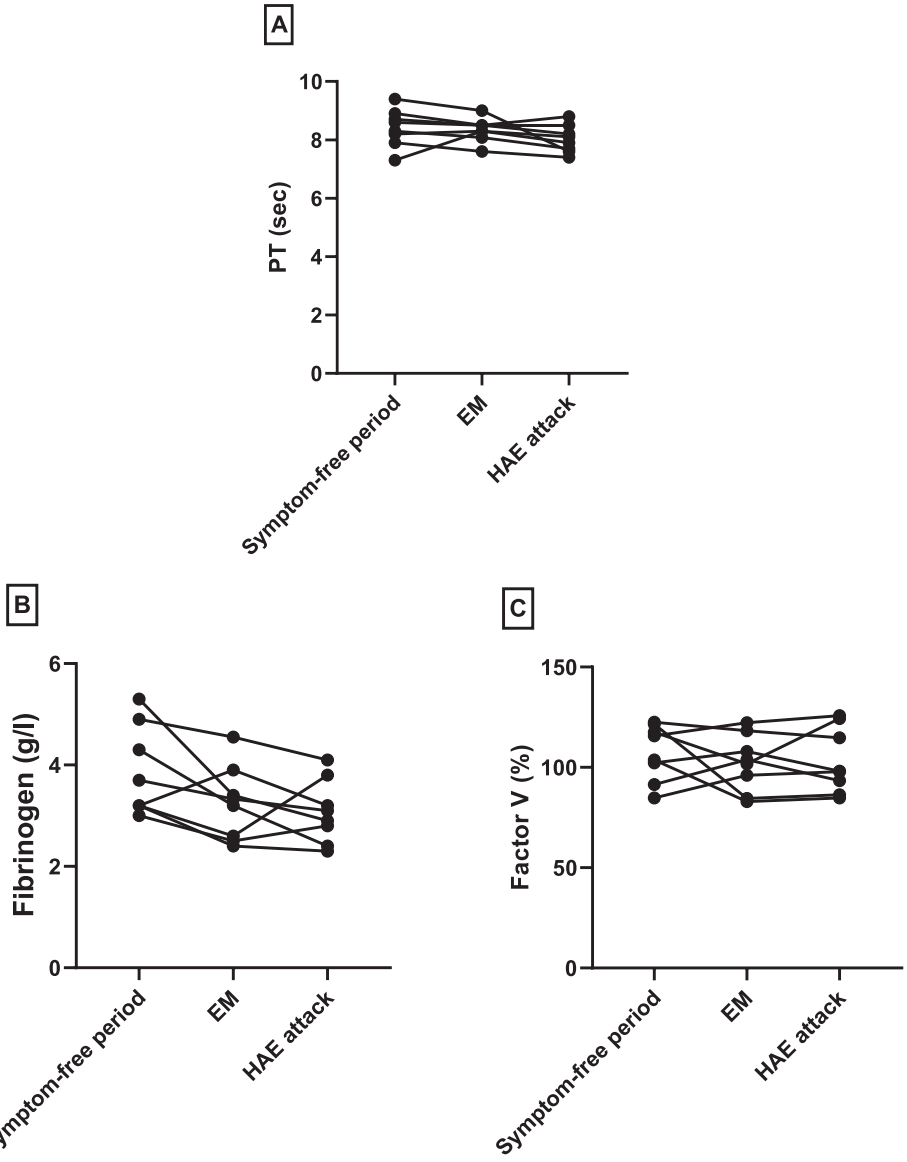

Fig. 3. Prothrombin time (A), fibrinogen (B), Factor V (C), Factor VII (D), Factor X (E), Factor XI (F), and Factor XII levels (G) were not significantly different in blood samples obtained in symptom-free periods, during EM, and during HAE attacks from 8 C1-INH-HAE patients.

review & editing. **Blanka Mező:** Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Resources, Software, Supervision, Validation, Writing - review & editing. **Nóra Veszeli:** Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Resources, Software, Supervision, Validation, Writing - review & editing. **Szabolcs Benedek:** Conceptualization, Data curation, Investigation, Methodology, Resources, Software, Supervision, Validation, Writing - review & editing. **Adrienne Fehér:** Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Resources, Software, Supervision, Validation, Writing - review & editing. **Ágnes Holdonner:** Data curation, Formal analysis, Investigation, Methodology, Supervision, Writing - review & editing. **Milos Jesenak:** Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Project administration, Resources, Software, Supervision, Validation, Visualization, Writing - review & editing. **Lilian Varga:** Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Project administration, Software, Supervision, Validation, Visualization, Writing - original draft, Writing - review & editing. **Henriette Farkas:** Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Software, Supervision, Validation, Visualization, Writing - original draft, Writing - review & editing.

Declaration of Competing Interest

K.V. Kőhalmi has received travel grants from CSL Behring and Shire. B. Mező, Sz. Benedek, A. Fehér, Á. Holdonner and M. Jesenak have no conflict of interest. N. Veszeli has received travel grants from CSL Behring. L. Varga has received travel grants from CSL Behring and Shire Human Genetic Therapies Inc. H. Farkas has received honoraria and travel grants from CSL Behring, Shire, Swedish Orphan Biovitrum, and Pharming; and/or served as a consultant for these companies and has participated in clinical trials/registries for BioCryst, CSL Behring, Pharming, and Shire.

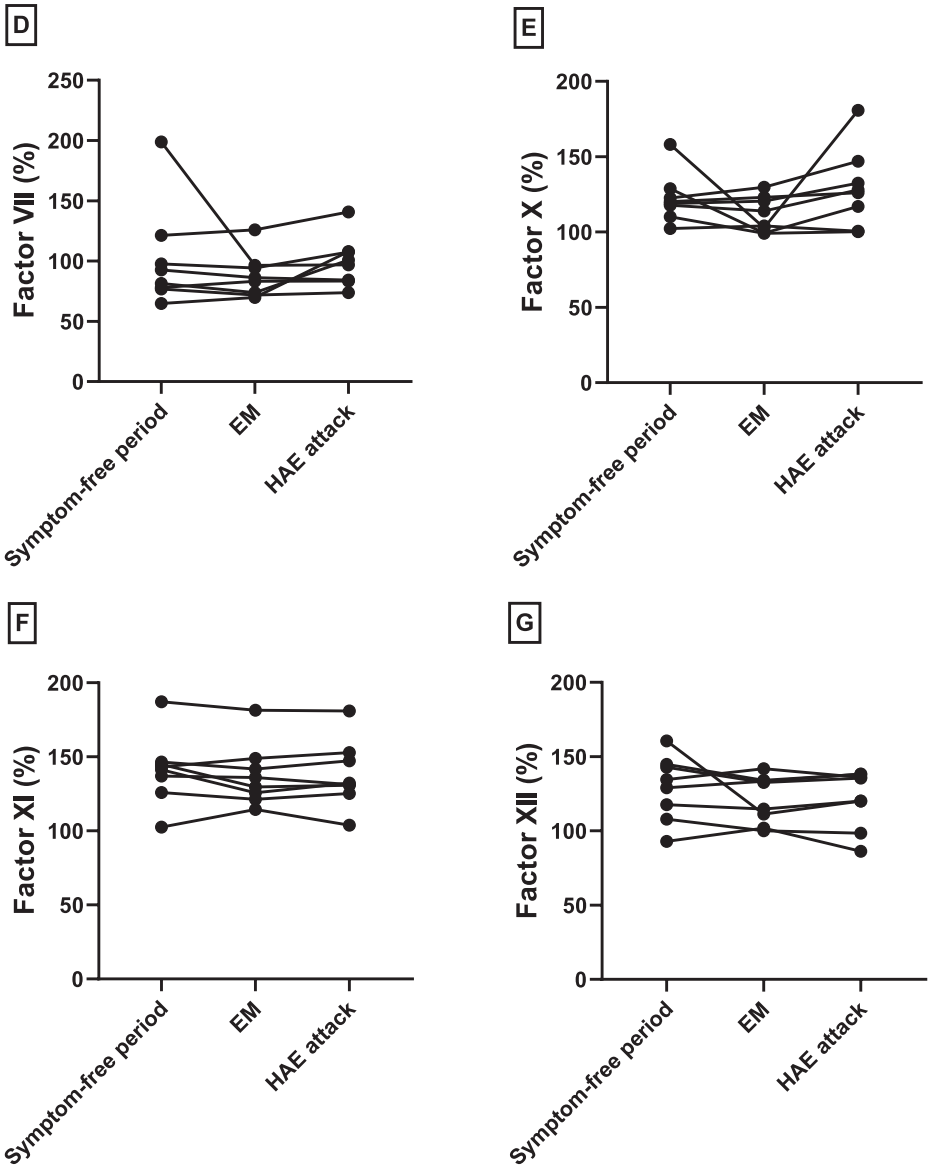

Fig. 3. (*continued*)

Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.intimp.2020.106293.

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