http://www.cjmb.org doi 10.34172/cjmb.2023.10

Open Access Original Article

Crescent Journal of Medical and Biological Sciences
Vol. 10, No. 2, April 2023, 61–66
eISSN 2148-9696

Determination of Factor II Codons Genotype in Southeastern Iranian Patients With Hereditary Deficiency of Factor II

Hamed Soleimani Samarkhazan[1], Shaban Alizadeh[1*], Ziba Majidi[2], Zahra Kashani Khatib[3], Majid Naderi[4]

Abstract

Objectives: Congenital prothrombin (factor II) deficiency is an inherited rare bleeding disorder with an autosomal recessive manner. The prevalence of this disorder is about one in 2 000 000 people in general population, but it is more common in areas with a high rate of consanguinity. To date, there is no report on the absence of prothrombin, which is a life-threating disorder. Considering the importance of factor II in body homeostasis, this study aimed to find any possible mutation of coagulation factor II codons in patients with inherited factor II deficiency in southeastern Iran.

Materials and Methods: This study was conducted on 12 patients with inherited deficiency of prothrombin. Early diagnosis was based on clinical symptoms, laboratory evaluation, and family history. Then, the function level of prothrombin was measured, the initial diagnosis of disease was confirmed, and polymerase chain reaction (PCR) analysis was performed. Finally, gene sequencing and genotyping of factor II was done.

Results: Molecular analysis indicated a point mutation in exon 7 in three patients and a frameshift mutation in exon 14 due to addition of a thymine base at position 1760-1761 in one patient, both of which have been reported for the first time.

Conclusions: Molecular methods performed on patients from Southeastern Iranian population in terms of coagulation factor II deficiency revealed a substitution mutation in exon 7 in three patients and a frameshift mutation in exon 14 in one patient, both of which were reported for the first time. Considering the significant difference between the clinical symptoms of the present study and previous studies, probably the type of mutations reported in this study (for the first time) caused these clinical symptoms, but statistical studies did not show any relationship between the type of mutation and the occurrence of clinical symptoms. And it needs more investigations on more patients, with a larger population.

Keywords: Factor II deficiency, Prothrombin, Congenital bleeding disorders, Blood coagulation factor

Introduction

Human prothrombin (factor II) is a 70 kDa glycoprotein synthesized in presence of Vitamin K in the liver. The gene encoding prothrombin (*F2*) is located on chromosome 11p11-q12 and contains 14 exons and 13 introns, including 20 210 base pairs. Prothrombin molecule includes four domains, including Gla-domain, kringle-1, kringle-2, and canonical protease domain that contains A and B chains (1). After degradation of prothrombin complex by prothrombinase complex (consisting of factors X and V, calcium, and phospholipid), kringle-1 and 2 domains are released and active thrombin is formed. Thrombin includes two polypeptide chains A (36 amino acids) and B (259 amino acids). It is a bifunctional enzyme. On the one hand, it is involved in coagulation cascade via activation of a number of clotting factors (I, V, VIII, XI, XIII) and plays an anticoagulant role by activating thrombomodulin/protein C pathway (2,3).

Hereditary deficiency of this factor, which has a prevalence of approximately one in 2 000 000 people in general population, can manifest in mild or sever mode. This autosomal recessive disorder can affect all the human races. The cause of hereditary deficiency of factor II, either mild or severe, is often a form of gene mutation that involves the coding gene of this factor. So far, more than 50 mutations have been detected in factor II mainly in the form of missense mutation (80%). However, other mutations impair the structure of prothrombin, including insertion/deletion (10%), nonsense (6%), and splice site (4%) mutations .

Complete lack (absence) of prothrombin is contrary to human life and no report has been mentioned in this regard (4,5). Severe bleeding is the most obvious clinical manifestation in hypo-prothrombinemia with <10% plasma levels of prothrombin in homozygous patients. In studies on patients with prothrombin deficiency, intracerebral hemorrhage (ICH, which is life threatening), hematoma, and spontaneous petechiae have been observed in 60% of patients, hemarthrosis in 42%, and gastrointestinal bleeding in only 12% of patients.

Received 6 June 2022, **Accepted** 20 December 2022, **Available online** 5 February 2023

[1]Department of Hematology and Transfusion Medicine, School of Allied Medicine, Tehran University of Medical Sciences, Tehran, Iran. [2]Department of Medical Laboratory Science, School of Allied Medicine, Tehran University of Medical Sciences, Tehran, Iran. [3]Laboratory Hematology and Blood Banking, High Institute for Research and Education in Transfusion Medicine, Tehran, Iran. [4]Department of Hematology and Transfusion Medicine, School of Allied Medicine, Zahedan University of Medical Sciences, Zahedan, Iran.
***Corresponding Author:** Shaban Alizadeh, Tel: +9888982909; Fax: +9888983037, Email: alizadehs@tums.ac.ir

Bleeding after tooth extraction and menorrhagia in 20% of homozygous women with menorrhagia are common symptoms. In patients with this defect, routine coagulation tests such as PT (prothrombin time) and aPTT (activated partial thromboplastin time) are variably prolonged.

This disorder is approximately 10 times more common in regions with a high rate of consanguineous marriages (6,7). So far, no studies have been carried out on coagulation factor II in Iran. Nonetheless, some studies evaluated patients with thrombosis and their association with G20210A polymorphism in this factor. Due to high rates of consanguinity in Iran, the incidence of factor II deficiency, such as autosomal recessive inherited disease, is expected to be high. Considering the importance of this factor in body homeostasis, this study aimed to find any possible mutations of coagulation factor II codons in patients with inherited factor II deficiency in southeastern Iran .

Key Messages

- Congenital coagulation factor II (prothrombin) deficiency is an autosomal recessive, rare bleeding disorder with variable clinical symptoms.
- Prothrombin codons PCR analysis can help us to determine probably mutation that cause disorder. In this study we detected 2 new mutation in prothrombin that cause deficiency.

Materials and methods

Study Design

In this study, 12 patients with factor II deficiency were selected from Ali Asghar Children's Hospital in Zahedan, Iran from 30 April to July 1, 2015. Routine coagulation tests of PT, PTT, and BT were performed for these patients. Prothrombin activity was measured in plasma for all subjects who had prolonged PT and PTT results; these subjects were candidates for determining the genotype of coagulation factor II codons.

Informed consent was obtained from all subjects. The questionnaires were completed by a physician through interviewing with the patients or their families (children under 16 years of age). The questionnaire included such information as age, gender, city of residence, age of diagnosis, family history, history of consanguineous marriage of parents, clinical symptoms, treatment, prophylaxis, and disease outcome. The study protocol was approved by the ethical committee of Tehran University of Medical Sciences, Iran.

The patients were divided into three groups based on the level of factor II activity as follows: <1%, 1-12%, and >12%. Moreover, based on clinical signs and bleeding symptoms, the patients were divided into four grades: Grade 0: patients without symptoms of bleeding, Grade 1: patients showing clinical symptoms following injury or drug injection, Grade II: patients with minor spontaneous bleedings such as ecchymosis, bruising, bleeding gums, epistaxis, and bleeding from minor injuries, and Grade III: patients with severe life-threatening spontaneous bleeding, including cerebral bleeding, gastrointestinal bleeding.

Blood Sampling and Isolation of DNA

Whole blood samples with ethylenediaminetetraacetic acid (EDTA) anticoagulant were collected from the patients. Frozen blood samples were dispatched on dry ice to the Hematology Department of Tehran University of Medical Sciences to detect the mutations. EDTA containing plasma of patients was stored at -70°C until use. Genomic DNA of patients was extracted from EDTA containing peripheral blood samples using genomic DNA extract kit (Viogene Company, UK) and was kept at -20°C in aliquots.

PCR Analysis

The extracted DNAs were analyzed by PCR for genetic mutation in prothrombin gene. Oligonucleotide primers (Table 1) were chosen according to previous studies and synthesized by cDNA synthesis kit (Sinaclon, Iran). All the exons, introns, and 5'UTR and 3'UTR regions of factor II were amplified by PCR using Eppendorf thermocycler. PCR reaction was conducted using specific shuttle primers for each exon. Products of PCR were fractionated on 2% agarose gel electrophoresis and stained by ethidium bromide.

DNA Sequencing

Sequencing was based on the Sanger method, also known as chain termination. In this method, DNA is sequenced in a single pattern. After PCR, amplified DNA must be isolated from other agents. Glycogen, ammonium acetate, and absolute alcohol were used for this purpose, which caused DNA to separate and precipitate in the tube. All the steps of purification and sequencing were performed in Pishgam Company. After receiving the sequencing results, the sequences were analyzed with CodonCode Aligner software (version 5.1.5) and statistical data were performed using the Statistical Package for the Social Sciences (SPSS) software, version 18.

Prothrombin Activity Assay

The level of plasma activity of factor II in all patients included in the study was determined by Zahedan Hemophilia Center. Outcomes were expressed as a percentage of the quantity of prothrombin in pooled normal plasma, which was random designated as 100%.

Statistical Analysis

Chi-square test was used to evaluate the correlation between factor II and severity of bleeding symptoms as well as correlation between mutations and clinical symptoms. Data were analyzed using SPSS version 18. A *P*

Table 1. Primers Used for PCR Analysis of Factor II Codons

Primer Type	Primer Sequence	Exon	Product Length
Forward Reverse	CATGTTAGTTCAACATTACCCAGAGG GCCAGCTCTGTGTGCTCTGTC	1	246
Forward Reverse	GTTCCTGAGGTCGCTGTTCCATGAC CTCTGTGGAAGTGTCTAGAGTTGGG	2	275
Forward Reverse	GTGAGGAGGAGCATAACATTTACT GTGGATGGCTAGGTGCAGCAGAAG	3	203
Forward Reverse	CACCAACATCCCATCCACCCTGAC CCATGTCCTGCTCCCCAAACCCG	4	171
Forward Reverse	GAAATAAGTCCCCAGGCTCCAAG GAACTTGAGGTACGCTTGCTCCCT	5	219
Forward Reverse	AGGGAGCAAGCGTACCTCAAGTTC AGCCCCCGGGCTTGGTCATGGGTC	6	241
Forward Reverse	GCGCCTGGCGGTGACCACACAT TCCCACTAGGATTTGTCCCTGCAACT	7	259
Forward Reverse	TGAGGAATGGCCCAGCCCAGTCCCG CAAGCAGTGAGGGGCAAGTCCT	8	249
Forward Reverse	AGGCAGGTGAGGTAGTGGGCATCCG AGGAGTGAATGGTAGCGCAGGGCTC	9	254
Forward Reverse	CTCATCCTCAGCTCCTAATGCT AGGACTCAGACCCCTGCCAGACAC	10	256
Forward Reverse	GTGAACCTGCAGCTTCTCCATTTCT TCTGCCCCTCAGCTAACAAGCAT	11	254
Forward Reverse	CAGGCGGCTCCTGTGGGGGTTG CTTGGGCCCCACTGTTCCCTCA	12	281
Forward Reverse	TGGACTCTCACCAGCTGTGTCTCGT CAGGCAACTGTTTCTAGATACTAGAG	13	242
Forward Reverse	CCTTGAACTTGACTCTATTGGAAACC ACGGGATTGGTTCCAGGAGCCCAGA	14	228

value <0.05 was considered statistically significant.

Results

Baseline Characteristics of Subjects

In this study, out of 12 included patients, seven (55.83%) were male and five (41.66%) were female. The mean age of male and female patients was 13.4 (range: 3-21) and 55.2 (range: 47-65) years, respectively. The mean age of disease diagnosis was 4.5 and 44.6 years in males and females, respectively. According to the report by Hemophilia Center, plasma level of factor II was 7-14% in all the patients. None of the patients had <1% factor level, nine patients had 1-12% factor activity, and three patients had >12% factor activity.

In the present study, gynecological examination of female patients showed that all the studied patients had menorrhagia and postpartum hemorrhage (100%) and two of them had ICH hemorrhage (40%). Out of 12 patients, there were five cases of epistaxis (41.6%), two cases of bleeding after tooth extraction (16.6%), two cases of deep hematoma (16.6%), and five cases of ecchymosis (41.6%). Joint, gastrointestinal (GI), and ICH bleedings were not observed in any of the patients. The characteristics of patients under study is shown in Table 2. Figure 1 shows clinical symptoms of patients with hereditary factor II deficiency.

Genetic Studies

In three patients (No. 3, 5, and 7), a point mutation was found in exon 7. The guanine (G) base at position 844 was substituted by adenine (A). This homozygous mutation, which is reported for the first time, is known as p.282 asp> asn, indicating substitution of asparagine for aspartic acid in amino acid 282. Figure 2 shows this mutation.

In one patient (No. 11), frameshift mutation was observed in exon 14. In this mutation, due to addition of a thymine (T) base to exon between 1760 and 1761 positions, the stop codon appeared prematurely, causing

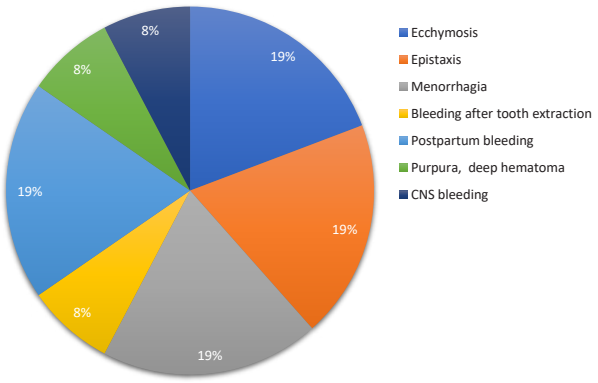


Figure 1. Clinical Symptoms of Patients With Hereditary Factor II Deficiency.

Table 2. The Characteristics of Patients

Patient	Age	Gender	Age at Diagnosis	Factor II Level	Family History	Blood Group	Clinical Symptoms
1	3	Male	2.5	12	-	O-	Epistaxis and ecchymosis
2	5	Male	2.5	12	-	O-	Epistaxis and ecchymosis
3	65	Female	59	7	+	AB+	Postpartum bleeding, menorrhagia, CNS bleeding
4	65	Female	59	7	+	AB+	Postpartum bleeding, menorrhagia, CNS bleeding
5	21	Male	4	9.6	One brother	B+	Bleeding after tooth extraction
6	47	Female	35	14	Two brothers and one sister	B+	Postpartum bleeding, menorrhagia
7	18	Male	8	9.7	One brother	B+	Purpura, deep hematoma and epistaxis
8	21	Male	4	9.6	One brother	B+	Bleeding after tooth extraction
9	49	Female	35	14	Two brothers and one sister	B+	Postpartum bleeding, menorrhagia
10	50	Female	35	14	Two brothers and one sister	B+	Postpartum bleeding, menorrhagia
11	6	Male	2.5	12	-	O-	Epistaxis and ecchymosis
12	20	Male	8	9.7	One brother	B+	Purpura, deep hematoma and epistaxis

a defective protein translation. It should be noted that this homozygous mutation has been observed for the first time. Figure 3 shows this mutation.

Correlation Between Mutation And Clinical Features

Clinical symptoms in patient No. 3 included menorrhagia, postpartum hemorrhage, and CNS bleeding. There was no correlation between mutation at position 844 of exon 7 and clinical symptoms ($P = 0.5$). The clinical symptoms in patient No. 5 included epistaxis and gum bleeding. There was no significant correlation between mutation in patent No. 5 and clinical symptoms ($P = 0.54$). Also, there was no significant correlation between mutation in position 844 of exon 7 and the level of factor II activity ($P = 0.24$), as well as severity of disease ($P = 0.5$).

Clinical symptoms in patient No. 11 were ecchymosis and nose bleeding. There was no significant correlation between frameshift mutation and nose bleeding, as well as ecchymosis ($P = 0.49$). Also, no significant correlation was found between frameshift mutation, severity of symptoms, and the level of factor II activity ($P = 0.55$).

Statistical studies did not show any relationship between mutation type and the incidence of clinical symptoms. Among clinical symptoms, a significant correlation was only found between factor II activity level, postpartum hemorrhage ($P = 0.018$), and menorrhagia ($P = 0.018$).

Discussion

This study was conducted on 12 patients with factor II deficiency from different cities in Iran in late 2016. It is believed that the actual quantity of patients with factor II deficiency is higher in this region because of the high rate of consanguineous marriages.

Congenital deficiency of factor II is a rare bleeding disorder with a prevalence of one in 2 000 000 people in general population. It is an autosomal recessive disorder affecting all the human races (8). This disorder is more prevalent (nearly 10 times higher frequency) in regions with a high propensity for consanguineous marriages. Caucasians, Middle East Asian immigrant population, as well as patients with latinohispanic background (Barcelona, Padua, Segovia, Puerto Rico, etc) account for nearly 70% of patients with factor II deficiency (9).

Severe bleeding is the most obvious clinical manifestation in hypo-prothrombinemia with <10% plasma level of prothrombin in homozygous patients. In studies on patients with prothrombin deficiency, ICH, hematoma, and spontaneous petechiae have been reported in 60% of patients. ICH is extremely dangerous in these patients. Hemarthrosis has been reported in 42%

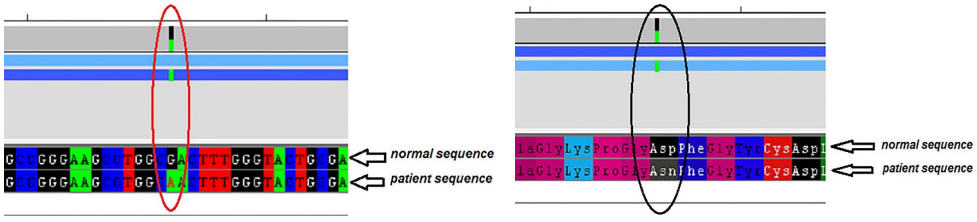


Figure 2. Mutation at Position 844 of Exon 7 (the Guanine (G) Base at Position 844 Substituted by Adenine (A)).

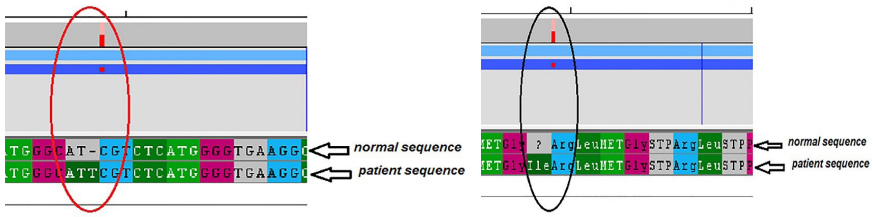


Figure 3. Frameshift Mutation in Exon 14 (Addition of a Thymine (T) Base to Exon Between 1760 and 1761 Positions).

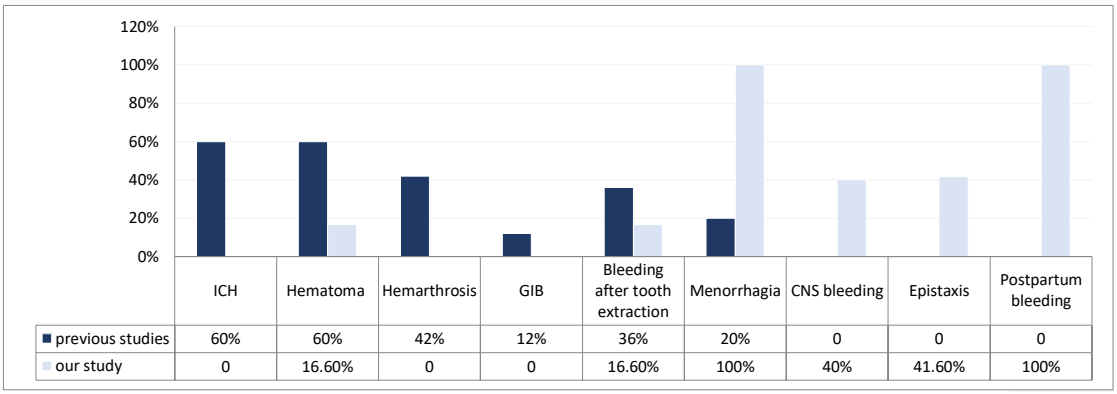
of patients while GI bleeding has been observed only in 12% of cases. The common symptoms also include bleeding after tooth extraction (in 36% of patients) and menorrhagia (in 20% of homozygous women). No symptoms are often observed in heterozygous cases of this disease with normal levels of prothrombin in plasma (40-60%) until tooth extraction or after surgery.

Congenital prothrombin deficiency was first described by Quick in 1947. Shapiro et al evaluated congenital dys-prothrombinemia in a large family and reported that plasma prothrombin concentration and its biological activity was half the normal level; it was concluded that the disease is inherited in an autosomal recessive manner. In another study, O'Marcaigh et al investigated the functional features and analyzed Dharan (Arg271 His) and Corpus Christi (Arg 382 Cys) mutations, as well as hypo-prothrombinemia (10-13). In 2009, Lancellotti et al began their research on congenital defects of prothrombin. They concluded that approximately 49 mutations can cause defects in prothrombin in the form of hypo-prothrombinemia and dys-prothrombinemia, and that the lack of human prothrombin is incompatible with life (14). In 2013, Lancellotti et al again conducted studies on congenital prothrombin deficiency and found some new mutations on FII gene (15). In 2014, Bafunno et al detected a new mutation causing dys-prothrombinemia. The level of FII activity was 0.82% in this mutation, which occurred in the form of *F2,* c.1090 T/A (p.Val322Glu) and showed normal antigenicity. In this disorder, activation of mutated factor II by Okarin caused mesothrombin aggregation, which caused impaired prothrombin maturation towards thrombin. Minor bleeding was observed in this study (16).

Among 12 patients with factor II deficiency in this study, mutation was found in only four cases, of which three were point mutation and one case was frameshift mutation. Also, 33.33% of patients had considerable mutation. The findings of this study are different from previous studies in terms of clinical symptoms. In previous studies, ICH, hematomas, and spontaneous petechiae were the most common findings, while epistaxis and ecchymosis were the main clinical symptoms in the present study. Epistaxis has not been reported in previous studies. Bleeding from joints, GI tract or ICH were not reported in our patients. In previous studies, 20% of homozygous women showed signs of menorrhagia, whereas 100% of women in this study had menorrhagia. ICH was observed in two (40%) out of five women under study and postpartum bleeding was observed in 100% of women, while there was no report of this type of bleeding in factor II deficiency. Bleeding after tooth extraction was observed in 16.6% of patients in this study, but it was seen in 36% of patients in previous studies (17,18). Figure 4 compares the clinical manifestations of factor II deficiency in this study and previous studies.

Statistical analysis did not indicate any relationship between mutation type and clinical symptoms.

Limited number of patients, lack of cooperation from some patients, and high cost of sequencing are some of the limitations of the present study. It is recommended to do the following for detailed information: 1) repeat the study on a larger population of patients to find clinical symptoms and type of mutation, 2) evaluate the level of factor II at the level of mRNA and assess its relationship with the level of factor II antigenic, and 3) study the epigenetic



	ICH	Hematoma	Hemarthrosis	GIB	Bleeding after tooth extraction	Menorrhagia	CNS bleeding	Epistaxis	Postpartum bleeding
previous studies	60%	60%	42%	12%	36%	20%	0	0	0
our study	0	16.60%	0	0	16.60%	100%	40%	41.60%	100%

Figure 4. Comparison of Clinical Findings in Previous Studies With Our Study.

modification of factor II in terms of methylation and its relationship with expression level of factor II.

Conclusion

Molecular methods performed on patients from Southeastern Iranian population in terms of coagulation factor II deficiency revealed a substitution mutation in exon 7 in three patients and a frameshift mutation in exon 14 in one patient, both of which were reported for the first time. Considering the significant difference between the clinical symptoms of the present study and previous studies, probably the type of mutations reported in this study (for the first time) caused these clinical symptoms, but statistical studies did not show any relationship between the type of mutation and the occurrence of clinical symptoms. And it needs more investigations on more patients, with a larger population.

Authors' Contribution

Conceptualization: Hamed Soleimani Samarkhazan and Shaban Alizadeh.
Methodology: Shaban Alizadeh, Hamed Soleimani Samarkhazan and Majid Naderi.
Validation: Hamed Soleimani Samarkhazan and Ziba Majidi.
Formal Analysis: Hamed Soleimani Samarkhazan and Zahra Kashani Khatib.
Investigation: Hamed Soleimani Samarkhazan and Shaban Alizadeh, Zahra Kashani Khatib and Ziba Majidi.
Resources: Hamed Soleimani Samarkhazan and Shaban Alizadeh.
Data Curation: Shaban Alizadeh and Majid Naderi.
Writing—Original Draft Preparation: Hamed Soleimani Samarkhazan and Ziba Majidi.
Writing—Review and Editing: Hamed Soleimani Samarkhazan and Ziba Majidi.
Visualization: Hamed Soleimani Samarkhazan, Zahra Kashani Khatib and Ziba Majidi.
Supervision: Shaban Alizadeh.
Project Administration: Shaban Alizadeh and Majid Naderi.

Conflict of Interests

The authors declare that there are no competing interests.

Ethical Issues

The study protocol was approved by the ethical committee of Tehran University of Medical Sciences, Iran.

Financial Support

This research was supported by Tehran University of Medical Sciences & Health Services (Grant No. 039-02-31-25343).

References

1. Chinnaraj M, Planer W, Pozzi N. Structure of coagulation factor II: molecular mechanism of thrombin generation and development of next-generation anticoagulants. Front Med. 2018;5:281.
2. McMichael MA. Overview of Hemostasis. Schalm's Veterinary Hematology. 2022:763-86.
3. Green D. Coagulation cascade. Hemodialysis Int. 2006;10(S2):S2-S4. doi:10.1111/j.1542-4758.2006.00119.x
4. Meeks S, Abshire T. Abnormalities of prothrombin: a review of the pathophysiology, diagnosis, and treatment. Haemophilia. 2008;14(6):1159-63.
5. Daneshi M, Naderi T, Tabibian S, Shams M, Rashidpanah J, Dorgalaleh A. Congenital prothrombin deficiency. Journal of Cellular and Molecular Anesthesia. 2018;3(4):146-54.
6. Pasmant E, Dumont B, Lacapere JJ, Dautzenberg MD, Bezeaud A. A severe neonatal presentation of factor II deficiency. Eur J Haematol. 2011;87(5):464-6. doi:10.1111/j.1600-0609.2011.01670.x
7. Dorgalaleh A, Farshi Y, Haeri K, Ghanbari OB, Ahmadi A. Risk and management of intracerebral hemorrhage in patients with bleeding disorders. Semin Thromb Hemost. 2022;48(3):344-355. doi:10.1055/s-0041-1740566
8. Jesudas R, Pipe SW. Congenital Bleeding Disorders. Concise Guide to Hematology. Springer; 2019. p. 95-110.
9. Peyvandi F, Duga S, Akhavan S, Mannucci P. Rare coagulation deficiencies. Haemophilia. 2002;8(3):308-21.
10. Peyvandi F, Cattaneo M, Inbal A, De Moerloose P, Spreafico M. Rare bleeding disorders. Haemophilia. 2008;14:202-10.
11. Girolami A, Ferrari S, Cosi E. A comment on congenital prothrombin abnormalities associated with thrombosis but not with bleeding. J Thromb Thrombolysis. 2021;51(2):513-5.
12. Shapiro SS, Martinez J, Holburn RR. Congenital dysprothrombinemia: an inherited structural disorder of human prothrombin. J Clin Invest. 1969;48(12):2251-9.
13. O'Marcaigh AS, Nichols WL, Hassinger NL, et al. Genetic analysis and functional characterization of prothrombins Corpus Christi (Arg382-Cys), Dhahran (Arg271-His), and hypoprothrombinemia. Blood. 1996;88(7):2611-2618.
14. Lancellotti S, De Cristofaro R, editors. Congenital prothrombin deficiency. Semin Thromb Hemost. 2009;35(4):367-381. doi:10.1055/s-0029-1225759.
15. Lancellotti S, Basso M, De Cristofaro R, editors. Congenital prothrombin deficiency: an update. Semin Thromb Hemost. 2013;39(6):596-606. doi:10.1055/s-0033-1348948
16. Bafunno V, Bova M, Loffredo S, et al. Mutational spectrum of the c1 inhibitor gene in a cohort of Italian patients with hereditary angioedema: description of nine novel mutations. Ann Hum Genet. 2014;78(2):73-82.
17. Peyvandi F, Mannucci P, Lak M, et al. Congenital factor X deficiency: spectrum of bleeding symptoms in 32 Iranian patients. Br J Haematol. 1998;102(2):626-8.
18. Piran S, Khatib R, Schulman S, et al. Management of direct factor Xa inhibitor–related major bleeding with prothrombin complex concentrate: a meta-analysis. Blood Adv. 2019;3(2):158-67.