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Association Between Val34Leu Polymorphism and Risk of Umbilical Cord Bleeding in Severe Congenital Coagulation Factor XIII Deficiency in Southeast of Iran

Majid Naderi [1]; Akbar Dorgalaleh [2]; Shadi Tabibian [2]; Peyman Eshghi [3]; Taregh Bamedi [1]; Shaban Alizadeh [2,*]

[1]Genetic Researcher Center in Non-Communicable Disease, Zahedan University of Medical Sciences, Zahedan, IR Iran
[2]Department of Hematology, Allied Medical School, Tehran University of Medical Sciences, Tehran, IR Iran
[3]Pediatric Congenital Hematologic Disorders Research Center, Shahid Beheshti University of Medical Sciences, Tehran, Iran

*Corresponding author: Shaban Alizadeh, Hematology Department, Allied Medical School, Tehran University of Medical Sciences, Tehran, IR Iran. Tel: +98-5413229688 Fax: +98-21883338998, E-mail: alizadehs@sina.tums.ac.ir

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Background: Factor XIII deficiency is a rare bleeding disorder that presents various life threatening clinical manifestations such as *central nervous system* (CNS) and umbilical cord bleeding. Thisdisorder occurs due to different mutations and polymorphisms among which Val34Leu has associated with the majority of clinical features.
Objectives: The current study aimed to evaluate the relationship between polymorphism and umbilical cord bleeding as the most common clinical feature in affected patients with FXIII deficiency in Sistan and Baluchistan province, Iran.
Patients and Methods: This case control study was conducted on 12 FXIII deficient patients with umbilical cord bleeding as the case group and 15 patients with FXIII deficiency without umbilical cord bleeding as the control group. All the patients and controls were evaluated for Val34Leu polymorphism. Eventually, the obtained data were analyzed by SPSS software version 19.
Results: Results of the current study showed no statistically significant association between FXIII Val34Leu and umbilical cord bleeding. In addition no statistically significant difference was found between the cases and the controls.
Conclusions: FXIII Val 34Leu polymorphism is not associated with umbilical cord bleeding.

*Keywords:*Factor XIII Deficiency; Umbilical Cord; Polymorphism, Genetic

1. Background

Congenital Coagulation factor XIII deficiency is a rare hemorrhagic disorder with estimated incidence of one per three to five million, transmitted in autosomal recessive manner (1). Although the exact prevalence of the disease is unclear but incidence of the disorder with increased rate of consanguine marriage is high in the region under study. Sistan and Baluchistan is located in the southeast of Iran, and with more than 350 cases has the highest prevalence of the disorder in the world (2). This disorder has wide spectrum of clinical presentations that range from mild bleeding feature to life threatening episode including central nervous system (CNS) bleeding. Life threatening clinical manifestations including umbilical cord bleeding, recurrent miscarriage and intracranial hemorrhage are the common features in the affected patients (1, 2). Umbilical bleeding as an early bleeding episode occurs a few days after birth and can be life threatening in homozygous patients with FXIII deficiency. This feature is considered as the most common clinical manifestation in the patients affected with FXIII deficiency and can be found in approximately 80% of the cases. Factor XIII deficiency occurs due to different mutations in genes of subunits of factor XIII (3, 4).

Val34Leu is one of the most common polymorphisms of factor XIII-A subunit which has shown to accelerate the rate of FXIII activation almost 2.5 times. Various studies have indicated the association of this polymorphism with a large number of disorders such as risk of thrombosis, myocardial infarction (MI), CAD, and intracranial hemorrhage (2, 5).

2. Objectives

The current study aimed to evaluate the association of Val34Leu polymorphism with umbilical cord bleeding (UC) as the most common clinical manifestation in patients affected with this feature in Sistan and Baluchistan.

Implication for health policy/practice/research/medical education:
Umbilical cord bleeding is a common and life threatening episode in patients with severe factor XIII deficiency. Identification of genetic risk factors can help health care system to prevent this diathesis. This study assessed the association between Val34Leu as a common polymorphism of factor XIII with risk of umbilical cord bleeding in patients with factor XIII deficiency.

3. Patients and Methods

3.1. Study Subjects

This case-control study was conducted on 12 factor XIII deficient patients with history of umbilical cord bleeding as the case group, and 15 factor XIII deficient patients without umbilical cord bleeding as the controls; the two groups were as similar as possible regarding age and sex. Written consent was obtained from each participant, according to the protocol approved by the Medical Ethics Committee of Zahedan University of Medical Sciences. Patients with abnormal clot solubility test in 5M urea or 1%monochloroacetic acid environments, patients with positive family history of FXIII deficiency, and also patients with clinical manifestations suspected to FXIII deficiency were included in the study.

3.2. Study Protocols

Each patient was examined by a physician and also interviewed by an expert staff to complete a questionnaire regarding the demographic data and previous medical history. All suspected patients to FXIII deficiency were referred to coagulation laboratory of Iranian Blood Transfusion Organization (IBTO). At the beginning, all patients were evaluated by routine coagulation tests including prothrombin time (PT), partial thromboplastin time (PTT), bleeding time (BT), and platelet count. Once no abnormality was observed in the tests, patient was candidate for assessment of clot solubility test in 5 M urea or 1% monochloroacetic acid environments. Then patients with abnormal clot solubility test were a candidate for molecular analysis.

3.3. DNA Extraction

Blood sample from each patient was collected in ethylene-diamine-tetra-acetic acid (EDTA) anticoagulant tube. Blood specimen was lysed with sodium dodecyl sulfate and genomic DNA was isolated from the leucocytes according to the standard protocol. DNA was purified using phenol-chloroform and ethanol precipitation (6).

3.4. Polymerase Chain Reaction

Following DNA extraction, genotyping factor XIII Val-34Leu Polymorphism was evaluated by polymerase chain reaction amplification of genomic DNA, PCR (polymerase chain reaction) test followed by restriction enzyme digestion polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP). Previously published primers were selected (7). For all PCR amplifications, positive control DNA samples of known genotype as well as a negative control without template DNA were considered.

3.5. Statistical Analysis

Data analysis was performed by the SPSS data-analysis package (version 18). Differences between the two groups were compared using an independent t-test , and a P value of 0.05 or less was considered statistically significant.

4. Results

4.1. Characteristics of the Study Population

This case- control study was conducted on 12 patients with UCB as the case, and 15 individuals without UC as the control groups. The mean age of patients in the case group was 8.5 ± 0.3 years and that of the control group was 8.2 ± 0.2 years. There was no statistically significant different between the age average of the case and control groups (P = 0.3). Among the patients three (25%) cases were male while nine (75%) were female. The blood group in the patients was as follow: 6 (50%): O, 3 (25%): B, 2 (17%): A, 1 (8.5%): AB. Results of the current study revealed that among the patients easy bruising and intramuscular haematoma were the most common clinical manifestations observed in 83% of the patients. Besides these features, post dental extraction bleeding and gum bleeding were observed in 16.6% of the patients. Poor wound healing, epistaxis; post-surgery bleeding and hemarthrosis were observed in 8.3%, 16.6%, 16.6% and 8.3% of the patients, respectively.

4.2. Val 34 Leu Polymorphism

Results of the current study indicated that no patients in the case and control groups were homozygote or heterozygote for this polymorphism. The distribution of this polymorphism did not differ between the two groups, therefore, there was no statistical difference between them (P > 0.05).

5. Discussion

Factor XIII deficiency is an extremely rare bleeding disorder worldwide that has the highest prevalence in Sistan and Baluchistan located in the south east of Iran (1, 2).

Table 1. Characteristics of Used Primers and Restriction Enzymes [a]

Polymorphism	Primers	Annealing, ˚C	PCR Product, bp	Digestion	Bands, bp
Val34Leu	5′CATGCCTTTTCTGTTGTCTTC3′	62	192	Dde I	161, 31
	5′TACCTTGCAGGTTGACGCCCCGGGGCACTA3′				

[a] Abbreviations: bp, base pair; Dde I, desulfovibrio desulfuricans 1; Leu, leucine; PCR, polymerase chain reaction; Val, valine

The disorder occurs due to different mutations among whichVal34Leu, Trp 187 Arg, Pro564Leu, and Trp204 Phe are more common. Val 34 Leu as one of the most common polymorphisms in factor XIII deficiency associated with different diseases such as intracranial hemorrhage, myocardial infarction, and thrombosis (3, 4). The study conducted by Catto et al. showed that Val 34 Leu polymorphism has a slightly higher incidence in patients with intracerebal hemorrhage (6). Reiner et al. evaluated the association of this polymorphism with hemorrhagic stroke (8). Factor XIII deficiency is presented with various life threatening clinical manifestations such as umbilical cord bleeding, intracranial hemorrhage, and deep soft tissue haematoma. Among the bleeding episodes of this disorder umbilical cord bleeding, as a diagnostic clinical manifestation, has the highest frequency, observed in 80% of all patients. The current study aimed to evaluate the association of this common polymorphism (Val 34 Leu) with the most common clinical features of the participating affected patients with FXIII deficiency. In fact the current study aimed to find a potential risk factor for this common and life threatening factor. This polymorphism was negative and none of the patients in the case and also control groups presented this polymorphism. The current study failed to find any significant relationship between this polymorphism and umbilical cord bleeding. In the similar study conducted by Anwar six FXIII deficient patients with umbilical cord bleeding were evaluated. In these patients Val 34 Leu was not reported as a responsible polymorphism while two polymorphisms including Glu 102 Lys and Ser295 Arg were reported (7). Finding established mutation in affected patients with different clinical manifestation is required to manage and prevent further spread of the disease, and also death of patients.

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Authors' Contribution

All authors had equal role in design, work, statistical analysis and manuscript writing.

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