Original Article

c.559 T>C as The Most Common Mutation of Factor XIII Deficiency in Iranian Patients is not Restricted to Southeast Iran

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Abstract

Background: Iran has a large group of patients with severe congenital factor XIII deficiency (FXIIID) and Trp187Arg mutation that is most disease causing mutations of FXIII in the world is only observed in the southeast of Iran in 352 patients with FXIIID. 743 patients with FXIIID was observed in 17 provinces of Iran, but Tehran city with more than 12 million population has no any registered patient with FXIIID. Here we described the first case with severe congenital FXIIID in Tehran Province with underline FXIII mutation.

MaterialsandMethods:A neonate with prolonged umbilical cord bleeding was referred to hemophilia center. The patient was screened by routine coagulation tests and by clot solubility test. After observation of normal routine tests and abnormal clot solubility patient was undergoing a full sequencing of FXIII-A gene. For confirmation of detected mutation in FXIII-A gene, exon 4 was amplified by PCR and cleaved by Eco130I restriction enzyme.

Results:We found the first case with severe congenital FXIIID in Tehran Province with Trp187Arg mutation in exon 4 of FXIII-A gene. Patient's parents were heterozygote for this mutation.

Conclusion: Trp187Arg mutation of FXIII-A is the most common mutation in Iranian patients with FXIIID and is not restricted to southeast of Iran.

Keywords: Factor XIII deficiency, Trp187Arg mutation, Tehran Province

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Introduction

Coagulation factor XIII (FXIII) is a tetrameric transglutaminase, consist of two subunits A (FXIII-A) with catalytic function and two B subunits (FXIII-B) as a carrier (A2B2) that held together by non-covalent bond. FXIII-A is synthesized by cells of bone marrow origin, whereas FXIII-B is produced by hepatocytes. In the final stage of blood coagulation, FXIII stabilizes fibrin clots and protects it from proteolytic degradation by the fibrinolytic system by formation of covalent crosslinks between γ-glutamyl and ε-lysyl residues of fibrin monomers after activation by thrombin (1-3).

FXIII deficiency (FXIIID) is extremely rare. Bleeding disorder occurs approximately 1 in 5 million prevalence in the general population (1, 4). Most affected patients have deficiency of A subunit.

Clinical manifestations of FXIII deficiency including umbilical cord bleeding, delayed wound healing, subcutaneous and soft tissue bleeding, recurrent abortion and pregnancy loss and intracranial bleeding as the main cause of death in affected patients (4-7). Most of bleeding episodes are seen in patients with severe deficiency (activity of FXIII is less than 1%). Clot solubility test in urea, acetic acid or monochloroacetic (MCA) acid has done for detection of FXIIID, but this test detects only severe form of FXIII deficiency and mild and moderate forms of FXIIID are diagnosed by quantitative assay such as amine incorporation and ammonia release assays (2, 8-10). In regions with a high rate of consanguineous marriage number of affected patients with rare bleeding disorders (RBDs) such as FXIIID are significantly higher (11, 12). The prevalence of FXIIID in Iran is very higher than the overall frequency in the rest of the world and the number of FXIIID in Iran was 473 patients in 2014. Although many studied have been performed in this country, but up to now any study about FXIIID has not been reported in Tehran Province (1). In This study, we assessed a new case of FXIIID in Tehran Province.

Methods

This study was performed in Tehran Province, central Iran. The study was approved by Iran University of Medical Sciences and a written consent was obtained from patients' parents. A neonate with unusual prolonged bleeding was referred to a physician. The neonate was referred to hemophilia laboratory. Initially a structural questionnaire was filled by interview of the patient's parents by a trained staff to obtain demographic data and any family history of FXIIID.

Then the patient was screened by routine coagulation tests, including Activated Partial Thromboplastin Time (APTT), Prothrombin Time (PT) (DiagnosticaStago -France kits and semi-automatic coagulation analyzer, STAGO, STart®), Bleeding Time (BT) (Ivy method) and platelet count (Sysmex kx_21 hematology analyzer). Since the factor XIII activity assay is not performed in Iran as a screening test for diagnosis of FXIIID, the patient was assessed by clot solubility test in an MCA acid environment (13). With observation of a normal routine coagulation tests and an abnormal clot solubility test patient was undergoing further investigations for the detection of underline FXIII-A gene mutation.

At baseline blood specimens with EDTA anticoagulants were drawn from all selected patients in order to isolate genomic DNA. DNA was obtained from the blood specimen after lysis with sodium dodecyl sulfate and proteinase K treatment of buffy coat. DNA was purified using phenol–chloroform and ethanol precipitation. The quality and quantity of DNA obtained were determined by spectrophotometer and also by means of *agarose*gel electrophoresis. Following DNA extraction, the coding region, intron/exon boundaries and 5' and 3' untranslated regions (UTR) of the FXIII gene were amplified by polymerase chain reaction (PCR). Amplified fragments were directly sequenced using an automated sequencer (Applied Biosystems, Foster City, CA). For confirmation of identified mutations, sequencing were repeated and in cases with a digestion site for the restriction enzyme (RE), amplified fragment was digested by appropriate RE under standard condition. After mutation detection, the patients' parents DNA was amplified with PCR and was digested by Eco130I (Fermentas Life Sciences, York, UK) according to the manufacturer's instruction. PCR and digestion conditions were described previously (14).

Results

The patient was a male neonate with prolonged cord bleeding. All routine coagulation tests were normal in this patient and clot solubility test was abnormal. Neonate did not receive any replacement therapy. Molecular analysis of FXIII-A gene revealed C.559T>C neucleotid exchange in exon 4 which lead to tryptophan to arginine substitution in FXIII A subunit (Trp187Arg).

This amino acid exchange affects FXIII-A chain structure and probable instability of this subunit and in the homozygous condition cause severe factor XIII deficiency. After detection of this mutation, tepatients' parent exon 4 product (a 513bp fragment) was cleaved by Eco130I and in both 4 fragments, including– 460, 392, 68 and 53 bp fragments was observed after digestion that was consistent with the

heterozygous state for Trp187Arg mutation.

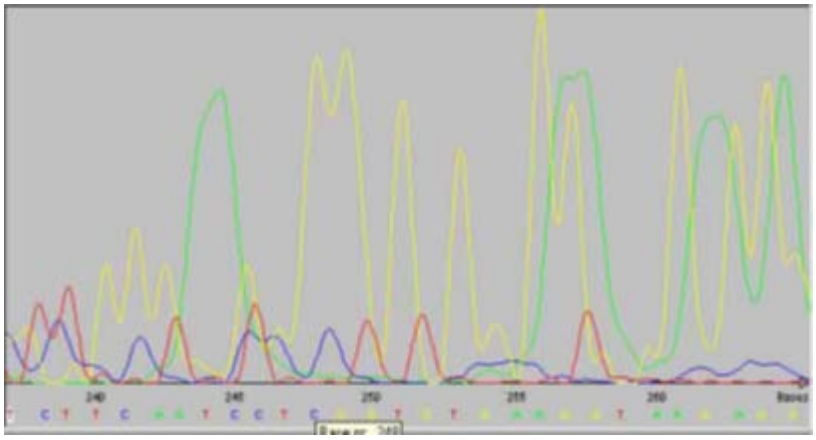

Fig. 1.Observed mutation in position of 559 with substitution of C instead of T

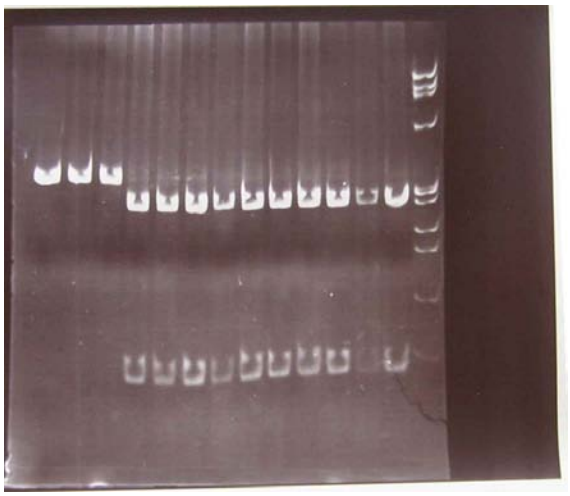

Fig. 2.PCR-RFLP for c.559T>C mutation confirmed the results of DNA sequencing

Discussion

FXIIID is an extremely rare hemorrhagic disorder with an estimated prevalence of 1 per 2 million in the general population. Iran as a Mideast country has the highest population of patients with FXIIID worldwide. FXIIID has an approximately 12 fold higher prevalence in Iran in comparison with an overall incidence of FXIIID in the world (1-4, 12). Among different provinces of Iran, Sistan and Baluchestan, southeast of Iran has the highest rate of FXIIID. This high prevalence of FXIIID in Iran is a result of the high rate of contagiousness marriage in this country (1, 12-14). Our previous study on FXIIID in Iran revealed 473 cases with severe congenital FXIIID in this country. Most of these cases (352 cases) were residents of Sistan and Baluchestan Province, southeast of Iran and all of patients in this area in except of 2 patients had Trp187Arg mutation in FXIII-A gene (1, 2,14-16). But this mutation was not observed in any other part of the country. Moreover, in our previous study, we did not find any patient with FXIIID in Tehran Province. In our study, we found patients with FXIIID in 17 provinces of Iran, but in Tehran Province as capital of Iran with the highest population among Iran provinces, we did not find any case with FXIIID and this case is the first one in this province (1-3). In this neonate we found Trp187Arg mutation in exon 4 that was same with only observed mutation in southeast of Iran. This finding was confirmed that Trp187Arg mutation is the most common in Iranian patients and is not restricted to southeast of Iran. Previously, we used this mutation for carrier detection and prenatal diagnosis (PND) only in southeast of Iran (1, 8-12). With this new finding, we can offer this mutation as the first step in

carrier detection and confirmation of FXIIID in suspected patient to FXIIID in all over the Iran (1-5). Since, in Iran, FXIII assay is not used for FXIII detection, a simple PCR-RFPL can used as a fast and reliable method for carrier detection and confirmation of FXIIID in suspected patients.

Conclusion

c.559T>C as the most common mutation of FXIII-A gene in Iranian patients is not restricted to southeast of Iran and can be used as the first screening mutation for diagnosis of disorder in Iran.

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Conflicts of Interest

The authors declare that there are no conflicts of interest.

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