Carrier Identification in Families of known Factor XIII Deficient Patients.

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ABSTRACT:

Objective: The aim of the study is to determine the prevalence of carriers within families affected by factor XIII deficiency, in interior Sindh population with the objective of understanding genetic distribution and potential implication for disease management and counseling.

Methodology: Cross-sectional study approved by Institutional Review Board (IREB) Baqai University was conduct at Department of Baqai Institute of Hematology at Baqai Medical University Karachi At Hemophilia Center Hyderabad and Research diagnostic lab Jamshoro The immediate family members of known carriers of factor XIII were enrolled. The Berichrom® Factor XIII assay was performed. The study highlighted the role of FXIII activity and the identification of mutations in FXIII Gene in the identification of carriers in FXIII deficient families, providing a simple, cost-effective approach to improve patient care and genetic counselling.

Results: A statistically significant difference in FXIII activity between carriers and non-carriers is shown by a one-way ANOVA (F = 19.44, p =.000) The variance in FXIII activity is mostly due to variations between groups rather than within-group variability, as indicated by the high F-value and low p-value.

Conclusion: This study concluded that Factor XIII and bleeding score are notable for identifying carriers in FXIII-deficient families also these measures to clinical practice can improve diagnosis, patient care, and genetic counselling

Keywords: Intracranial hemorrhage, coagulation tests, consanguinity, and factor XIII. Intracranial hemorrhage, coagulation tests, consanguinity, and factor XIII.

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Introduction:

Factor XIII (FXIII) is a protein critical to the blood clotting pathway and ensures the permanence and stability of fibrin blood clots. It also has pronounced functions in wound healing, and tissue regeneration, injury response, pregnancy, and maintenance of bone.[1] In circulation, FXIII exists in four subunits, two A subunits (FXIII-A) and two B subunits (FXIII-B). Enzymatic activity is found on the FXIII-A subunits and has been shown to have the capability of fibrin stabilization whereas FXIII-B subunits are in transport of FXIII-A as well as providing support to the de polymerization of FXIII-A functions to stabilize clots.[2] FXIII deficiency is a rare inherited bleeding disorder that can cause life-threatening bleeding even death primarily because of intracranial bleeding, hemorrhage beneath the umbilical cord and uterine pregnancy. On the contrary, patients who have severe lack of FXIII-B tend to have milder symptoms. Severe FXIII-A deficiency is approximately 1 of every 2 million people, whereas heterozygous FXIII-A is higher with a percentage going up to 3.5. People having heterozygous FXIII -A deficiency especially females are more susceptible to bleed during haemostatic stress events unlike most bleeding disorders.[3] Factor XIII deficiency is classified as a rare bleeding disorder (RBD), the overall incidence of which shows a range of about 3 5 per cent. The disorders have the autosomal recessive mode of inheritance, and with commanding cousin marriages in our society, they are more prevalent in Pakistan than in the western countries.[4,5] But here, there is no valid information on the prevalence of these rare bleeding disorders in developing country like Pakistan as well as other countries.

The FXIII-A is present in bone marrow and mesenchymal lineage-derived cells, which include platelets, megakaryocytes, monocytes, circulating and tissue macrophages, dendritic cells, chondrocytes, osteoblasts and pre-adipocytes. The A2B2 complex of FXIII is stabilized in plasma by the B subunits in a regulatory mechanism. Moreover, it is assumed that the functioning of FXIII can be regulated with the help of thrombin and some proteolytic enzymes secreted by granulocytes.[6,7]

The condition of FXIII deficiency is where this protein is lacking or less than 5 percent of the normal functioning is present. There is an autosomal recessive transmission pattern. With two carrier parents, the child has 25 percent probability of inheriting two mutated copies of the gene and suffer a deficiency, 50 percent chance of being a carrier or 25 percent chance of inheriting neither a mutated copy of the gene.[8]

Approximately 95 percent of all FXIII deficiencies are related to the mutation of FXIII-A gene. Cases that are severe tend to affect people with homozygous or compound heterozygous mutations, which causes extreme decrease in levels of FXIII, causing poor blood clotting. Other incidents that do not demonstrate the symptoms are those that have a single copy of the mutation (heterozygous). To be properly diagnosed, decreased or absent FXIII-A activity as well as mutations in F13A1 gene should be determined.[9,10]

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Health experts describe easy bruising, mucosal bleeding and muscular bleeding as common symptoms of FXIII deficiency. As a rule, FXIII activity levels of more than 30 percent suffice in a normal clotting process. Patients with homozygous mutations have a tendency to bleed through the umbilical stump in the newborn stage which is seen in nearly 80 percent of the newborns. There is a possibility of delayed diagnosis of female patients even at a later age.

The major complications of FXIII deficiency are repeated unexplained miscarriages and spontaneous intracranial hemorrhage. The main outcome of the condition, which leads to disability or death among the affected individuals, is intracranial bleeding, the incidence of which is reported as being about 30%.[11]

To identify carriers of FXIII deficiency in families that had already been affected, particularly in populations where consanguineous marriages are high, which increases the prevalence of the condition among the population was the aim of this study. Because the inheritance pattern of FXIII deficiency is autosomal recessive, the carrier status of FXIII poses a great danger to the occurrence of more children affected by it in such families. To help start early preventive treatment and lower the chance of severe bleeding cases, it is vital to detect the problem early.

Objectives:

The study aims to check FXIII levels in each sibling and the proband, to screen all family members for specific mutations in selected exons using the Sanger sequencing method based on mutations identified in the proband, and to determine the carrier rate within the family.

Methodology:

This cross-sectional analytical study was conducted in collaboration with Dr. Arshi Diagnostics, the Hemophilia Welfare Society Karachi (HWSK), and the Otaq of Dadu Village at the Hematology Department of Baqai University, following ethical approval. A total of 185 participants were recruited in this family-based study, with the sample size determined by the number of reachable index cases and their relatives; specifically, 30 index cases were targeted, with an average of 6–8 family members screened per proband (180–240 relatives), allowing estimation of local carrier frequency and identification of potential recurrent mutations. Inclusion criteria comprised siblings, spouses, and parents of probands or affected patients with genetic defects in FXIII A and FXIII B subunits, while individuals with other bleeding disorders or already diagnosed patients were excluded. Blood samples were collected via clean venipuncture into citrate tubes for coagulation/FXIII testing and EDTA tubes for DNA analysis, transported under cold conditions, and processed within two hours; plasma was separated by centrifugation and stored at −70°C, while EDTA blood was maintained at 4°C for DNA extraction within 24-48 hours.

Coagulation Screening Tests:

Coagulation assays were performed on the STA Compact analyzer (Diagnostica Stago, France), a fully automated coagulation analyzer employing a mechanical clot-detection (viscosity-based) methodology, using the manufacturer's reagents and internal quality control materials run with each batch for Screening.

1. **Prothrombin Time (PT)**. By adding a calcium thromboplastin reagent to citrated plasma and recording the time to clot formation PT was measured.
2. **Activated Partial Thromboplastin Time (APTT)**. For APTT incubating plasma with a phospholipid reagent and a contact activator (e.g., silica or ellagic acid), by following recalcification with calcium chloride, and recording the clotting time in seconds. established reference range Locally were used for interpretation.

Aim of both PT and APTT were primarily to exclude other coagulation factor deficiencies and to confirm that the intrinsic and extrinsic pathways were otherwise intact, since these tests remain normal in isolated FXIII deficiency.

3. **Urea Clot Lysis Assay (Screening Test for FXIII Deficiency)** qualitative screening method was used for the urea clot solubility test.

- Citrated plasma was recalcified with 0.2–0.25 M calcium chloride and allowed to clot at 37°C for 30–60 minutes.
- The formed clot was immersed in a 5 M urea solution (some protocols use 1% monochloroacetic acid as an alternative) and incubated at room temperature or 37° C.
- The clot was observed for dissolution at 1, 2, 6, 12, and 24 hours.

within 24 hours Dissolution of the clot (typically within 1–2 hours in severe deficiency) was interpreted as suggestive of FXIII deficiency, reflecting failure of cross-linking of fibrin monomers due to absent/deficient FXIII activity. Clots from normal individuals remained insoluble in urea for at least 24 hours. Given the low sensitivity of this test for partial/heterozygous (carrier) deficiency, it was used only as an initial screen, with the quantitative assay used for confirmation.

4. **Berichrom Factor XIII Assay (Quantitative FXIII Activity).** Quantitative FXIII activity was measured using the Berichrom FXIII assay (Siemens Healthineers, formerly Dade Behring), a photometric ammonia-release assay based on the principle of FXIII-mediated transamidation:
5. Plasma FXIII (Factor XIIIa, activated by thrombin and calcium) catalyzes the incorporation of a primary amine into a glutamine-containing peptide substrate, releasing ammonia.
6. Released ammonia reacts with 2-oxoglutarate and NADH in the presence of glutamate dehydrogenase (GLDH), causing oxidation of NADH to NAD+.
7. The rate of decrease in absorbance at 340 nm, measured photometrically, is proportional to FXIII activity in the sample.

Results were expressed as a percentage of normal pooled plasma activity (%), calculated against a calibration curve derived from serial dilutions of a reference plasma. This quantitative assay allowed discrimination between homozygous/compound heterozygous deficient patients (markedly reduced activity), heterozygous carriers (intermediate activity), and individuals with normal FXIII activity.

Results:

Based on the results of this study, which highlights demographic, clinical, and statistical data gained from in-depth research, families affected by Factor XIII (FXIII) insufficiency will have a complete understanding of factors influencing carrier identification.

Analysis of Bleeding Score and Age:

The distribution of gender of the participants is presented in Fig 1, which shows that there are 185 participants with a gender split of 102 females and 83 males. Figure 1 demonstrates the distribution of the bleeding score is right skewed with majority of the scores ranging between 2.5 and 5.0,

with few having scores higher than 10.0, indicating low overall bleeding scores in the sample. The bleeding score of each individual is continually recorded and ranges from 1-19, with a mean of 4.092. This indicates that while people generally bleed more or less, a small number are at risk of bleeding. The variance (7.051) and standard deviation (2.6554) suggest moderate variability, suggesting some stability in the bleeding patterns, although there are significant outliers. In current study, we observed the frequency of bleeding symptoms in the total participants and bruise was the most common bleeding symptom among our participants. There were about 63 individuals with this symptom. Gum Bleeding was the second most common bleeding symptom. Thirty-three people were reported with this symptom. 16 participants which were females complained about menorrhagia. Only one participant reported that he was bleeding during the circumcision. The frequency of bleeding symptoms is also represented as bar graph in Figure 2.

Fig No 1: Histogram showing distribution of bleeding score right skewed.

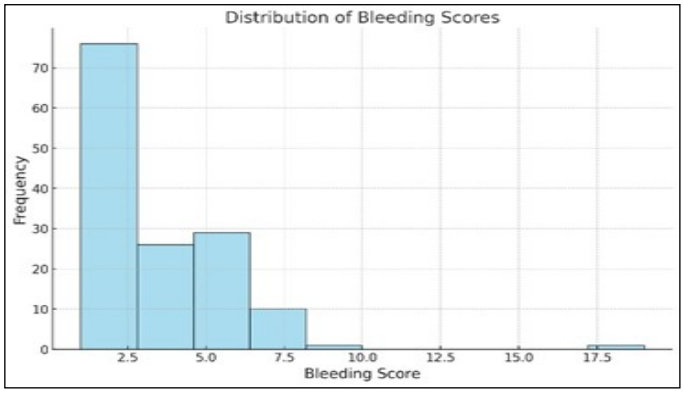
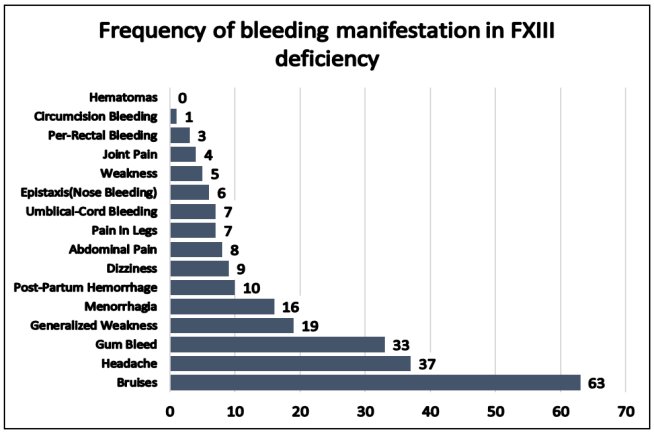


Fig No 2: Bar chart showing bleeding manifestations in factor X111 deficiency in frequency.



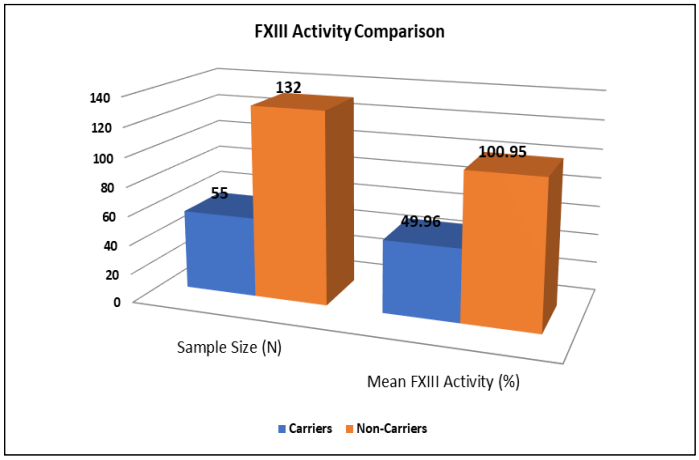
Out of 185 participants, 33 participants had this symptom.

1. **FXIII Activity Comparison by Carrier Status:**

There are significant disparities in FXIII activity between carriers and non-carriers. The mean FXIII activity in carriers is 49.96%, which is less than the 100.95% seen in non-carriers. This discrepancy highlights the different metabolic characteristics of carriers. A more uniform deficit pattern among carriers is shown by the much lower standard deviation for carriers (26.27%) compared to non-carriers (79.22%). Greater accuracy in predicting the mean FXIII activity of carriers is indicated by the decreased standard error (3.75% for carriers vs. 6.92% for non-carriers).

The bar graph in fig 3 compares FXIII activity of the carriers with that of non-carriers and reveals that the mean activity of FXIII (100.95%) among non-carriers is significantly higher than that among carriers (49.96%) and the non-carriers shows a larger sample size (N=132) as compared to carriers (N=55). bar graph compares FXIII activity between carriers and non-carriers, showing significantly higher mean FXIII activity (100.95%) and sample size (N=132) for non-carriers compared to carriers (49.96% and N=55).

Fig No 3: The bar graph compares FXIII activity of the carriers with that of non-carriers



The bar graph shows the distribution of the Factor XIII carrier status in a population, with a vast majority of non-carriers (N=132) and a few numbers of carriers (N=55). This means that the sample studied has more non-carriers than carriers.

Fig 4: pie chart depicting the number of patients identified as Normal, Homozygous and Heterozygous.

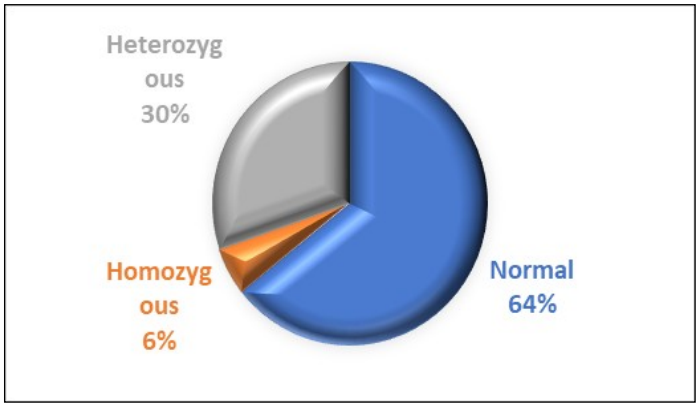
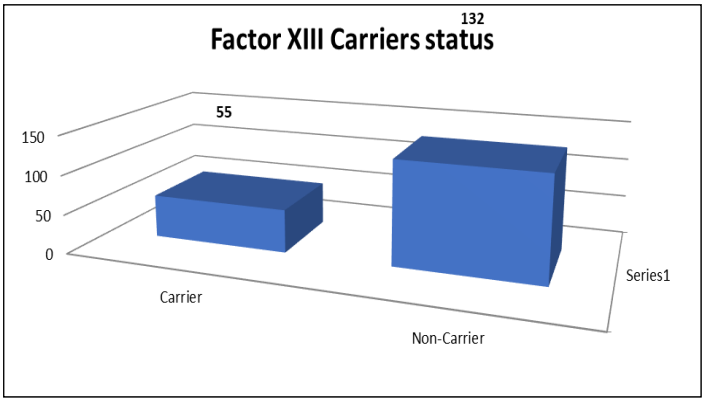


Fig No 5: The bar graph illustrates the distribution of Factor XIII carrier status in a population.



The bar graph in fig 5 illustrates the distribution of Factor XIII carrier status in a population, highlighting a significantly larger group of non-carriers (N=132) compared to carriers (N=55). This indicates that non-carriers are more prevalent in the sample studied.

2. Significance of One-Way ANOVA in Statistics:

These findings showed in figure 5 are further supported by a one-way ANOVA (F = 19.44, p =.000) indicating a statistically significant difference in FXIII activity between the two groups (carriers and non-carriers). The high F-value and low p-value suggest that there is greater difference between groups than within groups, thus the variance in FXIII activity is largely due to variations between groups rather than within groups.

Discussion:

Evaluation of the families for Factor XIII (FXIII) deficiency carriers is essential for improved clinical management, genetic counselling and early treatment. This study was to identify the clinical and demographic factors such as FXIII activity and bleeding scores which can help distinguish carriers from non-carriers. The results offer useful insights that can improve both the accuracy of diagnoses and clinical outcomes, paving the way for better methods to detect carriers.[12] The intergenerational aspect of this study is highlighted by the participation of 185 participants. whose ages vary from 1 to 85 years. The target demography usually involved in carrier identification and reproductive decision-making is represented by a mean age of 27.31 years. which is representative of a young adult population. The broad age spectrum will allow for a novel approach to studying FXIII activity and bleeding tendencies across generations. A useful viewpoint on inheritance patterns and the possibility of early diagnosis is answered by the inclusion of several generations. The bleeding score analysis has a range from 1 to 19 with a mean score of 4.092 indicating significant inter-family variability in the tendency to bleed. These results highlight the fact that whereas most people only have little bleeding, others suffer from serious problems. Such a heterogeneity is probably due to differences in FXIII activity level and to other factors that might influence activity levels, including lifestyle-related factors and primary clotting disorders. 136)

The mean activity levels (49.96% and 100.95%, respectively) of the tests for FXIII activity clearly distinguish carriers from non-carriers. This noteworthy distinction demonstrates the value of FXIII activity as a trustworthy biomarker for carrier identification.

Diagnostic results of the one-way ANOVA (F = 19,44, p =,000) supports the statistical significance of the differences in FXIII activity between carriers and non-carriers. This is consistent with the ability of the tests for FXIII activity to distinguish these groups. The difference between the groups (92,736.42) and within the groups (849,043.64) is very large, which indicates the reliability of XIII activity as a carrier identification marker. 64). The Nagelkerke R Square of 1.000 and a perfect match (-2 Log Likelihood = 0.000) resulted in a correct classification of 100% between carriers and non-carriers. The results indicate that FXIII activity and blooding scores are a sensitive and specific method of carrier identification and will have a significant impact in clinical practice and genetic counseling. Early carrier detection paves the way for pro-active measures such as reproductive planning, prenatal diagnosis and cascade testing of at-risk family members. The findings of this study have broader public health implications particularly in where there is a high prevalence of FXIII deficiency. The results of this study have public health implications, especially in areas of the world where FXIII deficiency is prevalent. Carrier identification programs have the potential to have a major impact for those with bleeding disorders and their families, which is to enhance their quality of life if they have an undiagnosed or less than optimal diagnosis or treatment. Awareness campaign, training of healthcare workers and setting up diagnostic centre should be given importance in public health initiatives for identification of carrier.[13,14]

While this study provides valuable information for the identification of carriers in families with known FXIII deficiency, some limitations should be noted to provide context for the results and guide future research.[15]

A significant disadvantage is that to identify the carriers is based on the measurement of FXIII activity. FXIII activity levels provide valuable information, but the level of activity may not always be exactly correlated with hereditary carrier state.[15] Environmental factors, comorbidities and laboratory technical factors can affect the levels of FXIII – this can lead to false positive or negative results. This investigation faced challenges in the form of a lack of resources for genetic tests, which are more definitive in determining carrier status, but because it was not always available in cases that were at times unclear, the findings were more difficult to determine.[14]

Conclusion:

This study concluded that Factor XIII and bleeding score are notable for identifying carriers in FXIII-deficient families also these measures to clinical practice can improve diagnosis, patient care, and genetic counselling. Early diagnosis, tailored treatment, and public health awareness are essential to reduce the burden of bleeding disorders and improve quality of life. standardized bleeding assessments, better control of confounding conditions, and studies in more diverse populations are still needed..

References:

1. Kleber C, Sablotzki A, Casu S, Olivieri M, Thoms KM, et al. The impact of acquired coagulation factor XIII deficiency in traumatic bleeding and wound healing. Crit Care. 2022 Mar 24;26(1):69. doi: 10.1186/s13054-022-03940-2. PMID: 35331308; PMCID: PMC8943792.
2. Watanabe H, Mokuda S, Tokunaga T, Kohno H, Ishitoku M, et al. Expression of factor XIII originating from synovial fibroblasts and macrophages induced by interleukin-6 signaling. Inflamm Regen. 2023 Jan 6;43(1):2. doi: 10.1186/s41232-022-00252-4. PMID: 36609460; PMCID: PMC9817275.
3. Dorgalaleh A, Jozdani S, Zadeh MK. Factor XIII Deficiency: Laboratory, Molecular, and Clinical Aspects. Semin Thromb Hemost. 2025 Mar;51(2):155-169. doi: 10.1055/s-0044-1796673. Epub 2024 Nov 29. PMID: 39613144.
4. Zafar L, Zafar T, Ahmed M, Sohail B, Waheed A. Treatment of female patients with inherited bleeding disorders in reproductive age: a single centre study from northern Pakistan: Treatment of Female Patients with Inherited Bleeding Disorders. Foundation University Medical Journal. 2023 Dec 31;6(1):26-30. doi:10.33897/fumj.v6i1.93
5. Khan N, Kashif N, Shah H, Baloch SK, Shah MA, Syed FN. Frequency of Rare Bleeding Disorders. Pakistan Journal of Medical & Health Sciences. 2023; Jun 17;17 (04):569-71. doi:10.53350/pjmhs2023174569

6. Hethershaw EL, Cilia La Corte AL, Duval C, Ali M, Grant PJ, Ariëns RA, Philippou H. The effect of blood coagulation factor XIII on fibrin clot structure and fibrinolysis. J Thromb Haemost. 2014 Feb;12(2):197-205. doi: 10.1111/jth.12455. PMID: 24261582.
7. Mutch NJ, Koikkalainen JS, Fraser SR, Duthie KM, Griffin M, et al. Model thrombi formed under flow reveal the role of factor XIII-mediated cross-linking in resistance to fibrinolysis. J Thromb Haemost. 2010 Sep;8 (9):2017-24. doi: 10.1111/j.1538-7836.2010.03963.x. PMID: 20586921; PMCID: PMC3071935.
8. de Moerloose P, Schved JF, Nugent D. Rare coagulation disorders: fibrinogen, factor VII and factor XIII. Haemophilia. 2016 Jul;22 Suppl 5:61-5. doi: 10.1111/hae.12965. PMID: 27405678.
9. Rijken DC, Abdul S, Malfliet JJ, Leebeek FW, Uitte de Willige S. Compaction of fibrin clots reveals the antifibrinolytic effect of factor XIII. J Thromb Haemost. 2016 Jul;14(7):1453-61. doi: 10.1111/jth.13354. Epub 2016 Jun 9. PMID: 27148673.
10. Muszbek L, Katona É. Diagnosis and Management of Congenital and Acquired FXIII Deficiencies. Semin Thromb Hemost. 2016 Jun;42(4):429-39. doi: 10.1055/s-0036-1572326. Epub 2016 Apr 12. PMID: 27071048.
11. Ariëns S, Huisman A, Hovinga ICLK, Urbanus RT, van Galen KPM, et al. Limited value of testing for factor XIII and α2-antiplasmin deficiency in patients with a bleeding disorder of unknown cause. Haemophilia. 2024 Jul;30(4):998-1002. doi: 10.1111/hae.15059. Epub 2024 May 29. PMID: 38812123.
12. Shahraki H, Dorgalaleh A, Fathi M, Tabibian S, Teimourian S, Mollanoori H, Khiabani A, Zaker F. How to Assess Founder Effect in Patients with Congenital Factor XIII Deficiency. Int J Hematol Oncol Stem Cell Res. 2020 Oct 1;14(4):265-273. doi: 10.18502/ijhoscr.v14i4.4480. PMID: 33603988; PMCID: PMC7876424.
13. Mangla A, Agarwal N, Maruvada S. Sickle Cell Anemia. [Updated 2023 Sep 4]. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2023 Jan-. Available from: https://www.ncbi.nlm.nih.gov/sites/books/NBK482164/
14. Bounaix L, Schroeder V, Fontana P . Management of anticoagulation and factor XIII replacement in a patient with severe factor XIII deficiency and recurrent venous thromboembolic disease: case report and review of literature. Research and Practice in Thrombosis and Haemostasis, 2024; 8(2). doi:10.1016/j.rpth.2024.102371
15. Dorgalaleh A, Othman M. Precision Medicine in Rare Bleeding Disorders. Semin Thromb Hemost. 2025 Mar;51(2):99-102. doi: 10.1055/s-0044-1800833. Epub 2024 Dec 5. PMID: 39638318.

Author's Contribution	
Hyder Ali Pehlvani	Conceived, Literature search, Manuscript writing, data collection, data analysis
Muhammad	Supervision, critical ap-
Muhammad Rizwan	Manuscript proof reading.
Ramzana Parveen	Data analysis, References