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Assessment of Plasminogen Activator Inhibitor- 1(PAI1) In Children with hemophilia A.

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Abstract

Deficiency of coagulation factor VIII causes the x-linked recessive condition known as hemophilia A. This investigation aims to establish a correlation among PAI-1 and the severity of bleeding in hemophiliac children. Twenty-five children with hemophilia A and twenty-five typically developing children served as subjects in a case control study. After carefully examining the data, we found that the PAI-1 Level was significantly lower in the cases group contrasted with the control group. Furthermore, a negative connection ($r=-0.616$, $p<0.001$) was found among the PAI-1 Level as well as the ISTH-BAT score, determined by the International Society of Thrombosis as well as Hemostasis-Bleeding Assessment Tool. Because PAI-1 level is a great predictor of hemophilia, we concluded that it was lower in haemophilia A patients in contrast to healthy controls.

1. Introduction :

The lack of activity of coagulation factors VIII (FVIII) as well as IX (FIX) is the underlying cause of the X-linked bleeding condition hemophilia A (HA) as well as hemophilia B (HB), correspondingly [1]. The incidence of hemophilia A (HA) is one in every 5,000 male live births, while that of hemophilia B (HB) is one in every 30,000[2]. The intrinsic pathway of

the coagulation cascade is impaired when factor VIII is inadequate, leading to an inadequate production of thrombin by the FIXa and FVIIIa complex. A remarkable propensity for defective clotting in reaction to trauma and, particularly in those with severe hemophilia, with spontaneous bleeding, is produced by this mechanism in conjunction with the activity of the tissue-factor pathway inhibitor[3].

Hemophilia A can cause mild to severe bleeding episodes in affected persons. Severe hemophilia A affects around 50% of people with the disorder. There is a higher incidence of post-traumatic hemorrhage, such as bleeding after dental or surgical procedures, and spontaneous bleeding is more common in cases with hemophilia A. The most common sort of spontaneous bleeding is intra-articular joint hemorrhage, which can lead to arthropathy and long-term disability. This condition may lead to a reduction in range of motion and intense pain. The majority of individuals with severe hemophilia A acquire persistent impairment, sometimes manifesting as hemophilic arthropathy, due to recurrent joint hemorrhages[4]. There is below one percent of the usual level of factor VII in the blood of persons who suffer from severe hemophilia [4]. Bleeding episodes frequently transpire spontaneously or subsequent to minimal trauma in severe cases. However, patients with a moderate form of hemophilia only experience a mild bleeding pattern, which is characterized by less spontaneous bleeding as well as less joint damage. The biological basis for these phenotypic distinctions is still unknown[5]. Hemophilia severity is defined by plasma FVIII or FIX activity levels. In the severe form, the factor level is below one percent of the normal value; in the moderate form, it is from one percent to five percent; and in the mild form, it is between five percent as well as forty percent.[6] There was no association between the real FVIII level and bleeding phenotype, and FVIII levels below the typical 1% were reported. It was also proposed that the bleeding tendency is caused by factors other than the concentration of FVIII[5]. One of the fibrinolytic inhibitors that tightly controls fibrinolysis to prevent the dissolution of fibrin clots before the damaged vessel is restored is the direct plasmin inhibitor alpha-2 antiplasmin as well as plasminogen activator inhibitor-1 (PAI-1), a fast-acting inhibitor of tPA and uPA[7]. PAI-1, which is released when platelets are activated, hinders the breakdown of thrombi that are rich in platelets and also aids in their proliferation by preventing continuous thrombolysis [8]. Deficiency of PAI-1 causes enhanced fibrinolysis, which in turn causes a mild to moderate bleeding phenotype commonly associated with trauma, surgery, or injuries [9]. One of the key regulators of the plasminogen/plasmin system is PAI-1, which is a member of the serpin family of chemical drugs. An essential component of this system

is the proteolytic cleavage mediated by plasminogen activators (PAs), which transforms the inactive zymogen plasminogen into the active enzyme plasmin. Blood clots are made of an insoluble fibrin meshwork, which plasmin mainly breaks down when tissue-type PA (tPA) mediates the process. The plasminogen/plasmin system extends its activity to pericellular proteolysis associated with activities including cell migration and tissue remodeling through urokinase-type A (uPA)-mediated plasminogen activation[1] . Since its discovery, the Numerous animal models of disease and human studies have examined the pathophysiological function of PAI-1. There is evidence that PAI-1 is linked to a number of diseases, such as inflammation, neurological disorders, aging, cancer, metabolic abnormalities, and cardiovascular disease (CVD). In order to delve deeper into the function of PAI-1 in illness models and investigate their possible therapeutic uses, a number of PAI-1 inhibitors have been created[1] .

2. Patients and Methods:

This was randomized research performed in Beni-Suef university hospital and Health insurance hospital, Pediatric Departments, Beni-Suef from July 2023 to Jan 2024.

I. Inclusion criteria:

Children who displayed recurrent bleeding symptoms regardless of their FVIII deficiency and were under eighteen years old were included in the research. They were then categorized into: Severe hemophilia, characterized by below one percent factor activity, corresponding to <0.01 IU/mL.

Moderate hemophilia is characterized by a factor activity level that varies from ≥1 percent to ≤5 percent of normal, corresponding to ≥0.01 as well as ≤0.05 IU/mL. Mild hemophilia is characterized by a factor activity level above five percent but below forty percent of normal (≥0.05 as well as <0.40 IU/mL)[1] .

II. Exclusion criteria: children older than 18 years old.

III. Twenty-five individuals diagnosed with hemophilia A and twenty-five healthy controls, matched for age and sex, were included in the research.

3. Methodology: All patients were subjected to the following:

1. Full history taking involving: ***Personal*** history involving name, age and sex, ***Present*** history involving onset, course as well as duration of the present symptom and ***Past*** history.
2. Clinical examination including: Anthropometric measures, Full examination of all systems (cardiac, respiratory, neurological, and abdominal), Vital signs and Bleeding assessment using ISTH/BAT to assess the severity of the disease[1] .
3. Investigations including plasminogen activator inhibitor- 1(PAI1) level using

thrombin activatable fibrinolysis inhibitor ELIZA kits.

4. Ethical Considerations: The parents were informed about the trial. Following clearance from the Faculty of Medicine's Research Ethics Committee, we made sure to get the parents' written informed consent before enrolling their children in the study. The University of Beni-Suef.

Approval number: FMBSUREC/06062023/ Hussien

5. Laboratory measurements:

- Blood samples: Peripheral venous blood samples were collected in tubes containing 3.2% (0.109m) sodium citrate. Subsequently, the tubes were centrifuged twice at 2200 X g for 10 min at room temperature and finally the citrated platelet-poor plasma (PPP) was aliquoted and stored at −80.
- PAI1 determination: -Technoclone TC11070, Vienna, Austria's TECHNOZYM® PAI-1 antigen ELISA reagent kit was used to assess plasma PAI-1 antigen levels. After being coated with 100 µL each well in 15-millimeter Na2CO3, 35 mm NaHCO3, pH 9.6, the 96-well microtiter plates from Nunc A/S in Roskilde, Denmark were incubated overnight at 4°C with 5 µg mL−1 of monoclonal anti-PAI-1 antibody. - A To block the plates after coating, 1% BSA in PBS (136.9 mm NaCl, 6.5 mm Na2HPO4, 2.7 mm KCl, 1.0 mm KH2PO4, pH 7.4) was applied for 1 hour at 37°C. The plates were incubated at 37°C for 1 hour after adding 100 µL of plasma sample, which had been fourfold diluted in 1% BSA/PBS. A 100-fold diluted POX-conjugated anti-PAI-1 monoclonal antibody was used to capture bound antigens for 1 hour at 37°C. To facilitate development, 100 µL of 3,3′,5,5′-tetra methylbenzidine (BioMerieux, Marcy l'Etoile, France) was added to each well of the plates and let to incubate at room temperature. Spectrophotometric readings were taken at 450 nm using a Victor3 1420 Multilabel Plate Counter (Perkin Elmer, Waltham, MA, USA) after 10 minutes, and the reaction was halted by adding 2 m H2SO4 (50 µL per well).

  Three times with 0.1 percent Tween20 in PBS, the wells were rinsed between each incubation phase. The results were presented as ng mL−1. The coefficients for inter- and intra-assay variance were 16.5 percent and 4.2 percent, correspondingly.
- Bleeding severity: Bleeding severity was assessed using The International Society on Thrombosis and Hemostasis Bleeding Assessment Tool (ISTH-BAT) score. This bleeding score takes the frequency and intensity of bleeding symptoms into consideration while making a thorough evaluation. On a scale from minus one to

plus four, the twelve bleeding things are rated. More severe or frequent bleeding is indicated by higher scores. After adding together all twelve factors, the BS can take on values among -3 (no bleeding) as well as 45 (severe bleeding)[1] .

6. Statistical Methods: Analysis of the data was done using statistical program for social science (SPSS). The quantitative variables were described in the form of mean standard deviation, median and range. Frequency and percentages were used to characterize the qualitative variables when suitable. We determined the P value, which was classified as non-significant if it was above 0.05, significant if it was below 0.05, very significant if it was above 0.05, or highly significant if it was below 0.05.

[4]4. Results:

In the cases group, participants' ages varied from two to Sixteen with a mean ± SD of 9.4±4.13 years, whereas in the control group, participants' ages varied from one to fifteen with a mean ± SD of 8.4±4.44 years. When comparing the ages of the two groups, no significant distinction was found (p>0.05).

Table (1): Comparison between cases group and control group as regards PAI-1 Level

	Cases group (n = 25)	Control group (n = 25)	U	P-value
PAI-1 Level				
Min – Max	0.0 - 3.7	1.3 - 7.6	-4.380	<0.001**
Mean ± SD	1.64 ± 0.90	4.31 ± 2.26		

U: Mann Whitney U Test

*P- value < 0.05 is significant **P- value < 0.001 is highly significant

This table showed that PAI-1 Level in cases group varied from 0 to 3.7 and the mean ± SD was 1.64±0.90, whereas in control group varied from 1.3 to 7.6 and the mean ± SD was 4.31±2.26. There was significant reduction in PAI-1 Level in cases group than control group.

Regarding severity of hemophilia (factor level u/dl), It was mild > 5 in 4 cases (16.0%), moderate 1-5 in 11 cases (44.0%), and severe < 1 in 10 cases (40.0%).

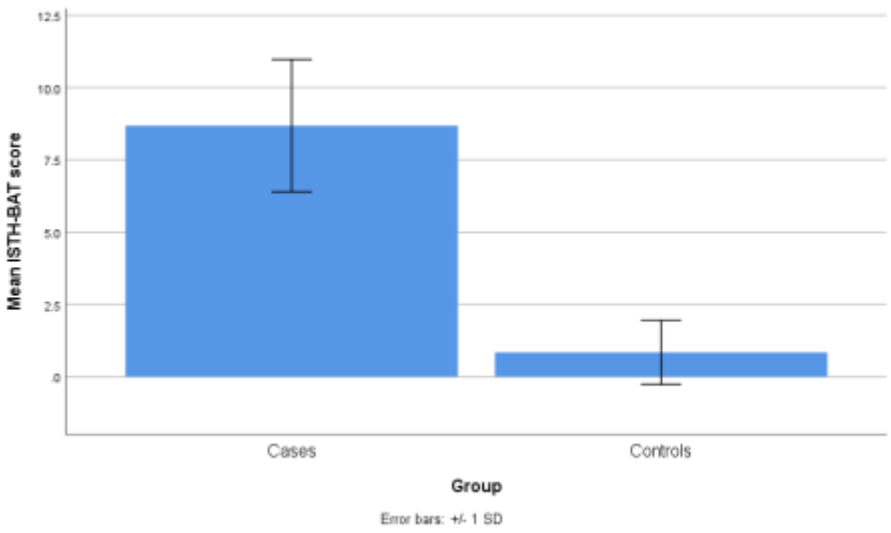

Figure (1): Comparison among cases group and control group as regards ISTH-BAT score.

This figure showed that PAI-1 Level in cases group ranged from 0 to 3.7 and the mean ± SD was 1.64±0.90, while in control group varied from 1.3 to 7.6 and the mean ± SD was 4.31±2.26. hence there was significant increase in ISTH-BAT score in cases group than control group.

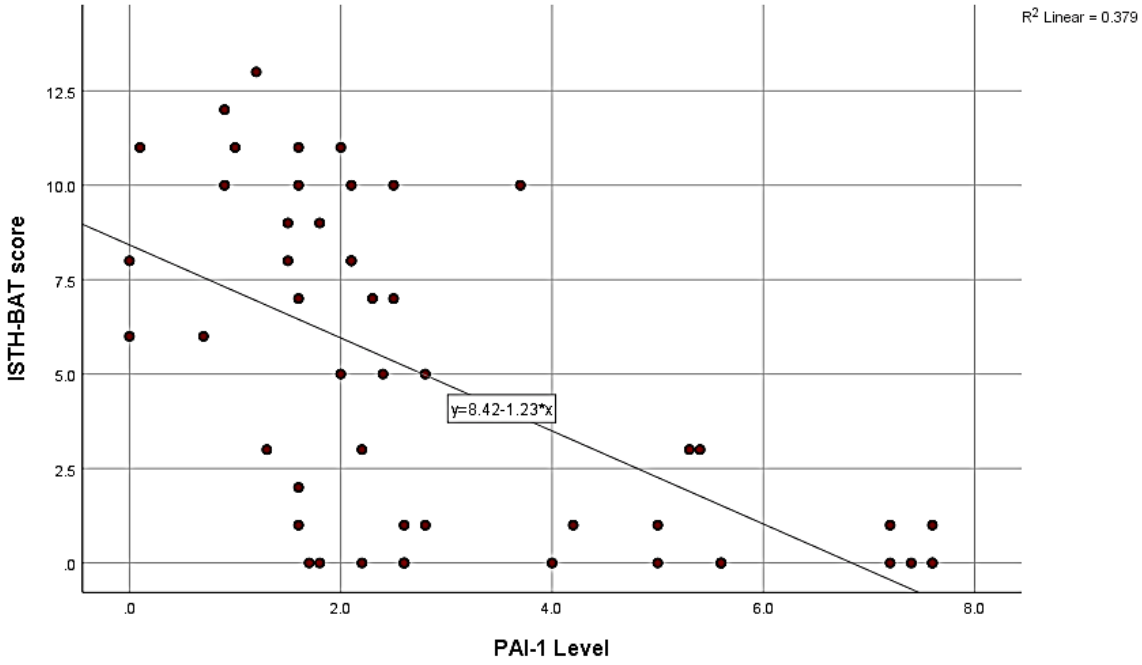

Figure (2): Correlation among PAI-1 Level and ISTH-BAT score

There was significant negative correlation among PAI-1 Level and ISTH-BAT score (r=-0.616, p<0.001**).

Our receiver operating characteristic (ROC) analysis showed that PAI-1 Level is a very good predictor of hemophilia, with a cutoff value below 2.1 as well as an area under the ROC curve of

0.861. The PAI-1 Level had sensitivity of 76%, specificity of eighty percent, positive predictive value of 79.2%, as well as negative predictive value of 76.9%.

Table (2): Receiver operating characteristic (ROC) Curve and diagnostic indices of the PAI-1 Level for prediction of Hemophilia

	AUC	P-value	95% Confidence Interval		Cut off Value	Sensitivity	Specificity	PPV	NPV
			Lower Bound	Upper Bound					
PAI-1 Level	0.861	0.001*	0.760	0.982	≤2.1	76%	80%	79.2%	76.9%

AUC: Area Under a Curve p-value: Probability value

4. Discussion:

Blood clotting time is abnormally long and spontaneous bleeding is common in hemophilia A, a bleeding condition caused by low activity or insufficiency of circulating factor VIII [1]. A deficiency in blood components essential for clotting characterizes this sex-linked recessive illness [1]. Musculoskeletal, soft tissue, and mucocutaneous hemorrhage are the hallmarks of these conditions, which otherwise present similarly in the clinic. The degree of hemorrhage is influenced by the activity level of the deficient component[1]. The objective of our investigation was to evaluate the level of PAI1 in children with hemophilia A. In the current thesis, the mean age was 9.4±4.13 years in cases group and 8.4±4.44 years in control group, all cases and control groups were males, with no significant distinction among both groups as regard age, sex and BMI; all p-values > 0.05. While, statistically significant variance was noted amongst both groups as regards family history. Also, significant decrease in weight and height percentile was noted in cases group (p<0.05).

In the same line was a previous study by Soliman et al. (2022)[1], 26 pediatric cases from the pediatric department's hematology unit. Also included in the research as a control group were fourteen people who appeared to be in good health. With p-values above 0.05, there were not significant gender or age disparities among the control and case groups.

In our study, regarding clinical data of patients' group, Age of first joint bleeding was <1 year in 9(36.0%), 1-5 years in 16(64.0%), most affected joint

was ankle, elbow and knee. Total hemophilia joint health score (HJHS) ranged from 2 to 44 and mean was 24.72±13.69, number of bleeding events per month ranged from 1 to 5 times with mean 2.76±1.20, while number of factor infusions per month ranged from zero to 12 times and the mean was 7.80±4.64.

In agreement with our results was study of Rehill et al. (2021)[1] age of first joint bleed in children patients with hemophilia varies from three months up to six years, for individuals whose first joint bleed occurs later in life, there is an inverse relationship between the intensity of the bleeding and the number of subsequent bleeds, as well as a decreased risk of orthopedic problems.

There was no disparity among the two groups with respect to any bleeding difficulties, as shown by the present data, which indicated the distribution of ISTH-BAT items in the cases and controls groups. Epistaxis, bruising of the skin, bleeding from small wounds, bleeding from the mouth, tooth extractions, muscle haematomas, and hemorrhage were all significantly more common in the cases group contrasted with the control group. Additionally, the ISTH-BAT score was significantly higher in the cases group in comparison to the control group ($p<0.05$).

Hemophilic patients exhibited much higher bleeding scores than controls in the study conducted by Soliman et al. (2022) that utilized the International Society of Thrombosis Bleeding Assessment Tool (ISTH/BAT) to evaluate bleeding [18]. According to Roy et al. (2019), the ISTH-BAT bleeding score for the six months before to prophylaxis varied among six and eighteen (Mean = 11.03, Median = 11). For the six months following prophylaxis, the ISTH-BAT bleeding score varied from five to seventeen (mean=9.94, Median= 10). Each patient group underwent a paired student t test to assess their ISTH-BAT based score before and after the 6-month prophylactic period. Children with hemophilia A showed a significant improvement in their ISTH-BAT based bleeding score contrasted with other groups (p value = 0.006)[2] .

The results show that in contrast to the control group, the cases group had a significantly lower PAI-1 level ($p<0.05$). It is consistent with our findings that those of Soliman et al. (2022), who found that, contrasted with controls, individuals with hemophilia had a significantly lower PAI 1 level and a higher TAFI level[18].

Liu et al. (2023) found that, the plasma levels of PAI-1 were

(29.43±5.51) µg/L in the thrombotic group, while the plasma levels of PAI-1 in the control group was (19.00±4.40) µg/L. The levels of PAI-1 in the thrombotic group were higher than those in the control group[2] . The results corroborated those of Soliman et al. (2022), who found a statistically significant association among PAI-1 levels and bleeding phenotype and a positive link between PAI-1 levels and controls. Therefore, it is possible that PAI-1 levels can be utilized to predict the likelihood of bleeding in hemophiliacs[18]. The severity of the clinical bleeding pattern is associated with low levels of PAI-1, in accordance with another study conducted by Dahlbäck et al. (2005)[2] . Heiman et al. (2017) reported that, Mild to moderate bleeding is a symptom of full plasminogen activator inhibitor 1 (PAI-1) deficiency that has not been addressed. Spontaneous bleeding does not occur; the most common causes of delayed bleeding are injuries, traumas, or surgical procedures[2] .

In the current study, there was significant negative correlation among PAI-1 Level and ISTH-BAT score (r=-0.616, p<0.001). This was in line with several previous studies (Olsson et al., 2016; Saes et al., 2020) which demonstrated that, PAI-1 Level had a negative correlation with ISTH-BAT score[2 2 4 5] .

5. Conclusion and Recommendations

In conclusion, our study suggested that, significant decrease in PAI-1 Level in cases group than control group. A statistically significant negative correlation was noted between plasminogen activator inhibitor 1Level and ISTH-BAT score that's why PAI-1 Level is an excellent predictor for Hemophilia. A characteristic of hemophilia is the ability to bleed into joints. However, these findings require confirmation by larger, more-powered study with larger sample size. across multiple institutions to confirm the generalizability of these results on the advantages of PAI-1 Level as an excellent predictor for Hemophilia.[2 3]

6. References:

1. Müller, J., Miesbach, W., Prüller, F., Siegemund, T., Scholz, U., & Sachs, U. J. (2022). An update on laboratory diagnostics in haemophilia A and B. Hämostaseologie, 42(04), 248-260.
2. Raso, S., Lambert, C., Boban, A., Napolitano, M., Siragusa, S., & Hermans, C. (2020). Can we compare haemophilia carriers with clotting factor deficiency to male patients with mild haemophilia? Haemophilia, 26(1), 117-121.
3. Pezeshkpoor, B., Oldenburg, J., & Pavlova, A. (2022). Insights into the molecular genetic of hemophilia A and hemophilia B: the relevance of genetic testing in routine clinical practice. Hämostaseologie, 42(06), 390-399.
4. Gholami, M. S., Valikhani, M., Dorgalaleh, A., Mousavi, S. H., & Pezeshkpoor, B. (2018). Hemophilia A. Congenital Bleeding Disorders: Diagnosis and Management, 103-137.
5. Semeraro, F., Mancuso, M. E., Ammollo, C. T., Dirienzo, L., Vitulli, A., Santagostino, E., ... & Colucci, M. (2020). Thrombin activatable fibrinolysis inhibitor pathway alterations correlate with bleeding phenotype in patients with severe hemophilia A. Journal of Thrombosis and Haemostasis, 18(2), 381-389.
6. Grabowski, E. F., Van Cott, E. M., Bornikova, L., Boyle, D. C., & Silva, R. L. (2020). Differentiation of patients with symptomatic low von Willebrand factor from those with asymptomatic low von Willebrand factor. Thrombosis and Haemostasis, 120(05), 793-804.
7. Kim, J. Y., & You, C. W. (2019). The prevalence and risk factors of inhibitor development of FVIII in previously treated patients with hemophilia A. Blood research, 54(3), 204-209.
8. Mochizuki, L., Sano, H., Honkura, N., Masumoto, K., Urano, T., & Suzuki, Y. (2023). Visualization of Domain-and Concentration-Dependent Impact of Thrombomodulin on Differential Regulation of Coagulation and Fibrinolysis. Thrombosis and haemostasis, 123(01), 016-026.
9. Duque, P., Mora, L., Levy, J. H., & Schöchl, H. (2020). Pathophysiological response to trauma-induced coagulopathy: a comprehensive review. Anesthesia & Analgesia, 130(3), 654-664.
10. Vercauteren, E., Gils, A., & Declerck, P. J. (2013, June). Thrombin activatable fibrinolysis inhibitor: a putative target to enhance fibrinolysis. In Seminars in thrombosis and hemostasis (Vol. 39, No. 04, pp. 365-372). Thieme Medical Publishers.

11. Bouton, M. C., Geiger, M., Sheffield, W. P., Irving, J. A., Lomas, D. A., Song, S. & Lucas, A. R. (2023). The under-appreciated world of the serpin family of serine proteinase inhibitors. EMBO Molecular Medicine, 15(6), e17144.
12. Hazendonk, H. C. A. M., van Moort, I., Mathôt, R. A. A., Fijnvandraat, K., Leebeek, F. W. G., Collins, P. W., ... & OPTI-CLOT study group. (2018). Setting the stage for individualized therapy in hemophilia: What role can pharmacokinetics play?. Blood reviews, 32(4), 265-271.
13. Minhas, A., Younas, A., Eiman, H., Rasool, I., Fatima, M., Imran, A., & Chughtai, A. S. (2023). The Role of ISTH Bleeding Assessment Tool in Diagnosing Patients with Suspected Bleeding Disorders. Journal of Haematology and Stem Cell Research, 3(2), 94-98.
14. Shahbazi, M., Ahmadinejad, M., & Teimourpour, A. (2024). Utility of the international society on thrombosis and hemostasis-bleeding assessment tool in the diagnosis of patients who suspected of platelet function disorders. Blood Coagulation & Fibrinolysis, 35(1), 8-13.
15. Chaves, D. G., da Silva Santos, B. A., Zucherato, L. W., Dias, M. M., Lorenzato, C. S., de Oliveira, A. G., ... & Rezende, S. M. (2024). High levels of anti–factor VIII immunoglobulin G4 and immunoglobulin G total are associated with immune tolerance induction failure in people with congenital hemophilia A and high-responding inhibitors. Research and Practice in Thrombosis and Haemostasis, 8(4), 102436.
16. Moretti, L., Bizzoca, D., Buono, C., Ladogana, T., Albano, F., & Moretti, B. (2021). Sports and children with hemophilia: Current trends. Children, 8(11), 1064.
17. Hassab, H. M., Saad, H. R., & Abdel Ghany, H. M. (2022). Quality of life and clinical assessment of joint health in children with hemophilic arthropathy. Egyptian Rheumatology and Rehabilitation, 49(1), 22.
18. Soliman, M., Osman, N., Hefnawy, S., & El Hawy, M. A. (2022). Assessment of plasminogen activator inhibitor-1 (PAI1) and thrombin activitable fibrinolysis inhibitor (TAFI) in Egyptian children with hemophilia A. Pediatric Hematology and Oncology, 39(4), 318-328.
19. Rehill, A. M., McCluskey, S., O'Donnell, J. S., Dockal, M., Preston, R. J., & iPATH Study Group. (2021, March). Heterogeneity in bleeding tendency and arthropathy development in individuals with hemophilia. In

Seminars in Thrombosis and Hemostasis (Vol. 47, No. 02, pp. 183-191). Thieme Medical Publishers, Inc.

20. Roy, S., & De, A. K. (2019). Effect of prophylactic management of hemophilia on bleeding episodes. Indian Journal of Hematology and Blood Transfusion, 35(3), 496-501.
21. Xue, F., Dai, J., Chen, L. X., Liu, W., Zhang, H. Q., Wu, R. H., ... & Yang, R. C. (2024). Report on the diagnosis and treatment of hemophilia in China (Version 2024). LabMed Discovery, 100007.
22. Dahlbäck, B., & Villoutreix, B. O. (2005). Regulation of blood coagulation by the protein C anticoagulant pathway: novel insights into structure-function relationships and molecular recognition. Arteriosclerosis, thrombosis, and vascular biology, 25(7), 1311-1320.
23. Heiman, M., Gupta, S., Lewandowska, M., & Shapiro, A. D. (2017). Complete plasminogen activator inhibitor 1 deficiency.
24. Olsson, A. (2016). Bleeding tendency and health-related quality of life in carriers of haemophilia.
25. Saes, J. L., Verhagen, M. J., Meijer, K., Cnossen, M. H., Schutgens, R. E., Peters, M., ... & Schols, S. E. (2020). Bleeding severity in patients with rare bleeding disorders: real-life data from the RBiN study. Blood advances, 4(20), 5025-5034.