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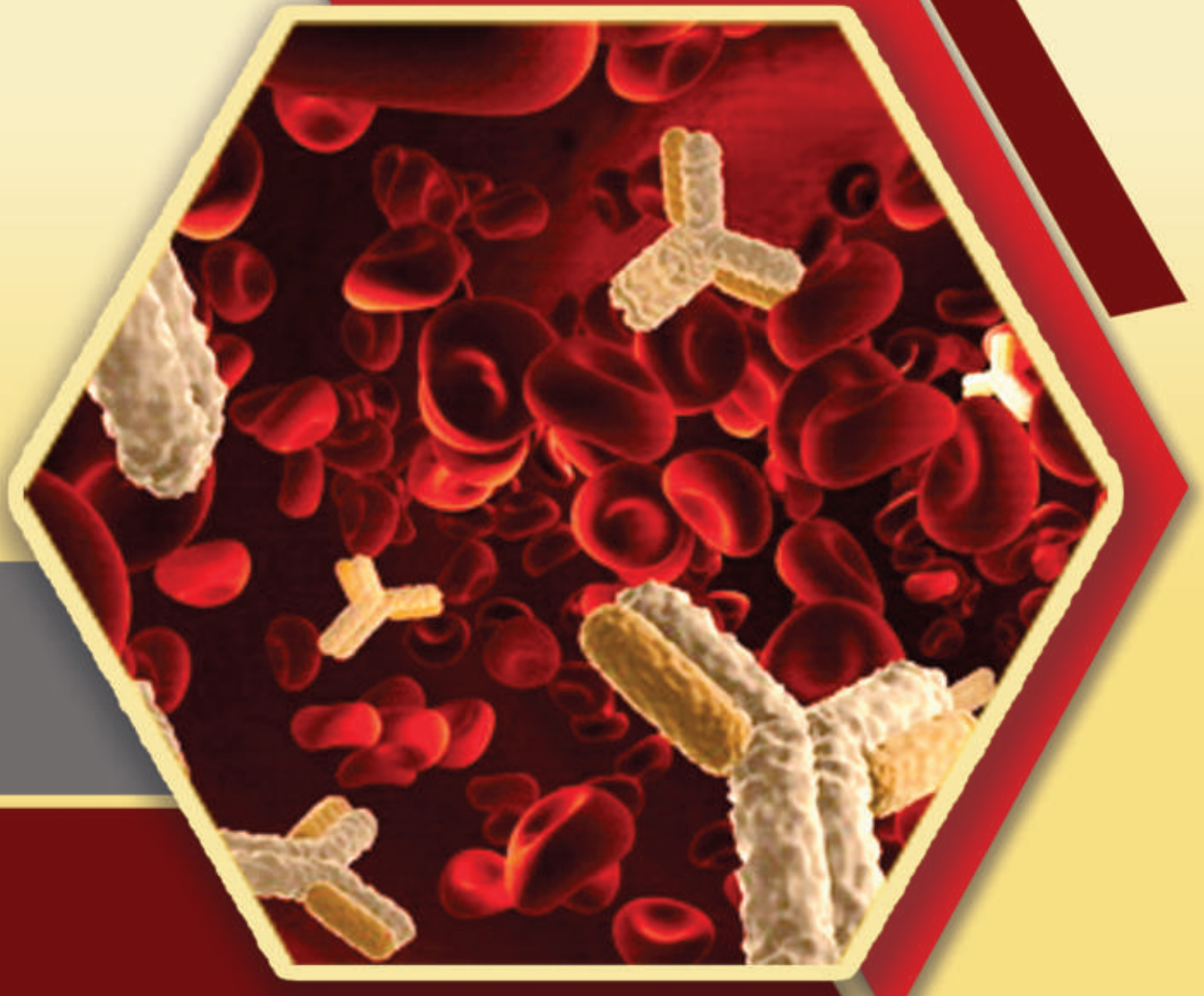

ICMR - NATIONAL INSTITUTE OF IMMUNOHAEMATOLOGY

INDIAN COUNCIL OF MEDICAL RESEARCH

NATIONAL INSTITUTE OF IMMUNOHAEMATOLOGY

ICMR-NIIH, Centre for Research, Management and Control of Hemoglobinopathies, Chandrapur, Maharashtra

Digital Inaugaration of ICMR-NIIH, Centre for Research, Management and Control of Hemoglobinopathies, Chandrapur by Honourable Prime Minister, Mr. Narendra Modi on 16th February, 2019. Chief Minister of Maharashtra Mr. Devendra Fadnavis, Minister of Road Transport and Highways of India, Mr. Nitin Gadkari, Former Governor of Maharashtra, Dr. Vidyasagar Rao and Former member of Lok Sabha & MoS Home Affairs, Mr. Hansaraj Gangaram Ahir attended the event.

Inauguration Ceremony and unveiling of Plaque of the ICMR-NIIH, Centre for Research, Management and Control of Hemoglobinopathies, Chandrapur, Maharashtra on 8th March 2019.

Centre for Research, Management & Control of Heamoglobinopathies at Chandrapur

A Centre for Research, Management & Control of Heamoglobinopathies at Chandrapur under ICMR-National Institute of Immunohaematology (ICMR-NIIH), Mumbai for Research and Training in Haemoglobinopathies, in particular Sickle Cell Disease (SCD) is to be developed at New Chandrapur, Maharashtra on the four acre land acquired by Indian Council of Medical Research.

ICMR- NIIH has been working on red cell genetic disorders including haemoglobinopathies and red cell enzymopathies and red cell membranopathies since its inception in 1957. Diagnostic services for these disorders are now being provided to over 1000 patients a year. Community screening programmes for both tribal and non tribal populations has also been undertaken in different states like Maharashtra, Gujarat, Madhya Pradesh, Chhattisgarh, Rajasthan, Orissa and Tamil Nadu. Large multicentre studies have been co-ordinated for thalassemia and SCD which has led to human resource development and helped to establish several new centres particularly in medical colleges for screening, diagnosis and prevention of these disorders. Several research projects have led to a better understanding of the pathophysiology and molecular pathology of these disorders resulting in improved management. An indigenous molecular diagnostic kit has also been developed for the detection of the common mutations causing haemoglobinopathies among Indians. This Institute was the first to establish 2nd trimester Prenatal Diagnosis for haemoglobinopathies in the country and by now we have been able to provide both 1st and 2nd trimester prenatal diagnosis to over 4000 couples at risk for severe haemoglobinopathies which has prevented the birth of nearly 1000 severely affected babies.

Haemoglobinopathies in particular SCD are a major health problem in the Vidarbha region of Central India. Chandrapur, Gadchiroli, Yavatmal, Wardha, Amravati, Gondia, Bhandara and Nagpur districts have huge tribal populations and other weaker sections of society where carrier frequencies of the sickle gene can be as high as 25 to 30% in many of these communities. There are around 12000 SCD patients in Vidharbha region and over 3000 SCD patients in Chandrapur alone who require optimum management and there may be many more who are undiagnosed. Estimated numbers of sickle cell carriers in Vidharbha is approximately 4,00,000. b-thalassemia patients are also seen in this region. There is a lack of diagnostic and management facilities in most of these places. Realizing the magnitude of the problem, a Centre for Research, Management & Control of Heamoglobinopathies is planned by ICMR at Chandrapur under ICMR-National Institute of Immunohaematology, Mumbai. This centre will play a lead role in the development of the National Haemoglobinopathies Control Programme.

The major objectives of this centre are as follows:

1. To undertake Basic Research Programmes that will benefit the local population.

2. To undertake Translational Research Work and Community Control Programmes in this under served region.
3. To serve as a Teaching and Training Centre for Haemoglobinopathies for the country.
4. To develop Human Resources who could work in different regions of the country where haemoglobinopathies is a major health burden.
5. Supporting the state and district administrations by confirming diagnosis offering prevention programmes by Prenatal Diagnosis and New born screening programme for early diagnosis and comprehensive care.
6. Strengthen the capabilities of Govt. Medical Colleges in the region through collaborative research.

Presently this centre has started functioning in a project mode since December 2015 from an interim facility in an old and unused TB Sanatorium at Govt. Medical College, Chandrapur.

Visit of the Secretary, Department of Health Research & Director General, ICMR Dr. Balram Bhargava to the proposed ICMR-NIIH Building site at Nahur and ICMR-NIIH Mumbai on 11th March, 2019.

Fibrinolysis and risk of Venous Thrombosis: An overview

Aniket Prabhudesai

Haemostasis (normal blood flow) in physiology is maintained mainly by three cascades. These involve the clotting cascade, the natural anticoagulation system and the fibrinolytic cascade. Blood clots are not normally formed in the body. When there is any injury to a blood vessel, clotting cascade (coagulation plasma proteins and platelets) gets activated to form a clot over the injury to prevent any further blood loss. However, this clot forming mechanism cannot continue forever. Our body physiology uses mechanisms to limit the clot to the site of an injury and ultimately removes the clot when the injury has been healed. These mechanisms include natural anticoagulation system which slows down this clotting process and the fibrinolytic cascade which resolves the blood clot thereby maintaining haemostasis.

Blood clots may occur in veins or arteries. In Venous Thrombosis (VT), blood clots may form in the veins of lower (leg) or upper (arm) extremities or in other uncommon veins such as cerebral sinus, hepatic or retina. VT is thus a potentially dangerous condition that can lead from preventable morbidity to mortality if left unattended and untreated. VT occurs with an incidence of approximately 1.75 per 1000 hospital admissions in Indian population (1).

THROMBOPHILIA

In 1856, Virchow proposed a triad to account for the cause of thrombosis- a) Vascular wall injury, b) Abnormal blood flow (stasis) and c) Hypercoagulability. Disturbances in any of these could lead to thrombosis (2). Some individuals may have a higher tendency to form blood clots, known as thrombophilia. Thrombophilia can be hereditary due to various predisposing genetic mutations or it can be acquired due to secondary to various etiologies such as antiphospholipid syndrome, hyperhomocysteinemia, use of oral contraceptives, pregnancy or post-partum, obesity, surgery or trauma and travel. The most common inherited thrombophilias include deficiencies of natural anticoagulants Protein C, Protein S, or Antithrombin along with Factor V Leiden mutation (mutated form of coagulation factor V). Natural anticoagulants Antithrombin neutralize thrombin whereas Protein C and S carry out the proteolytic inactivation of procoagulant cofactors Va and VIIIa via activated protein C (APC) complex and thus act like natural blood thinners. In Indian population, the above common thrombophilia markers are investigated in patients who develop VT and are prevalent in about one-third of patients (3-5). Remaining almost two-third of patients with evidence of thrombosis, however, are not found to have these conventional thrombophilia markers, though many of them have strong history of recurrent thrombosis, family history and thrombosis in unusual sites.

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FIBRINOLYSIS

Compared with coagulation, fibrinolysis has somewhat been neglected. Fibrinolytic system is responsible for dissolution of blood clots and the maintenance of one's vascular system. The fibrinolytic system contains an inactive plasminogen enzyme, whose conversion to active plasmin enzyme is mediated by tissue-type plasminogen activator (tPA) released by endothelial cell. Plasmin formed degrades fibrin strands into soluble fibrin degradation products (FDPs). However, this process cannot continue forever otherwise it may lead to premature clot dissolution and manifest in bleeding. To prevent such an event, inhibition of the fibrinolytic system occurs at the level of plasminogen by plasminogen activator inhibitor (PAI-1) or by thrombin-activatable fibrinolysis inhibitor (TAFI), or at the level of plasmin by alpha 2-antiplasmin (A2-AP) formerly known as plasmin inhibitor (PI) (Figure 1).

(Figure adapted from chapter 'An Insight into the Abnormal Fibrin Clots — Its Pathophysiological Roles' by Payel Bhattacharjee and Debasish Bhattacharyya, 2013)

Deficiency of precursor plasminogen, less production of fibrinolysis activator (tPA) or its release and/ or increased inhibition of fibrinolysis (inhibitors- PAI-1, TAFI, A2-AP) may lead to reduced fibrinolysis (6). This phenomenon is also known as hypofibrinolysis. There are a few global assays such as the Euglobulin clot lysis time (ECLT) and dilute whole blood clot lysis assay which estimate the overall fibrinolytic potential. However, these tests are found to be less sensitive and they do not estimate all the components of fibrinolytic components. Hence, it becomes necessary to ascertain the

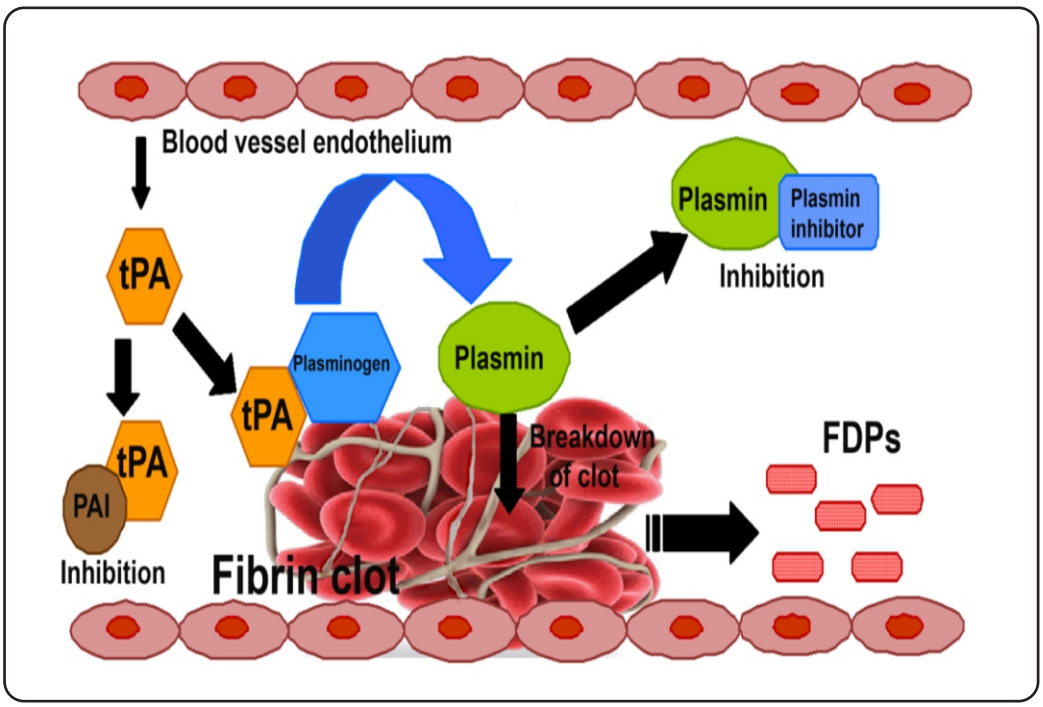

Figure 1: *Mechanism of Fibrinolysis process*

role each component of the fibrinolytic system plays in the development of venous thrombosis.

ROLE OF PAI-1 IN THROMBOSIS

PAI-1 is the primary inhibitor in plasma, released by endothelial cells, which rapidly inactivates tPA. High or increased PAI-1 level has been shown to establish a hypofibrinolytic state which is linked to increased risk of venous thrombosis (7). Animal studies support this finding that elevated PAI-1 level contributes to the development of venous and not arterial thrombosis (8). The gene for PAI-1 has several polymorphic loci. The 4G/5G insertion deletion polymorphism at position -675 in the promoter region of the PAI-1 gene is the most frequently studied polymorphism of PAI-1 in which possession of a 4G allele may contribute to defective fibrinolytic activity due to elevated PAI-1 in patients and thus possibly to an increased VT risk (9). This is because the polymorphism affects PAI-1 gene transcription with lower levels of plasma PAI-1 seen in the presence of the 5G allele (10). Possession of the 4G allele may thus be an increased risk factor for development of thrombosis in the vessels of several internal organs including the portal veins and deep veins (11).

ROLE OF TPA IN THROMBOSIS

Plasminogen is converted to the active enzyme plasmin by tissue-type plasminogen activator (tPA) released from endothelial cells. Most (95%) of circulating tPA antigen is coupled with PAI-1 and only 5% of total tPA circulates in free form. There is limited and conflicting data on the role of tPA in venous thrombosis. In the GAIT study, a strong genetic correlation between tPA levels and overall thrombosis risk was established (12). The study also established a significant association between variations found in tPA gene and tPA phenotype. Studies previously have focussed on monitoring the tPA release rate (and not the tPA level) and found reduced tPA release to associate with VT risk (13).

ROLE OF PLASMINOGEN IN THROMBOSIS

Plasmin, the active enzyme, is produced from its zymogen Plasminogen on cleavage of a single Arg-Val peptide bond at amino acid position 560–561. Plasminogen abnormalities have been described in patients with familial or recurrent venous thrombosis but these are rare and the association could be coincidental (14). The levels of plasminogen have been found to associate with elevated plasma clot lysis time, which is known to increase risk of thrombosis (7). Plasminogen deficiency is inherited in an autosomal recessive manner. Plasminogen deficiency, however, has been associated with development of ligneous conjunctivitis (15) than VT.

ROLE OF TAFI IN THROMBOSIS

On activation TAFI suppresses fibrinolysis by removing C-terminal lysine residues from partially degraded fibrin strands preventing the binding of plasminogen and tPA and making the clot more

resistant to lysis (16). High TAFI levels appear to be associated with venous thrombosis with almost two-fold increased risk (7, 17). Few variants in the TAFI gene (rs3742264 and rs1926447) have been shown to affect TAFI plasma levels, function, and stability and therefore may be functionally relevant to thrombotic disease risk (17-18).

ROLE OF A2-AP IN THROMBOSIS

A2-AP is the fast acting inhibitor of plasmin which competitively inhibits the binding of fibrin to plasminogen by strongly binding to lysine binding site of plasminogen. It is unclear whether A2-AP contributes to thrombosis risk. Only a few case reports have reported high A2-AP level to associate with thrombosis risk (19-20). Studies investigating role of A2-AP level in imparting the risk of VT are scarce and have failed to show an association, probably due to less number of patients.

INDIAN SCENARIO

There is lacuna in data on comprehensive study of the fibrinolytic defects as causative factors in Indian patients with venous thrombosis. There are only a handful reports on the study of fibrinolytic defects as causative factors in Indian patients. Association of PAI-1 4G/4G polymorphism with VT risk was reported by Akhter et al (21) whereas other studies have only tested one marker of fibrinolysis in their studies on less number of samples (22-23). Comprehensive study of all the individual components of the fibrinolysis cascade and its association with venous thrombosis risk has never been checked in Indian scenario.

EXPERIENCE AT NIIH

339 venous thrombosis patients (age <50 years and not on any anticoagulation therapy) during years 2014-2018 were recruited and studied for conventional thrombophilia markers along with fibrinolytic markers. We found conventional thrombophilia markers account for the cause of VT in 18.58% cases and impart a 7.38-fold risk of VT ($p=0.0009$). We also noted a strong association between impaired fibrinolysis and VT risk. Overall, 28.6% VT patients had at least one abnormal fibrinolytic marker. Possession of an abnormal fibrinolytic marker imparted a 3.24 fold risk of VT ($p=0.0006$). Elevated PAI-1 level was a major contributor in the battery of the fibrinolytic markers studied accounting for 17.7% (60 cases) of VT (24). A 3.37-fold overall VT risk was found to be associated with high PAI-1 level ($p= 0.0063$). Variant (4G) of PAI-1 promoter polymorphism 4G/5G was found to be associated with higher mean PAI-1 level ($p<0.0001$) and an increased VT risk (24-25). 4.43% VT patients were found to have low tPA level. High A2-AP level, high TAFI level was observed in 4.13%, 1.77% VT patients respectively. Low plasminogen level was observed in 0.89% VT patients. 21 (6.19%) VT patients were found to have a combination of thrombophilia and abnormal fibrinolytic marker and such a possession imparted a 13.57 fold increased risk of first VT. When a combination of both fibrinolytic parameters and conventional thrombophilia markers was

studied, 41% VT cases were found to possess either of the one or both markers. Thus, a combination of both thrombophilia and fibrinolysis markers will facilitate a comprehensive investigation and we plan to include fibrinolytic markers in the present thrombophilia profile that our institute offers to evaluate the root cause of venous thrombosis.

CONCLUSION

Venous thrombosis (VT) seems to be a multifactorial disease. Combination of multiple risk factors- inherited or acquired thrombophilia (prothrombotic tendency), environmental factors and impaired fibrinolysis (abnormalities in fibrinolysis pathways) may lead to a thrombotic phenotype. Fibrinolysis is often regarded as a secondary phenomenon responsive to the coagulation cascade, and not much attention has been given to the fibrinolytic system as a risk factor for thrombotic disease. Impaired fibrinolytic function is increasingly being reported to be the cause of several kinds of thrombotic diseases. Studying fibrinolytic cascade in VT patients will help to identify whether the patient is at risk of further clotting, thereby helping to determine an appropriate course and length of treatment to prevent future clots. Such testing may help to identify relatives who don't currently have symptoms but may be at risk. However, the testing should be performed in young age patients, those with familial history or recurrence of thrombosis and those with unusual site of thrombosis.

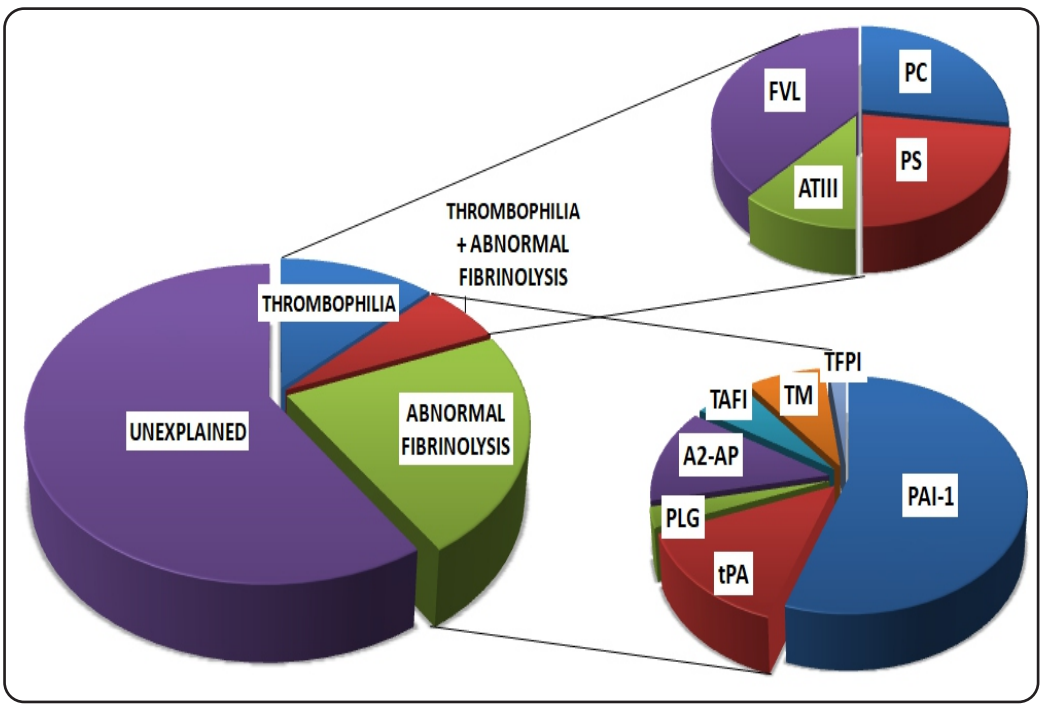

Figure 2: *Summary of the laboratory findings in the VT patients observed at NIIH. Pie charts show the distribution of the various thrombophilia and abnormal fibrinolysis markers found in these VT patients.*

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फिब्रिनोल्यिसस के कारन थ्रॉम्बोसिस का जोखिम

मनिषा पटवर्धन

रक्तस्तम्भन (HAEMOSTASIS) यह प्रक्रिया तीन क्रियाविधियों पर अवलंबित है। जब कभी शरीर की किसी रक्तवाहिनी मे किसी कारन घाव हो जाता है तब शरीर की क्लॉटिंग क्रियाविधि (प्लाज्मा क्लॉटिंग फैक्टर्स और प्लेटलेट्स) (CLOTTING CASCADE) सक्रिय हो जाती है और उस घाव पर गांठ बना देती है ताकि वहां से और खून न बहे। यह क्लॉटिंग क्रियाविधि हमेशा सक्रिय नहीं रह सकती। शरीर की प्राकृतिक थक्कारोधी क्रियाविधि (NATURAL ANTICOAGULANT SYSTEM) इस खून जमने की प्रक्रिया को धीमा कर देता है और फिब्रिनोल्यिसस क्रियाविधि (FIBRINOLYTIC CASCADE) रक्तवाहिनी का धाव भरने पर खून की गांठ का विघटन कर देता है।

वीनस थ्रॉम्बोसिस (VENOUS THROMBOSIS) पैर या बांह की रक्तवाहिनी, मस्तिष्क, पेट या आंख की रक्तवाहिनी मे खून जमने से होता है। ऐडी ली और साथियो को उनके अनुसंधान मे यह पता चला की भारत देश मे हर १०००० हस्पताल मरीजों मे से १७.५ को वीनस थ्रॉम्बोसिस होता है। कुछ लोगो मे थ्रॉम्बोसिस होने (खून जमने) का ज्यादा प्रमाण देखा गया है। उसे थ्रोम्बोफिलीया कहा जाता है। थ्रोम्बोफिलीया मे आनुवंशिक घटकों (HEREDITARY) जैसे प्राकृतिक थक्कारोधी क्रियाविधि घटकों प्रोटीन सी, प्रोटीन एस या एंटीथ्रोम्बीन की कमी तथा डीएनए मे आनुवंशिक परिवर्तन (MUTATION) जैसे फॅक्टर फाय लीडन म्युटेशन का समावेश है और संप्राप्त (ACQUIRED) घटकों एंटिफॉस्फोलिपिड सिंड्रोम, ज्यादा होमोसिस्टेइन, गर्भनिरोधक का इस्तेमाल, गर्भावस्था, बुढ़ापा, मोटापा, सर्जरी, अभिघात का भी समावेश हो सकता है।

भारत मे कई प्रयोगशालाओं मे थ्रोम्बोफिलीया के जांच होते हैं। उनमे थ्रोम्बोफिलीया मार्केर्स प्रोटीन सी, प्रोटीन एस, एंटीथ्रोम्बीन व फॅक्टर फाय लीडन म्युटेशन की जांच होती है। लेकिन केवल एक तिहाई थ्रोम्बोसीस मरीजों मे ये चार मे से कम से कम एक मार्कर की कमी होने की पुष्टि होती है। बचे दो तिहाई मरीजों मे यह मार्कर सही होते है हलाकि इनमे से कुछ मरीजों मे थ्रोम्बोसिस की पुनरावृत्ति होती है, पारिवारिक थ्रोम्बोसिस का ज्ञात होता है या असामान्य रक्तवाहिनी मे खून की गांठ होती है।

फिब्रिनोल्यिसस क्रियाविधि मे टिशू प्लास्मिनोजेन एक्टीवेटर (tPA) यह घटक प्लास्मिनोजेन को सक्रिय प्लास्मीन एंजाइम मे परिवर्तित कर देता है जो खून की गांठ को घुला देता है। यह प्रक्रिया को काबू मे रखने के लिये तीन फिब्रिनोल्यिसस इन्हीबीटर- प्लास्मिनोजेन एक्टीवेटर इन्हीबीटर- १ (PAI-1), एंटीप्लास्मीन (A2-AP) और थ्रोम्बीन एक्टीवेटेबल फिब्रीनोलीसीस इन्हीबीटर (TAFI) साथ काम करते है। प्लास्मिनोजेन की कमी, फिब्रिनोल्यिसस एक्टीवेटरकी कमी या फिब्रिनोल्यिसस इन्हीबीटर के बढ़ने से और कई अन्य प्लास्मा फॅक्टर्स मे दोष होने से फिब्रिनोल्यिसस प्रक्रिया ठीक से काम नही करती है। हाल ही के वर्षों में, दोषित फिब्रिनोल्यिसस प्रक्रिया और थ्रॉम्बोसिस रोग मे कोई संबंध होने का शोध चल रहा है और शोधकर्ताओं की इसमे रूचि बढ़ रही है। इन फिब्रिनोल्यिसस फॅक्टर्सके डीएनए (GENE) मे पौलीमोरफिस्म और म्युटेशन का तापस हो रहा है। फिब्रिनोल्यिसस प्रक्रिया या फॅक्टर्स मे दोष होने से भी थ्रॉम्बोसिस हो सकता है ऐसा कहना कुछ शोधकर्ताओं का है और इसे उन्होने साबित भी किया है।

इस कारण थ्रोम्बोफिलीया के चार मार्केर्स के साथ-साथ फिब्रिनोल्यिसस फॅक्टर्सची की भी जांच हो तो थ्रोम्बोफिलीया होने के कारणों का पूर्णतः पुष्टिकरण हो जाएगा।

भारत देश मे इस विषयी ज्यादा अनुसंधान नहीं हुआ है। और जो भी जानकारी उपलब्ध है वह बहुत कम है और सर्वग्राही फिब्रिनोल्यिसस प्रक्रिये पर उपलब्ध है लेकिन फिब्रिनोल्यिसस के अलग अलग घटको की थ्रोम्बोसिस मे क्या भूमिका है उसपर ज्यादा जानकारी नहीं है।

एन आय आय एह (NIIH) मे अध्ययन

हमने ३३९ भारतीय वीनस थ्रॉम्बोसिस मरीजों मे थ्रोम्बोफिलीया के चार मार्केर्स और फिब्रिनोल्यिसस फॅक्टर्सची की जांच की । इनमेसे सिर्फ १८.५८% मरीजों मे थ्रोम्बोफिलीया मार्कर्स (प्रोटीन सी, प्रोटीन एस, एंटीथ्रोम्बीन की कमी या फॅक्टर फाय लीडन म्युटेशन) मौजूद थे । लेकिन जब फिब्रिनोल्यिसस फॅक्टर्सची की भी जांच हूई तब लगभग ४१% मरीजों में थ्रोम्बोसिस होने का कारण पता चला। खून मे ज्यादा प्रतिशत प्लास्मिनोजेन एक्टीवेटर इन्हीबीटर- १ (PAI-1) का होना सबसे ज्यादा घातक था जो १७.७% मरीजों मे पाया गया । पौलीमोरफिस्म PAI-1 4G/4G के होने से खून में ज्यादा मात्रा में PAI-1 बनता है यह भी पता चला इस अध्ययन मे । ४.४३% मरीजों मे tPA की कमी, ४.१३% मे ज्यादा TAFI, १.७७% मे ज्यादा A2-AP और ०.८९% मरीजों मे प्लास्मिनोजेन की कमी भी पायी गयी । हमारे इस अध्ययन मे हमने यह साबित किया है की थ्रोम्बोफिलीया के साथ-साथ फिब्रिनोल्यिसस फॅक्टर्सची की जांच हो तो थ्रोम्बोफिलीया होने के कारणों का पूर्णतः पुष्टिकरण होता है और इसलिए हम हमारे संस्थान मे यह फिब्रिनोल्यिसस मार्कर्स का थ्रोम्बोफिलीया जांच मे समावेश करने वाले है ।

निष्कर्ष

वीनस थ्रॉम्बोसिस मरीजों मे थ्रोम्बोफिलीया के साथ फिब्रिनोल्यिसस क्रियाविधि का अध्ययन करने से यह पहचानने में मदद मिलेगी कि क्या मरीज को भविष्य मे खून की गांठ बनने का खतरा है जिससे इसे रोकने के लिए एक उचित उपचार निर्धारित करने में मदद मिलेगी। इस तरह के परीक्षण से उन रिश्तेदारों की पहचान करने में मदद मिल सकती है जिनके पास वर्तमान में लक्षण नहीं हैं लेकिन जोखिम हो सकता है। हालांकि, यह जांच युवा उम्र के रोगियों, थ्रोम्बोसिस की पुनरावृत्ति, पारिवारिक थ्रॉम्बोसिस इतिहास या असामान्य रक्तवाहिनी मे खून की गांठ वाले मरीजों मे किया जाना चाहिए।

Award Winning Abstract

"Junior Investigator Award" at the Annual meeting of Thane Haematology Group, held on 31st March 2019.

Title: Prenatal Diagnosis of NADH cytochrome b5 reductase deficiency causing Recessive Congenital Methemoglobinemia (RCM) type II.

Anuja Kulkarni, Vinod Gupta, Prashant Warang, Rati Devendra, Prabhakar Kedar

Introduction: Type II recessive congenital methemoglobinemia (RCM) is a rare disease due to generalized NADH-cytochrome b5 reductase (cytb5r) deficiency. For the patients with strong clinical history of Type II RCM, an efficient prenatal screening is essential to prevent the occurrence of this disease. In this study prenatal screening of three fetuses for absence or presence of Type II RCM using molecular marker Gly76Ser and Gln77X in CYB5R3, which are already associated with Type II RCM was performed.

Methodology: Two chorionic villus sample and one amniotic fluid were referred to NIIH for prenatal diagnosis. Genomic DNA was isolated from it by using Qiagen DNA extraction kit according to the manufacturer's instructions. PCR was performed and the products were analyzed by gel and sequencing (BigDye Terminator v3.1 Cycle Sequencing Kit on a 3730 DNA Analyzer ,Applied Biosystems). For the parents and siblings, along with molecular analysis, methemoglobin level and activity was also measured.

Results: Parents of 3 fetus were Heterozygous for RCM Type II (Gly76Ser) while parents of 1 fetus were Heterozygous for RCM Type II (Gln77X) having intermediate NADH-Cyb5r activity (50-50% activity) and normal MetHB level (less than 2%). In all cases one of the earlier children has been already diagnosed with RCM Type II mutation causing severe mental retardation and neurological disabilities, methemoglobin levels from 34.1% to 65% and cytb5r activity from 0 to 10%. One of the prenatal analyses showed splice-site mutation p.Gly76Ser in homozygous state while in another one it was found in heterozygous state. In third case stop codon mutation p.Gln77X at nucleotide position 229 in exon 4 was seen in heterozygous condition. In the fourth case normal wild type genotype for the CYB5R3 gene was found. In second Gly76Ser heterozygous case the child was born with full term vaginal delivery. Methemoglobinemia level of the child on 5th, 40th and 70th day of life was found to be 4.51%, 8.29% and 10% respectively.

Discussion: The first CVS sample presented a homozygous Gly76Ser mutation and hence the parents were advised for termination of the fetus. Second CVS sample showed heterozygous for the same mutation and the parents were advised to continue pregnancy. After birth of this child Methemoglobin levels and activity displayed the Type I RCM symptoms however no clinical symptoms of Type II RCM were seen. In third case the fetus had normal genotype; hence it was advised to continue pregnancy. Prenatal diagnosis has been reported earlier globally in Type II RCM, however no reports have been yet published on the prenatal diagnosis in Indian population. Annual training programme in Immunohaematology: The training programme was held from 27th March to 26th April, 2019. Nine medical officers and eight technicians from all over India attended the training programme.

Conclusion: We reported the prenatal diagnosis first time in the Indian families in RCM Type II due to homozygous Gly76Ser and Gln77X mutation associated with severe mental retardation and neurological disabilities.

Annual training programme in Immunohaematology: The training programme was held from 27th March to 26th April, 2019. Nine medical officers and eight technicians from all over India attended the training programme.

Swaacha Bharat Pakhwada conducted at NIIH from 1/4/2019 to 15/4/2019

5th International Conference on Primary Immunodeficiency disorders held on 9th-11th March 2019 at Leela Hotel, Sahara, Andheri east, Mumbai.

20th Indo-US flowcytometry workshop on Diagnosis of Primary Immune Deficiency disorders held on 7th-8th March 2019 at NIIH, Mumbai. Dr. Paul Wallace discussing with participants

20th Indo-US flowcytometry workshop on Diagnosis of Primary Immune Deficiency disorders held on 7th-8th March 2019 at NIIH, Mumbai. A total of 60 clinicians and technicians had participated in the workshop

Dr. H.M Bhatia oration was awarded to Dr. Anand Deshpande on 11th Feb, 2019 on " My journey with in Transfusion Medicine along with NIIH - PPP!!!". Dr. Sudeep Gupta was the cheif guest at this occasion.

Dr Anuja Kulkarni, Research Associate received “Junior Investigator Award” at the Thane Haematology Group Annual meeting held on Sunday 31st March 2019.

Dr. Anita Nadkarni, Scientist F received the prestigious Dr. J G Parekh oration award in 42nd Annual conference of Mumbai hematology group held at Mumbai on 15 - 17th March, 2019

Mr. Aniket Anand Prabhudesai, Ph.D. (Applied Biology) Student registered with University of Mumbai, under the Guidance of Dr. Bipin P. Kulkarni, Scientist D, having worked on the topic 'Disorders of Fibrinolysis as a cause of thrombophilia in Indian patients with deep vein thrombosis (DVT)', successfully completed his Viva Voce examination on the 23rd of April, 2019, at the University of Mumbai.

Foundation Day programme (18 Feb, 2019)

Annual Training Program In Immunohaematology

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