Florence Nightingale

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Review

Diseases predisposing to thrombosis

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ABSTRACT

Blood is a liquid tissue that is noncoagulated under physiological conditions. Hemostasis, on the other hand, is an important factor in maintaining fluidity, keeping the blood non-coagulated. Thrombosis is a condition that occurs as a result of an abnormal clot forming in the vein blocking blood flow. The causes of thrombosis are multifactorial. The involvement of many acquired and hereditary factors in the formation of thrombosis occurs through different mechanisms. Thrombus formation in the arterial and venous systems is thought to originate from different etiologies. Thrombophilia, on the other hand, describes the conditions in which the tendency to coagulate in the blood increases. In addition to hereditary causes, many diseases such as cancer and diabetes can increase the risk of thrombophilia. This compilation provides an overview of hemostasis and thrombosis, as well as determining the causes and risk factors of clotting by examining the mechanism of blood clot formation, describes the status of thrombosis studies within *in vitro/in vivo* studies observing thrombosis formation, therapeutic effects that may break the chain between antithrombotic therapy and bleeding risk, and provides discussions on effective drug delivery systems. This review provides a contemporary view of the most common inherited or acquired causes of thrombophilia and their role in the development of venous and arterial thrombosis.

Keywords: Arterial, hemostasis, thrombophilia, thrombosis, venous.

Blood is a tissue found within the circulatory system, which plays a crucial role in physiological systems such as cellular communication and the immune system. It is transported through metabolism, hormones, and gas exchange, and under normal conditions, it exists in a liquid state. The average human carries approximately 70-80 milliliters of blood per kilogram of body weight (70-80 mL/kg).[1] This ratio corresponds to approximately 1/13 of the human body weight. When the structure of blood is examined, it is observed to consist of a liquid portion called plasma and the presence of blood cells. Approximately 50-60% of the total blood volume is liquid, of which about 90% is water; the rest is composed of ions, glucose, amino acids, various proteins, hormones, and other metabolites.[2] Blood cells consist of erythrocytes, leukocytes, and thrombocytes; the remaining portion after removing fibrinogen and coagulation factors from plasma is called serum. The structure of blood should remain non-coagulated to perform physiological processes. Various factors contribute to the fluidity of blood flow. These include the negative pressure in the thoracic cavity, the pumping power of the heart, the contraction in the vascular system, and the valves in the venous vessels. Additionally, the smooth surface composed of endothelial cells within the blood vessels, through which blood circulates, is another factor that ensures the fluidity of blood flow.[2,3]

HEMOSTASIS

Hemostasis refers to the mechanism that maintains the non-coagulated state of blood in the circulatory system. To preserve the fluidity and liquid state of blood, enzymatic reactions lead to coagulation in the event of injury or damage. During coagulation, numerous co-factors are involved. Simultaneously, through physiological mechanisms, bleeding is stopped due to trauma,

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and the formed clot is eventually cleared, reopening the blood vessel. This function is accomplished by the balanced operation of hemostatic and fibrinolytic activities.[4] If the coagulation mechanism outweighs this balance, thrombosis formation is observed. On the other hand, a positive change in the fibrinolysis mechanism leads to bleeding. The hemostasis system has two distinct characteristics. The first one is surface-dependent, where the hemostasis mechanism becomes functional on the phospholipid layer, resulting in clot formation at the site of injury or trauma. Therefore, not the entire circulatory system is affected by coagulation.[5] The second characteristic is that every mechanism present in the system has an opposing inhibitor, and there are numerous feedback loops. Therefore, hemostatic and fibrinolytic activities form an interacting and continuous mechanism. The hemostasis mechanism consists of three different elements. These systems encompass vascular endothelium, platelets, and the coagulation system.[4-6]

Vascular endothelium

Endothelial cells, which have numerous essential biological functions, form a thin layer surrounding the walls of blood vessels. They have an approximate width of 10-15 µm and a length of 20-25 µm. They align along the long axis of the blood vessels, creating a single-layered structure with elongated nuclei arranged in a polygonal pattern. Therefore, the vascular endothelium not only provides a non-thrombogenic surface but also acts as a selectively permeable barrier between tissues and blood.[7] Endothelial cells, which play a significant role in hemostatic and fibrinolytic activities, possess anticoagulant and antiplatelet properties, as well as initiating properties for coagulation. The endothelial surface is intact, and blood flows continuously. Therefore, under normal conditions, spontaneous activation of the coagulation system and platelets is prevented.[8] In processes like trauma, when this surface is disrupted, the coagulation system and platelets are activated. The outer membrane of endothelial cells is composed of a proteoglycan layer called the glycocalyx, which contains dermatan sulfate, heparan sulfate, and heparin. Due to its content, the surface facing the blood exhibits a smooth property, and this outer membrane is responsible for antithrombotic functions.[9] Antithrombin III (AT-III) is a plasma protein that serves as a natural anticoagulant and is produced by heparan sulfate. AT-III exhibits its anticoagulant effect by inhibiting thrombin, which is the responsible enzyme for converting clotting factors such as factor XIIa, FXIa, FXa, FIXa, and fibrinogen into fibrin. Additionally, there is another membrane receptor called thrombomodulin on the outer membrane, which interacts with thrombin to accelerate the activation of protein C (PC), a potent coagulation inhibitor.[10] In this mechanism, active PC increases the concentration of plasminogen activators and inactivates factor V (FV) and FVIII, thereby controlling thrombosis. Protein S (PS) acts as a cofactor in the anticoagulant activity of active PC and is synthesized by endothelial cells. Protein C is also involved in the secretion of tissue plasminogen activator (t-PA). Besides t-PA, which is a crucial substance in the conversion of plasminogen to plasmin, urokinase synthesized in the kidneys and endothelial cells is another important activator in this process. Plasmin, as a proteolytic enzyme, is responsible for the breakdown of fibrin. Plasminogen activator inhibitor-1, synthesized by endothelial cells, is responsible for neutralizing urokinase. This inhibitor facilitates the inactivation of t-PA.[11] Prostacyclin (PGI2), which acts as a vasodilator and an inhibitor of platelet aggregation, is synthesized and secreted from endothelial cells to inhibit platelet activation. It is also responsible for increasing the diameter of blood vessels, promoting vasodilation. In this mechanism, adenylate cyclase is stimulated by PGI2 binding to receptors on the platelet membrane, leading to an increase in cyclic adenosine monophosphate (cAMP) levels in platelets. Endothelial cells partially synthesize FVIII, which is called von Willebrand factor (vWF) when it forms a bimolecular complex with circulating FVIII. This complex exhibits procoagulant properties.[12] The vWF plays a significant role in hemostasis and facilitates the adhesion of active platelets to collagen and subendothelial components by binding to a specific receptor on the platelet membrane. The increase in cAMP levels prevents platelet aggregation, shape differentiation, and binding to vWF and collagen, thereby inhibiting thrombus formation. Collagen molecules like type 4 collagen are also crucial factors in

the interaction between subendothelium and platelets after trauma. They are synthesized by endothelial cells and play a critical role in hemostasis.[10-13]

Platelets

Platelets are one of the blood cells representing the final stage of cell development, with a round or oval shape and an approximate diameter of 2-4 μm. They lack a nucleus. After the occurrence of tissue damage due to trauma, platelets interact with various endothelial structures and are one of the main elements for the continuity of hemostasis. Platelets are formed through the fragmentation of elements called megakaryocytes, which differentiate from bone marrow stem cells. They have a membrane covered with a glycocalyx and consist of a double-layered lipid membrane, where functionally important proteins are embedded. Endoplasmic reticulum remnants and canal systems are concentrated near the membrane, while granules of varying densities are located in the inner region. Platelets are rich in mitochondria, and the synthesis of different enzymes takes place in the Golgi apparatus and endoplasmic reticulum. Additionally, they store calcium (Ca+2).[14] Platelets have the ability to contract, change shape, and bind to other platelets or different sites due to the presence of contractile proteins in the cytoplasmic region, such as actin, myosin, and thrombosthenin. In platelets, secretion can occur, and this is facilitated by secretory granules containing various substances. These granules consist of alpha (α)-granules, lysosomes, and dense bodies. Alpha-granules contain various proteins specific to platelets, such as fibrinogen, FV, beta (β)-thromboglobulin, platelet factor 4, FVIII, and growth factors. These granules can neutralize heparin. On the other hand, lysosomes contain acid hydrolases.[15] Platelet growth factors mainly consist of transforming growth factor-β, endothelial cell growth factor, and platelet-derived growth factor. These growth factors are contained within the α-granules of platelets. Another type of secretory granule, the dense bodies, contains substances responsible for platelet metabolism and includes storage molecules such as adenosine diphosphate (ADP) and adenosine triphosphate (ATP).[16] Both ATP and ADP are responsible for the formation of membrane activities and provide the necessary energy for cellular functions. Platelet cells in the circulatory system circulate in the blood for about 10 days, and under normal physiological conditions, they move independently from the endothelium without damaging the vascular endothelial lining. On the 10th day, the reticuloendothelial system phagocytoses platelets to eliminate them. However, in certain blood disorders resulting from trauma, vascular damage triggers a platelet response. Platelet adhesion is stimulated by the damage to endothelial cells that bind with subendothelial structures such as collagen.[17] The interaction between vWF and the glycoprotein 1b receptor on the platelet membrane regulates platelet adhesion to the vessel wall. As a result of this interaction, dense bodies and α-granules undergo a shape change, facilitating the release of their components within the surface system. These components are then deposited around and inside the membranes. Calcium is an ion released from dense bodies, and it is essential for the blood clotting mechanism. ADP and thromboxane A2 (TXA2), also known as a potent vasoconstrictor, are critical factors that affect platelet aggregation. TXA2 causes vasoconstriction and narrowing of blood vessel diameter. The aggregation of platelets forms either a platelet plug or a hemostatic plug. The changes that occur support the interaction of coagulation factors with the surface, leading to the formation of fibrin. Depending on the extent of damage in the blood vessel, in addition to the platelet plug, a blood clot may also be necessary for the coagulation mechanism. For example, in cases of significant damage due to trauma, both platelet plug and blood clot may be required.[18]

Coagulation system

Coagulation is a process that causes the flowable blood to solidify, making it unable to flow further. The coagulation process is regulated by different plasma proteins. In the enzymatic reaction series catalyzed by the active form of coagulation factor, various factors such as phospholipids, cofactors, and Ca+2 ions play a role, along with coagulation factors. There are three main groups of proteins in the coagulation system: fibrinogen group, contact group, and prothrombin group. These proteins control the activation and inhibition of each other in the coagulation system. The fibrinogen group includes factors; I, V, VIII, and XIII. The contact

group, which is involved in the intrinsic pathway, consists of factors XI, XII, prekallikrein (PK), and high-molecular-weight kininogen (HMWK). Finally, factors II, VII, IX, and X are stable factors of the prothrombin group and are synthesized in a vitamin K-dependent manner.[19]

The coagulation reaction develops according to three different pathways. These pathways are called the intrinsic, extrinsic, and common pathways. The intrinsic pathway is composed of procoagulant structures present in the circulating blood, while the extrinsic system utilizes tissue factor, FIII, and enzymes originating from body tissues, along with enzymes found in the circulating blood.[20] In both pathways, a complex called prothrombin activator complex (PAC) is synthesized, which converts prothrombin into active thrombin and consists of ions and cofactors. The conversion of fibrinogen to fibrin is catalyzed by activated thrombin.[21]

Intrinsic pathway

The pathway involving FXI, FXII, HMWK, and PK is the coagulation pathway where contact activation takes place. Contact activation is initiated by vascular trauma, and physiologically, these stimuli lead to the formation of the intrinsic pathway. When collagen is exposed due to damage, FXII comes into contact with it and is converted into an activated form, a proteolytic enzyme. Inactive FXII, upon contact with PK and HMWK proteins, becomes activated. The activation of FXII has a significant impact on the blood coagulation mechanism.[22] The activated FXII can enzymatically convert the inactive zymogen of FXI into active serine protease FXIa, and this effect is demonstrated in the presence of PK and HMWK. The activated coagulation factor FXIa, in the presence of free calcium, phospholipids, and activated FVIII, converts the zymogen of FIX into active FIXa. The role of active FIX is to convert FX into FXa, which is an important initiator in the formation of the PAC along with free calcium, phospholipids, and activated FVIII. Subsequently, the PAC is formed when FXa binds to platelet phospholipids and FV. This complex leads to the cleavage of prothrombin to thrombin. As mentioned earlier, thrombin catalyzes the conversion of fibrinogen to fibrin. This pathway is also known as the contact activation pathway.[23]

Extrinsic pathway

Another coagulation mechanism that plays a role and is also known as the extrinsic pathway is the tissue factor pathway. It begins when tissue thromboplastin (tissue factor), which is produced as a result of tissue damage, enters the circulation. One distinct feature of tissue thromboplastin compared to other coagulation factors is that it is found not only in the circulatory system but also in the kidney, brain, spleen, and vascular endothelial cells. The tissue factor is composed of both lipid and protein elements.[24] The protein part of the tissue factor interacts with different phospholipids to catalyze the conversion of FVII to its active form, FVIIa. The activated FVII, in the presence of ionized calcium, converts FX to FXa. Subsequently, the PAC is formed by FXa with the help of FV and released phospholipids. The activated FX, acting as a protease, cleaves prothrombin to thrombin. The activated thrombin, in turn, catalyzes the conversion of fibrinogen to fibrin.[23,24]

Common pathway

Both the intrinsic and extrinsic pathways converge at the final activation of FX. Therefore, during the conversion of FX to FXa, both pathways come together in the common pathway. FXa combines with procoagulants such as FII, FV, platelet phospholipids, and Ca+2 ions to form a group of molecules called the prothrombinase complex. This complex catalyzes the conversion of prothrombin to thrombin. Thrombin exerts its proteolytic effect on fibrinogen, resulting in the polymerization of fibrin monomers and the formation of fibrin fibers. FXIII is also activated by thrombin in the presence of Ca+2 ions, and the coagulation process concludes with fibrin stabilization.[25]

THROMBOSIS

Thrombosis is the term used to describe the pathological process in a living organism's vascular system where an abnormal formation called "thrombus" occurs, and the hemostatic response continues without being properly regulated from the beginning.[26] The factors involved in hemostasis are regulated to maintain the fluidity of blood while being ready to respond effectively and rapidly to vascular

damage by forming a clot to prevent blood loss. When hemostatic elements deviate from their normal values and hemostasis cannot be achieved, pathological processes may occur, leading to either bleeding due to insufficient clotting or thrombus formation due to excessive hemostasis. Thrombus forms actively and circulates in the bloodstream. The process of thrombus formation is different from static blood clotting, and thrombocytes and polymorphonuclear leukocytes are stored within the fibrin mesh. The interaction of vascular, humoral, and cellular factors results in the development of thrombosis in the blood flow.[27]

The pathophysiology of thrombosis is not solely attributed to the precipitation involving all elements of the coagulation system. It can be summarized as follows:[28]

1. Initiation of coagulation at the endothelial and subendothelial regions,
2. Formation of fibrin clot and activation of mechanisms that limit thrombosis during the fibrinolytic process,
3. Formation of pathological thrombus due to imbalance between fibrinolytic process, coagulation, and anticoagulant processes.

After the formation of a pathological thrombus, a part or the whole thrombus can lead to embolism. There are three different factors that can cause thrombosis: vascular injury, changes in the composition of blood, and stagnation of blood flow. Any of these factors, alone or in combination, can contribute to thrombus formation. Abnormalities in blood components can be observed in platelet functions (primary hemostasis), coagulation (secondary hemostasis), and fibrinolysis stages.[29] When there is an abnormal increase in coagulation factors, an increase in platelet count, and an enhancement in platelet aggregation and adhesion, thrombosis and hypercoagulability conditions can occur. Hypercoagulability refers to the shift in the hemostatic balance towards a prothrombotic state. Examples of hypercoagulability include changes such as an increase in the concentration of clotting factors or a decrease in fibrinolytic activity in the blood.[30]

Thrombosis is a commonly encountered and multifactorial disease in the population. Its incidence is about 1% in adults, while in children, it is observed at a rate of 1 in 100,000 per year. Both genetic and acquired factors can play a role in the development of thrombosis. Thrombophilia is a term used to describe a tendency towards thrombosis. Thrombosis can occur in the arterial and venous systems due to different etiologies.[31]

Arterial thrombosis

Arterial thrombosis is the most common type of thrombosis that leads to disease and death. Atherosclerotic vascular disease, which causes damage to the endothelium, is the most well-known factor leading to arterial thrombosis. Arterial thrombosis is also known as white thrombus. Large and medium-sized arteries such as the dorsal aorta, iliac arteries, and coronary arteries are affected by this condition. Lipids and lipoproteins start to accumulate in the subendothelial layer of the arteries due to changes in the arterial walls. These accumulations alter the non-thrombogenic properties and promote platelet adhesion and aggregation. As a result, fibrin accumulation leads to the formation of a platelet plug. Total vascular occlusion occurs when fibrin accumulation progresses due to the advanced activation of the clotting system. Acute myocardial infarction and cerebral vascular damage are well-known examples of arterial thrombosis.[31,32]

Venous thrombosis

Venous thrombosis is the formation of blood clots within the veins due to abnormalities in the coagulation system or slows blood flow (stasis) in the circulatory system. It is commonly observed in the deep veins of the legs and is known as deep vein thrombosis. However, it can rarely occur in other veins, such as those in the brain, retina, mesentery, and liver. Unlike arterial thrombosis, it is also called "red thrombus" and is composed of a mixture of fibrin, platelets, and red blood cells. These thrombi have the potential to detach from the blood vessel wall and enter the circulation, leading to embolism. Almost all genetic factors that predispose individuals to hereditary thrombosis also increase the risk of venous thrombosis. The fact that individuals carrying genetic thrombosis risk factors may

not experience thrombosis events is evidence that thrombosis is influenced not only by genetic factors but also by acquired factors.[33]

GENETIC FACTORS ASSOCIATED WITH THROMBOSIS

The possibility of thrombotic events arising from genetic factors has been a topic of discussion since 1965. The completion of deficiencies in the coagulation system in the late 1960s shed light on the understanding of hereditary thrombosis.[34] Although various genetic mutations and protein deficiencies causing thrombosis have been identified, the reason why about 40-60% of individuals with hereditary thrombosis develop blood clots cannot be fully explained. Disturbances in natural inhibitory mechanisms have been observed to be a significant factor in the etiology of hereditary venous thrombosis. In this context, the best-known factors include factor V Leiden (FVL), also known as activated protein C (APC) resistance, PC and PS deficiencies, the prothrombin G20210A mutation, and AT-III deficiency. In addition to these factors, an excess of FVIII and hyperhomocysteinemia are also considered other risk factors.[35]

Factor V Leiden Mutation

Factor V is a glycoprotein molecule with a single chain, produced by megakaryocytes, leukocytes, and the liver. It has a molecular weight of 330,000 daltons and consists of 2,196 amino acids. Approximately 80% of FV is found in the plasma in a free form, while the remaining 20% is present in platelets. The FV gene is located on the q21-25 region of the 1st chromosome and contains 25 exons. Factor V is composed of proteins with a domain structure consisting of internal repeats, arranged as A1-A2-B-A3-C1-C2.[35] The A domain contains 350 amino acids, the B domain contains 836 amino acids, and the C domain contains 150 amino acids. The functional unit responsible for coagulation is the B subunit, which is activated by thrombin. Activated factor V (FVa) has a heterodimeric structure and consists of N-terminal and C-terminal regions, each composed of 23 parts. Both subunits are held together in a non-covalent manner with Ca.[36]

Factor V functions as a cofactor in both the intrinsic and extrinsic pathways. In the intrinsic pathway, FV binds to thrombocyte phospholipids and FXa; in the extrinsic pathway, it combines with FXa and phospholipids, which are part of tissue thromboplastin, to form the prothrombinase complex. As a result, prothrombin is converted into thrombin. Thrombin converts fibrinogen to fibrin, and it also binds to the endothelial membrane protein thrombomodulin. This interaction changes thrombin's procoagulant structure into an anticoagulant form. Subsequently, thrombin plays a role in the activation of protein C. Activated protein C is a serine protease enzyme that inactivates FVIIIa and FVa, thus promoting anticoagulation and preventing further blood clot formation.[37]

The presence of FVL mutation increases the incidence of venous thrombosis, stroke, and pulmonary embolism. FVL carriage has been observed in 20% of all cases and in 50% of selected thrombosis cases in patients with venous thrombosis.

Antithrombin III reficiency

Antithrombin III is a natural anticoagulant that plays a significant role in the coagulation system by inhibiting thrombin and other clotting factors. It irreversibly inhibits FXIa, FIXa, and FXa. Additionally, AT-III accelerates the dissociation of the FVIIa-tissue factor complex and participates in the inhibition of FVIIa. Therefore, it is known as the most potent inhibitor in fibrin formation. Antithrombin III is a 65,000 dalton α-2 globulin molecule synthesized in the liver and found in human plasma at a concentration of 196 g/mol. The gene region encoding AT-III is located on chromosome 1, at the q23-25 region. Both hereditary and acquired factors contribute to its deficiency. In cases of hereditary deficiency, there is an increased risk of pulmonary embolism and venous thrombosis. The hereditary deficiency follows an autosomal dominant inheritance pattern and can be classified into two types.[38]

In type 1 deficiency, there is a reduced biological synthesis of the AT-III molecule. The decreased synthesis leads to a reduction in its activity. The cause of this deficiency may be due to deletions, insertions, or single nucleotide polymorphisms during the synthesis of the AT-III gene. On the other hand, type 2 deficiency is related to defects at specific locations in the gene regions. Molecularly, AT-III appears to be normal,

but functionally, it is defective. These different variants are classified into three types: type 2 reactive site defect, type 2 heparin binding site defect, and type 2 pleiotropic effect defects.[39,40]

Protein C deficiency

Protein C is a vitamin K-dependent protein, weighing 62,000 D, synthesized in the liver and plays a role in hemostasis. The coding region of the gene is located on chromosome 2, in the q13-14 region, and is 11 kb in length.[41] The activation of the PC occurs by thrombin. Thrombin binds to an endothelial cell receptor known as thrombomodulin, which causes thrombin to lose its ability to convert fibrinogen to fibrin. Activated protein C in the presence of PS breaks down and inactivates FVIIIa and FVa, thus inhibiting coagulation. The presence of PS enhances this effect. There are two types of inherited PC deficiency: quantitative, known as type 1, and functional, known as type 2. In type 1, both the activity and antigen levels of PC are reduced to about 50% of the normal value. In type 2, the antigenic level of PC is normal, but it is functionally inactive. Patients with PC deficiency are often observed to have type 1 heterozygosity. Acquired PC deficiency can occur in liver disease or vitamin K deficiency. Additionally, intravascular coagulation, deep vein thrombosis, and infections can lead to PC deficiency.[42]

Protein S deficiency

Another vitamin K-dependent protein, PS, with a molecular weight of 70,000 dalton, is synthesized in the liver, endothelial cells, megakaryocytes, and Leydig cells. It is found in the plasma and also in the α-granules of platelets. The gene encoding PS is located on the 3rd chromosome at the p11.1-11.2 region and spans 80 kb in length. In plasma, PS exists in two different forms. Approximately 35-50% of PS is inactive and is associated with the complement regulatory protein C4b-binding protein. The remaining portion is free and possesses co-factor activity. In the inactivation of FVa and FVIIIa by APC, PS acts as a cofactor.[43]

Elevated levels of FVIII, FIX, and FXI

An increase in FVIII, FIX, and FXI levels can lead to an increased predisposition to thrombosis. Elevated levels of FVIII are associated with deep vein thrombosis in patients and can also be seen as a hereditary condition. However, the specific mutation causing the increase in FVIII levels has not yet been identified.[44]

Prothrombin 20210 mutation

Prothrombin; a vitamin K-dependent, single-chain glycoprotein with a molecular weight of 71,000 dalton, plays a role in the conversion of fibrinogen to fibrin in its active form. It is produced by the liver and contains about 10% carbohydrates. The gene encoding prothrombin is located in the 11th chromosome at the p11-12 region and is 21 kb in length.[45] The gene consists of 13 introns and 14 exons, and it encodes 579 amino acids. In studies conducted on the prothrombin gene, a single nucleotide change mutation (G20210A) was identified in the 3'-untranslated region of the gene at the 20210th nucleotide, where guanine is replaced by adenine. As a result of this mutation, plasma prothrombin levels increase, leading to a predisposition for venous thrombosis. Among factors contributing to thrombosis, the G20210A mutation is the second most common risk factor and increases the risk of clot formation by 2-4 times.[46]

Hyperhomocysteinemia

The difference between hyperhomocysteinemia from other hereditary factors is that it carries the risk of causing both venous and arterial thrombosis. It is also referred to as methionine metabolism disorder. The thromboembolic effects of hyperhomocysteinemia were described by Falcon in 1994 and Fermo in 1995. Later in 1996, Heijer reported it as a risk factor for recurrent venous thrombosis and deep vein thrombosis. The potential of causing arterial thrombosis was first described by D'Angelo and Selhub in 1997.[47] Under normal conditions, the fasting plasma concentration of homocysteine is 5-15 µmol/L. In mild, moderate, and severe hyperhomocysteinemia, these values are respectively 15-30 µmol/L, 30-100 µmol/L, and above 100 µmol/L. The causes of hyperhomocysteinemia include both genetic and acquired factors. Acquired factors may involve deficiencies of folate, vitamin B6, and vitamin B12, while genetic factors may be associated with a gene found in the 11q23 chromosome region. Elevated homocysteine levels increase the risk of thrombosis by up to 3-4 times.[48]

Elevation of lipoprotein (a)

Lipoprotein (a) is located on the 6th chromosome and is closely positioned with the plasminogen gene. It is independent of low-density lipoprotein metabolism and is synthesized in the liver. Under normal conditions, elevated lipoprotein levels in adults pose a risk for venous thrombosis due to its antifibrinolytic effect, and it is also considered a factor in cardiovascular diseases (CVD). While lipoprotein (a) is present in about 13% of the general population, it is observed in about 30% of individuals with thrombosis.[49]

ACQUIRED RISK FACTORS FOR THROMBOSIS

In addition to genetic factors, various acquired factors are also considered among the risk factors that increase the risk of thrombosis. The presence of one or more of these factors leads to a significant increase in the risk of thrombosis. Acquired risk factors for thrombosis include advanced age, obesity, physical inactivity, pregnancy, smoking, and stress, as well as diseases such as cancer, inflammation, hypertension, congestive heart failure, hypertriglyceridemia, and diabetes.[50]

Cancer and thrombosis

In 1865, Armand Trousseau[51] described an increased risk of thrombosis in patients with cancer. Thrombosis was also reported as a complication of cancer. Therefore, the coexistence of both conditions is referred to as Trousseau's Syndrome. This syndrome, characterized by migratory and recurrent thrombosis in superficial veins such as the arm and chest wall, is seen as a variant of venous thromboembolism. Venous thromboembolism and arterial thrombosis are complications of cancer and are frequently observed in cancer patients. It can also serve as a hidden precursor of cancer.[52] Trousseau's Syndrome is frequently associated with the lungs and pancreas. Tumor cells protect themselves from the immune system and chemotherapeutics by creating fibrin/thrombus aggregates, which facilitate their adhesion to the vascular wall. This process is also observed in metastatic cancer types and accompanies metastasis. Tumors can enhance fibrinolytic activity either through plasminogen activator or surface-dependent mechanisms.[53] As a result of activity, connective tissue is invaded, and a part of the metastasis occurs through this process. In *in vivo* animal experiments, it has been observed that coagulation, fibrinolysis, and platelet activity are responsible for pathological tumor growth, and if these pathways are pharmacologically inhibited, metastatic activity declines. Although the pathogenesis of hypercoagulability due to tumor cells has not been fully explained, some abnormalities in blood composition, abnormalities in blood vessel walls, and changes in blood flow are considered among the reasons.[51-54]

COVID-19 and thrombosis

The first case of severe acute respiratory syndrome coronavirus 2, known as coronavirus disease-2019 (COVID-19), was reported in 2019, and in March 2020, it was declared the beginning of a pandemic. Alongside mild or life-threatening symptoms caused by COVID-19, studies have also shown an increased risk of thrombosis in observed cases. COVID-19 leads to a hypercoagulable state strongly associated with mortality. Widespread macro or microvascular thrombosis is also linked to other symptoms observed in COVID-19 patients.[55] According to studies, the proinflammatory state that is highly prevalent in COVID-19 has been linked to the formation of thrombosis as a part of antiphospholipid syndrome, with a role attributed to lupus anticoagulant. Therefore, COVID-19 is a disease that induces a prothrombotic state, and the frequency of major thrombotic events is concerning due to its unique prothrombotic pathophysiology. COVID-19 cases have shown micro and macrovascular thromboembolic or *in situ* thrombotic complications in the lungs, spleen, brain, intestines, and periphery. Frequent thrombus formation during hemodialysis, stroke as a symptom in previously healthy young patients, and reports of arterial and venous thromboembolism despite prophylactic or full therapeutic anticoagulation have been documented in COVID-19 cases.[56] Thrombotic events have been identified in asymptomatic patients as well. Thrombosis has been observed both in the acute setting and in the weeks following the critical illness phase, suggesting that the prothrombotic state may persist for several weeks or even longer after hospital admission.[57]

Stress and thrombosis

Psychological stress induces various changes in the organism, affecting homeostasis and hematopoiesis.[58] The stress-induced hyperactivation of the hypothalamic-pituitary-adrenal axis and the autonomic nervous system can trigger cellular and molecular changes in coagulation factors, platelets, endothelial function, sterile inflammatory response, and redox balance. Therefore, the risk of CVD may increase due to psychological stress. Understanding the mechanisms underlying the relationship between stress and the underlying pathology of CVD, which is thrombosis, is highly valuable.[59] While there is no definitive evidence that both acute and chronic stress alone is sufficient to trigger acute coronary thrombotic events, it has been suggested that in the presence of other risk factors, stress may lead to a greater risk of CVD. The stress response is characterized by autonomic and neuroendocrine dysfunction, platelet activation, coagulation abnormalities, impaired fibrinolysis, endothelial dysfunction, and inflammation, which contribute to a prothrombotic state.[60]

THE ROLE OF *IN VIVO* AND *IN VITRO* STUDIES IN THROMBOSIS

Since thrombosis formation is multifactorial, its background is illuminated through the evaluation of personal lifestyle and genetic factors. Although some of the genetic factors that carry the risk of thrombosis have been identified today, a significant proportion of patients still cannot be thoroughly examined as to why they develop thrombus.[61] Under normal conditions, blood, which is in a liquid and flowing state, can lead to arterial and venous thrombosis by forming clots, and it can also pose a life-threatening risk by causing venous thromboembolism. Thrombophilia is a term used to describe a predisposition to thrombosis.[62] Currently, thrombophilic disorders are influenced by multiple acquired factors as well. Although the risk of thrombosis varies in adults and children, therapeutic evaluation should be effectively conducted to prevent or treat thrombus formation in patients without causing fatalities. Factors such as maintaining a good quality of life, a stress-free environment, healthy nutrition, and maintaining a healthy weight, along with an active lifestyle, can help reduce the occurrence of thrombosis.[63] In addition to that, there are therapeutic agents available that can effectively reduce or treat thrombosis. Investigating the pathogenesis and studying *in vivo/in vitro* thrombus formation is crucial for the effectiveness of treatment. *In vitro* studies have been conducted to quantitatively observe artificial thrombus formation in plasma or circulating blood.[64] It has been observed that thrombus formation occurs behind the mesh placed in the flowing fluid. The pressure changes during thrombus formation allow for continuous monitoring of the process. The resulting thrombosis has the characteristics of a white thrombus but constitutes less than approximately 1% of the plasma and blood volume. In these studies, platelets play a crucial role in the initial stage of thrombosis. With this model, it is also possible to investigate thrombolysis, which takes about 10 min to occur. However, *in vitro* experiments may not be sufficient to track different physiological and pathological processes in complex biological systems. Therefore, *in vivo* studies are also an effective method to observe thrombosis formation factors and the formation stages.[65] For instance, it has been demonstrated that new technologies incorporating intravital video microscopy with vascular window analysis can be utilized to directly visualize arterioles and venules to observe thrombus formation in a live mouse. These systems provide suitable platforms for studying the roles of platelets, blood coagulation proteins, endothelium, and vascular wall in order to understand their functions. *In vivo* studies allow for biochemical and cell biology investigations. Platelet activation, adhesion, secretion, and aggregation, as well as thrombin generation and fibrin formation initiated by tissue factor, have often been studied using *in vitro* systems.[65,66] Therefore, considering the complexity of the hemostatic system and thrombosis, reevaluating thrombus formation through *in vivo* processes, especially using intravital microscopy and genetically modified mice, has been deemed appropriate.

ANTITHROMBOTIC AGENTS AND NANOTECHNOLOGY

Thrombus formations form outside the physiological healthy hemostatic responses and can cause significant medical problems

for affected individuals. Once the thrombosis formation stage is safely examined, the next topic of discussion is identifying effective therapeutic agents. Antithrombotic agents are divided into two categories: drugs that interrupt the coagulation cascade or reduce the synthesis of coagulation factors (anticoagulants) and drugs that inhibit platelet function (antiplatelet agents).[67] The third class of agents consists of thrombolytic drugs that act to promote the dissolution of formed thrombi. Although anticoagulants are mainly used for the prevention and treatment of venous thrombosis, they also have some activity against arterial thrombosis.[68] Different examples of anticoagulants include desirudin, apixaban, betrixaban, edoxaban, and heparin. Antiplatelet agents are effective in the prevention and treatment of arterial thrombosis. For instance, aspirin is an irreversible inhibitor of cyclooxygenase 1 that blocks platelet activation and aggregation throughout the lifespan of the platelets. However, current standard care therapies for thrombosis treatment are systemic in their therapeutic design, and as such, they interfere with the patient's physiological hemostasis.[69] Examples of serious clinical side effects commonly associated with current therapies include bleeding complications. Therefore, there is a significant demand for new therapeutic interventions that can offer improved therapeutic efficacy while reducing or preventing these debilitating complications. Recent advancements in nanotechnology provide an opportunity to meet this clinical demand by developing new and enhanced drug delivery systems. In this context, various nanocarriers, affinity ligands, and polymer coatings optimize the pharmacokinetics of antithrombotic agents and target drug delivery to sites of thrombosis.[70] In addition, new antithrombotic agents with the ability to detect pathological changes in the vascular microenvironment, such as altered hemodynamic forces and expression of inflammatory markers, have been developed. One example of such studies is the use of recombinant fusion proteins for the delivery of antithrombotic agents, which has shown promising results. Therefore, a comprehensive evaluation of thrombosis factors, effective monitoring of formation processes, and the utilization of new and different therapeutic approaches are essential on the agenda to minimize the potentially fatal risk of thrombosis.[71]

In conclusion, thrombosis is a condition that can arise from external factors such as smoking, a sedentary lifestyle, as well as acquired factors stemming from diseases like cancer and cardiovascular diseases, or genetic factors like high lipoprotein (a) levels, PC, and PS deficiencies. Genetic factors involve genetic mutations and deficiencies of related proteins; however, the reasons why approximately 40-60% of patients forming thrombi are prone to thrombosis remain unknown. Thrombosis refers to the formation of clots in the blood, which under normal circumstances should remain in a non-coagulated state. These clots can also form due to traumatic injuries. The hemostatic system operates through hemostatic-fibrinolytic activity, which protects the flow of blood and ensures its coagulation when necessary. Thrombosis can manifest in different forms, including arterial and venous, and the formation of thrombi can lead to life-threatening conditions like venous thromboembolism. The list of diseases that can cause thrombosis is continuously being illuminated, and thrombus formation can result from a combination of multiple factors rather than a single factor. COVID-19 is one of the most recent examples that carries a risk of thrombotic complications. Investigating thrombus formation through *in vitro* and *in vivo* methods is crucial in understanding its underlying mechanisms. Therefore, the exemplary studies conducted should be reinforced with *in vivo* analyses. Additionally, nanotechnological approaches can be used to enhance the effectiveness of antithrombotic agents and provide efficient treatment options after the background of thrombosis risk is clarified.

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