Problem 5. Pathophysiology of the Hemophilia A

In order to workout this problem study pages 32-5, 53, 555-6 and 556 of your Pathophysiology (5th edition) textbook.

A 22-year old medical student took a light blow to the right knee while playing soccer. Two hours later his knee became swollen, very painful with a diffuse hematoma. The pain and the swelling eventually made him immobile. The clinical examination showed hematros of his right knee. The patient had a history of prolonged bleeding incidents on two occasions during his childhood - after the tooth extraction; however, nobody paid attention. Laboratory tests indicated a reduced activity of factor VIII and a prolonged activated partial thromboplastin time (APTT) while the factor IX, von Willebrand factor and prothrombin time remained within the physiological values.

Table 1 shows the relevant laboratory indicators. The edematous knee was immobilized and with analgesic therapy, the swelling regressed within four days. On the third day, most of the blood was aspirated from the knee synovial cavity while the remaining hematoma spontaneously reabsorbed. Since this was a case of newly discovered coagulopathy with mild clinical features, a coagulation status for the entire family was determined. Patient’s mother had a normal partial thromboplastin time while the factor VIII activity was only 0.49 (49%). The father and the two brothers had no coagulation disorders. Patient’s sister had the factor VIII activity of 0.51 (51%) and her APTT remained within physiological range. His grandfather on the mother’s side died from a gun shot wound because they were “unable to stop the bleeding” while the mother’s sister is a carrier of the disease.

<table>
<thead>
<tr>
<th>Indicator</th>
<th>Measured value</th>
<th>Normal levels</th>
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<tbody>
<tr>
<td>Platelets</td>
<td>375</td>
<td>150-450 x 10^9/L</td>
</tr>
<tr>
<td>Bleeding time</td>
<td>5</td>
<td>&lt;10 min</td>
</tr>
<tr>
<td>Activated partial thromboplastin time (APTT)</td>
<td>37,5</td>
<td>22-33 s</td>
</tr>
<tr>
<td>Prothrombin time (PT)</td>
<td>11,7</td>
<td>11,5-13,5 s</td>
</tr>
<tr>
<td>Fibrinogen</td>
<td>3,7</td>
<td>1.5-4,5 g/L</td>
</tr>
<tr>
<td>Activity of factor VIII (VIIIa)</td>
<td>0,12 (12%)</td>
<td>0,50-2,00 (50-200%)</td>
</tr>
<tr>
<td>Activity of factor IX (IXa)</td>
<td>0,97 (97%)</td>
<td>0,50-1,40 (50-140%)</td>
</tr>
<tr>
<td>von Willebrand factor</td>
<td>1,37 (137%)</td>
<td>&gt;0,60 (&gt;60%)</td>
</tr>
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</table>

The test is used to show the platelet function in hemostasis. After a superficial incision on the forearm, the newly formed blood droplets are collected with cotton pads. The bleeding time is measured from the onset of bleeding until the new blood droplets cease to form. During the test, the pressure cuff is used to maintain the upper arm pressure constant at 40 mmHg.

The test is used to assess the intrinsic pathway of coagulation. Standard phospholipids together with kaolin (substance with electronegative potential that activates factor XII) are added to citrated plasma; APTT is the time of clot formation after excessive Ca^{2+} addition. APTT is the cumulative indicator of function for all coagulation factors except for factor VII.

The test is used to assess the extrinsic pathway of coagulation. Standardized tissue extracts are added to the citrated blood; PT is the time of the clot formation after excessive Ca^{2+} addition.
Exercise A: Repetitions of relevant knowledge TYPE I

1. In terms of genetic relations within a family with the occurrence of hemophilia A, all of the following claims are correct except for one:

   a) All sons of hemophilic individuals are genetically and phenotypically healthy in terms of coagulation status, since the sons inherit their X chromosome from the mother and not from the father.

   b) All daughters of the hemophilic fathers inherit the defective X chromosome from their father, which makes them obligatory carriers of the disease; and sometimes they also develop the phenotypic manifestation of the disease.

   c) Daughters of the hemophilic individuals pass on the defective gene to 50% of their daughters which makes them carriers; and 50% of their sons manifest the disease in phenotype.

   d) Hemophilic phenotype remains expressed throughout the lifetime and the severity of clinical symptoms and the laboratory indicators are similar among the affected family members.

   e) Thrombocytopenia is the basic cellular mechanism which initiates the phenotypic expression of the genetically inherited coagulation disorder - hemophilia A.

2. Pathogenesis of hemophilia A can be correctly described by all of the following claims, except:

   a) Deficiency of coagulation factor VIII reduces the enzymatic activity of the factor X activating complex, in which factor VIIIa acts as a cofactor of the molecular enzyme complex.

   b) Women - carriers of the genetic disorder (patient’s mother and sister) are phenotypically healthy since the by half reduced “healthy” factor VIII protein is still sufficient for normal hemostasis and is clinically manifested as a recessive expression of the aberration.

   c) Hemophilia A is a sexually transmitted disease and so its’ clinical manifestation is determined by the concentration of androgen hormones in the plasma.

   d) Severe clinical features develop if the factor VIII activity drops below 1% of the normal activity which is manifested by spontaneous bleedings, 1-5% activity is manifested with moderate clinical features and 6-30% is manifested with mild clinical form of the coagulopathy.

   e) Prolonged, hard to stop bleeding after tooth extraction, circumcision or after a superficial mechanical trauma are the most common initial signs that point to the milder forms of the disease.

3. The molecular pathophysiological basis of the factor VIII dysfunction is described by following statements, except:

   a) Von Willebrand factor is a multimer protein, of molecular weight 1-20 million daltons, which stabilizes factor VIII within the plasma and enables complex to
bind to the platelets; and its dysfunction can cause clinical features resembling the factor VIII dysfunction itself.

b) Half-life of the factor VIII in plasma is 10-12 hours; however, the development of neutralizing antibodies caused by substitution therapy significantly reduces this time, thereby causing a therapeutic resistance.

c) There are four types of genetic disorder (genetic inversion, insertions of repeat sequences, complete or partial deletions and point mutations) which manifest by the identical damage to the intrinsic pathway of coagulation activation which laboratory manifests as the prolonged APTT (Table 1).

d) Dysfunction of factor IX that causes hemophilia B, on the molecular level, causes a dysfunction of factor VIII since the tenase complex VIIIa/IXa becomes dysfunctional and therefore the factor X activation becomes impaired.

e) Since this is an X chromosome-linked genetic disorder, phenotypic expression is dominant and expressed only in male.

4. All of the following pathogenetic mechanisms are responsible for the patient’s condition, except:

   a) Mechanical trauma to the tissue during the soccer game damaged the endothelium of blood vessels, which led to bleeding since the platelet response was unable to correct the damage by itself.

   b) The bleeding resulted from a dysfunctional internal mechanism of coagulation system activation, due to insufficient function of factor VIII (Table 1).

   c) Activity of factor VIII measuring 0.12 (12%), physiological activity of factor IX and normal concentration of von Willebrand factor are laboratory indicators that point to the diagnosis of hemophilia A (compare Table 1).

   d) Karyotype analysis of the patient’s genome could show a shortened chromosome X along with a normal appearance of all other chromosomes.

   e) A bleeding tendency develops due to a functional insufficiency of the tenase complex leading to the omitted proteolytic cleavage of the coagulation factor X and subsequently insufficient fibrin and clot formation.

5. Disorder of a cascade chain reaction of coagulation factor activation in hemophilia A includes all of the following changes except for one:

   a) There is a reduced binding of factor VIII and IXa which reduces intrinsic pathway of coagulation.

   b) In the laboratory tests, the activation of factor XII by the exogenous particles of kaolin does not lead to a sufficient activation of prothrombin which is manifested as a prolonged APTT (see Table 1).

   c) In hemophilia, the amplification effect of the cascade reaction on the factor X activation is absent, which causes a reduced fibrin synthesis from fibrinogen.

   d) Molecular transformations of factor VIII in hemophilia include a reduction of the molecular weight, changed binding site or a reduced plasmatic concentration.
e) Von Willebrand factor and factor XII in the patient are within the physiological range, since they are not the substrates of the enzyme complex composed of factor VIII, and therefore their lysis does not occur.

**Exercise B: Algorhythmic workout of the pathogenesis**

Arrange the following terms in causative order:

1. Prolonged APTT (See Table 1)
2. Brothers with a normal coagulation status;
3. Partial deletion of coagulation factor VIII gene;
4. Hemarthros;
5. Sport injury to the knee and lower leg;
6. Activity of factor VIII 0.12 (12%);
7. Mother, a carrier of the defective X chromosome, produces X* and X gametes;
8. Reduced activity of factor X at the surface of platelets;
9. Reduced cleavage of peptide bonds between Arg-Ile on factor X;
10. Reduced activity of a molecular complex VIIIa/IXa;
11. Deficient thrombin substrate;
12. Formation of a X*Y zygote;
13. Sister, a carrier of the disease;
14. Cessation of bleeding;
15. Sister inherited mother’s X chromosome which is identical to the one in the patient;
16. Prolonged bleeding after a tooth extraction;
17. Bleeding into the joints, muscles and hematomas;
18. Hepatocytes produce defective factor VIII;
19. The history record of grandfather’s bleeding after being wounded;
20. Swelling of the knee and reduced mobility of the joint;
21. Mother’s sister is the carrier of the disease;
22. Therapy by immobilization and analgesics;

Note: (x* stands for X chromosome with a deletion in location of the factor VIII gene)

**Exercise C: Feedback integration of the problem TYPE V**

1. Treatment of hemophilia A using derivatives of plasma (fresh frozen plasma, concentrated factor VIII, cryoprecipitate), carries a risk of contracting a hepatitis or immunodeficiency (HIV-1) virus which is a consequence of using large number of donors (1000-5000) because during therapy, approximately 5-15% of the patients develop neutralizing antibodies that make the therapy more difficult (claim 3b).
2. Severity of the clinical features directly correlates to the activity of factor VIII (claim 2d); and clinically the coagulopathy is manifested when the activity falls below 30% (<0.30) of the physiological values because in women carriers of the disease, the activity of factor VIII is approximately 50% (see introduction) which is sufficient for physiological hemostasis.

3. Reduced activity of factor VIII can be increased by administering synthetic analogue of vasopressin (e.g. 1-desamino-8-D-arginine vasopressin, DDAVP) which increases a release of von Willebrand factor from the endothelial cells and thereby contributes to the stability and adhesiveness of factor VIII to the thrombocytes (claim 3a) because inhibitors of fibrinolysis like ε-aminocaproic acid inhibit the clot lysis by inhibiting the binding of plasmin to fibrin which can be used in hemophilia treatment, particularly in the prevention, when preparing the patient for a surgical procedure.

Additional questions:

4. Factor VIII gene is composed of 186 kb, forms 26 exons and the gene product, the protein, has a relative molecular weight of 330 kD. What are the reasons for a slow advancement towards biotechnologic production of synthetic factor VIII e.g. gene therapy?

5. What genetic laboratory test can be used to analyze the samples of chorionic villi to determine prenatally which child inherited X chromosome from the mother who is a carrier of the disease?