Case study:

Mistake Diagnosis of Thrombocytopenia

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Abstract:

Background: Thrombocytopenia is a condition in which platelet (PLT) count becomes less than normal range in some abnormal cases. The normal range of thrombocytopenia varies from 150.000 to 450.000 cell/cm^3. There are some cases that lead to pseudothrombocytopenia. The most common causes of Pseudothrombocytopenia involving; instrument errors, hemodilutiona, heparin-inducedthrombocytopenia, immune-mediated destruction of platelets and using Ethylenediaminetetraacetic acid (EDTA) as an anticoagulant. Pseudothrombocytopenia is phenomenon that platelet count turnto below the lower limit (150.000 cell/cm^3) in vitro. This phenomenon typically caused by using anticoagulant that named EDTA which leads to platelet aggregation. Generally, pseudothrombocytopenia has no sign and symptoms or clinical significance, but false low platelet count may leads to unnecessary extra diagnostic tests and treatment. The false result of low platelet count leads to treating by platelet transfusion; this could be harmful in patients with increased intravascular platelet activation. Recently, this phenomenon named as EDTA-dependent pseudothrombocytopenia. Four woman visited the laboratory for routine blood investigations, sever thrombocytopenia appeared. In this report, two of these cases will be discussed including how can differentiate between real thrombocytopenia and pseudothrombocytopenia.

Objective: To avoid laboratory mistakes and how solve this problem in the laboratories and what must do to write a true report.

Results: The results of complete blood count (CBC) showed very severe thrombocytopenia and during collecting information's from all of them, no sign and symptoms or family history was detected. After replacing EDTA with sodium citrate as an anticoagulant it finally proved that the patient has pseudo thrombocytopenia.

Conclusion: It is very important to differentiate the real low platelet count with pseudothrombocytopenia. Thus, re-test of CBC with other anticoagulant (Sodium citrate) and blood smear also must be performed.

Keywords: Pseudothrombocytopenia, EDTA-dependent pseudothrombocytopenia, Platelet aggregation.

Introduction:

Thrombocytopenia is a common hematological disorder characterized by abnormally low number of platelets in circulating blood from multiple causes [(1)]. There are different causes (Pseudothrombocytopenia; hemodilution; increased consumption; decreased production; increased sequestration; immune-mediated destruction of platelets) either alone or in combination make thrombocytopenia. Using EDTA anticoagulant [(2, 3)] and heparin [(4, 5)] in routine hematological investigations is one of the causes of

pseudothrombocytopenia. There are two types of heparin induce two types of thrombocytopenia, type 1 pseudothrombocytopenia and type 2 pseudothrombocytopenia. The first type is callednon-immune disorder; in which presents within the first 2 days after exposure to heparin and the patient' status return to normal condition naturally. While, the second type is an immune mediated disorder which happens 4 to 10 days after heparin exposure and it can be seen in about (0.3) to (5%) of individuals. The lattermakes a "Heparin-PF4" immune complex. Therefore, the body produced auto-antibodies against the Heparin-PF4 complex; this antibody binds to this complex and destroys the platelet [6].

Using anticoagulant EDTA, and calcium chelator are listed in safe and reliable anticoagulants for a routine complete blood count test since these types of anticoagulant can keep blood cell counting and sizing. Nonetheless, in some cases platelet clumping occurs leading to false low results [3, 7]. It has also been reported in disease-free individuals. Platelet clumping in the presence of EDTA leads to mistake in platelets count resulting from the presence of an autoantibody against glycoprotein IIb/ IIIa located on the surface of cell membrane of platelets. Moreover, wrong diagnosis of platelet count (thrombocytopenia) leads to list of unnecessary extra investigations in addition to unnecessary treatments such as bone marrow biopsy, surgery, spleenectomy, steroid therapy, and platelet transfusion. Thus, when platelet count is too low, pseudothrombocytopenia should be considered [3, 7, 8]. The probability of platelet clumping by using EDTAis about one in 1000 normal adults and is slightly more common in women [3].

Case 1

A 28-year-old pregnant female attended for routine complete blood picture as pregnant manipulation. Her platelet count was 57.000 cell/cm3. She had no sign and symptoms of bleeding tendency or hematologic personal problem or family history. The result of clotting and bleeding time were within normal range. The expiry date of EDTA tube was checked and no clot blood also found in the tube that used for the test. The blood redraw for more laboratory investigations such as; prothrombin time (PT), Partial thromboplastic time (PTT), International Normalized Ratio (INR) and serum fibrinogen and collected in sodium citrate tube, during this time CBC test repeated for being sure about the result while it was surprisingly different because the total count of platelet was 245.000 cell/cm3. The results of PT, PTT, INR and serum fibrinogen were normal. After the case considered as a rare situation, blood smear prepared to obtain more information. The blood smear shows huge number of platelet clumping using blood that collected in EDTA.K3 tube, while there was no platelet clumping seen in the smear that collected in sodium citrate tube. Thus, it was not necessary for the patient to transfer platelets and other unnecessary medical process avoided, figure (1, 2).

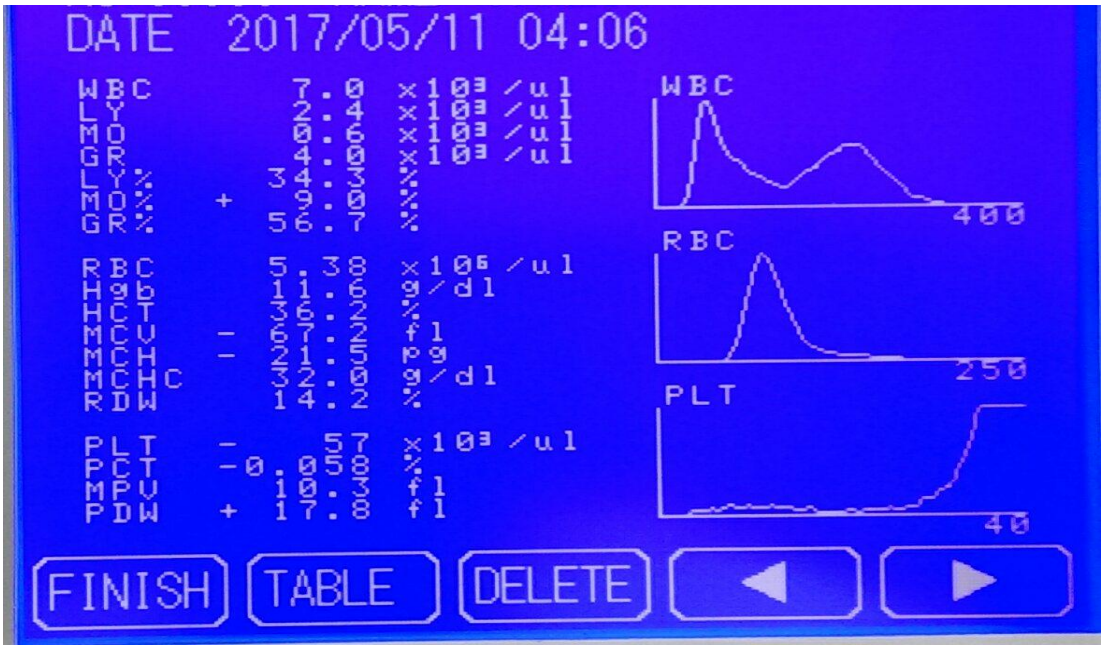

Figure (1): Shows the reading of blood counter using anticoagulant EDTA.

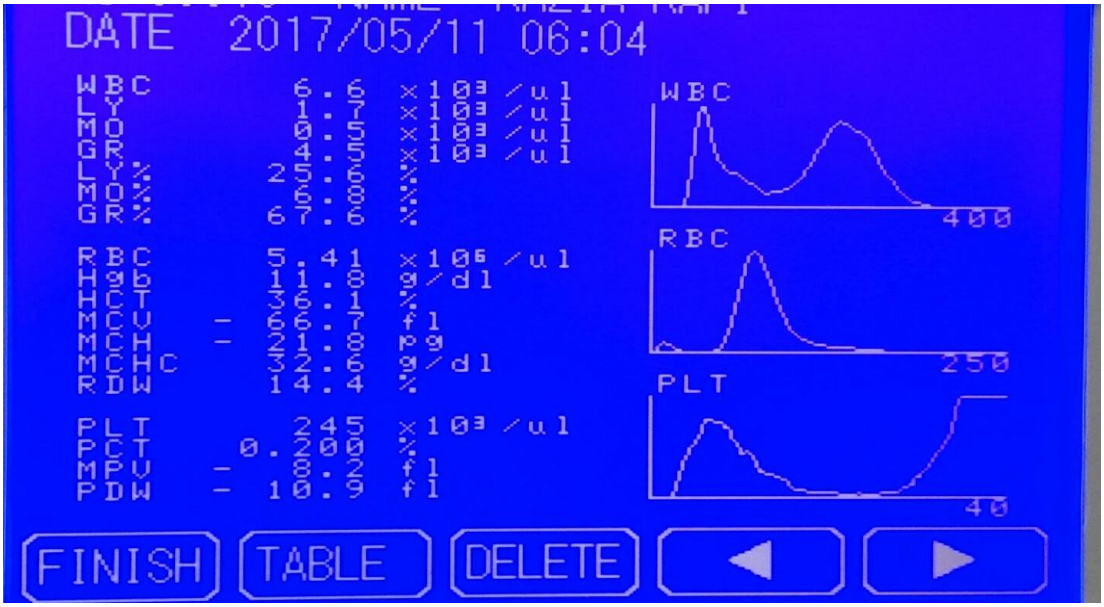

Figure (2): Shows the reading of same sample by using same blood counter using anticoagulant sodium citrate.

Case 2

Another female also attended for routine complete blood picture she was 33 year-old. Her platelet count was 33.000 cell/ cm3 without any sign and symptoms of bleeding tendency or hematologic personal problem or any family history. The result of clotting and bleeding time were normal. Another blood sample was draw for more laboratory investigations such as; PT, PTT, INR and serum fibrinogen and collected in sodium citrate tube, and CBC test repeated. The result of total count of platelet was 179.000 cell/ cm3 and the results of PT, PTT, INR, serum fibrinogen were normal and the blood smear shows huge number of platelet clumping using blood that collected in EDTA.K3 tube. Compare with blood smear of the sample that taken with sodium citrate tube, there are no platelet clumping was detected. Thus, it was not necessary for the patient to transfer platelets and other unnecessary medical process avoided, figure (3, 4).

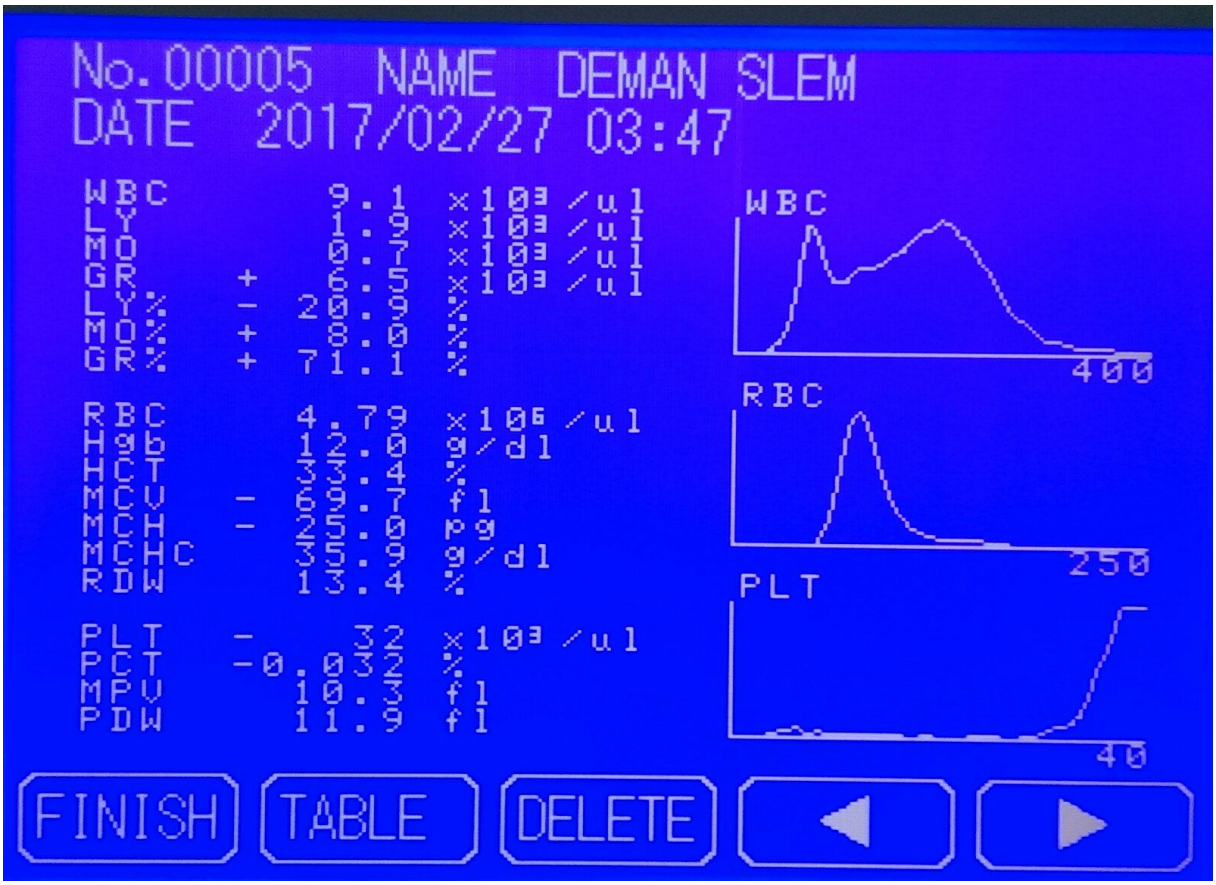

Figure (3): Shows the reading of blood counter using anticoagulant EDTA.

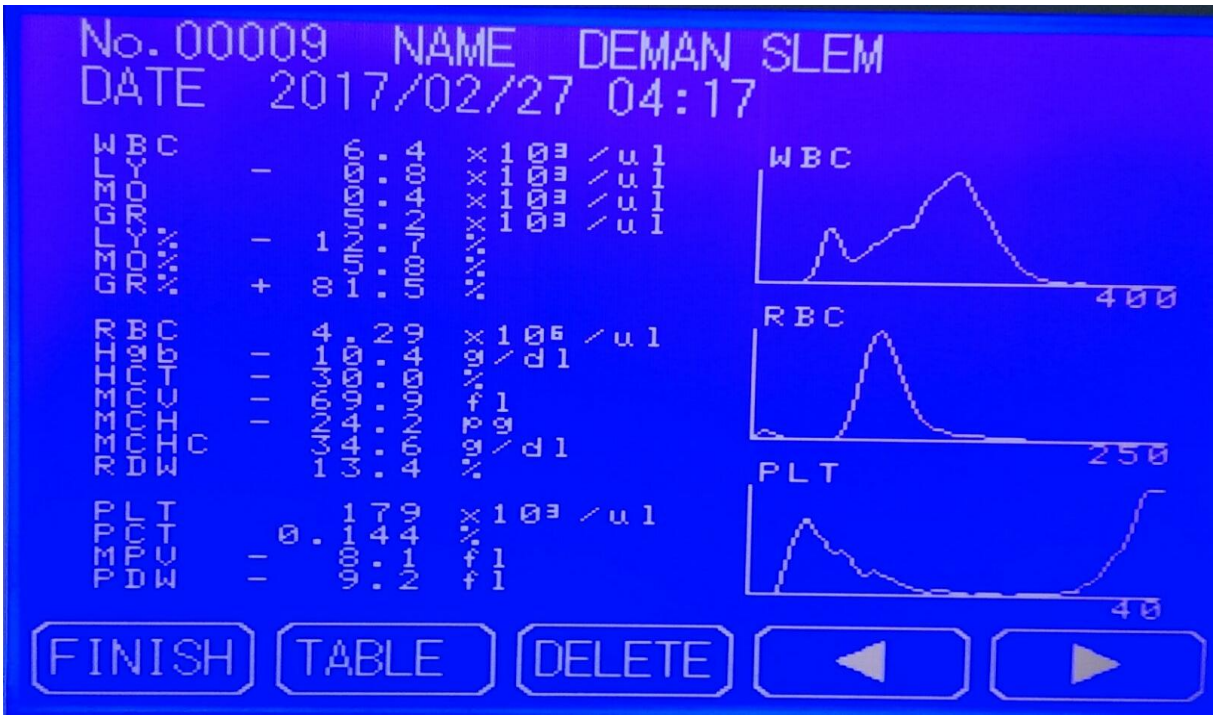

Figure (4): Shows the reading of same sample by using same blood counter using anticoagulant sodium citrate.

Case 3 and 4

After these two cases and during about 45 days other two cases have been attended to the laboratory. Both of cases were have same problem. The slightly differences have been seen in the age, number of platelets, white blood cells and other blood parameters.

Discussion:

Platelets play a vital role in preserving vessel wall integrity. Decreased platelet number count below normal range may possibly leads to defect of primary hemostasis and bleeding [9]. There are many reasons result to the destruction of platelets such as; hemolytic uremic syndrome, immune thrombocytopenia, and disseminated intravascular coagulation. In addition, there are other causes that belong to decreased production of platelets in bone marrow including; leukemia, sepsis, human immunodeficiency virus, and decreased production of thrombopoietin, hemodilution, or the use of certain drugs such as; valproic acid, methotrexate, pantoprazole, and heparin. While using EDTA as anticoagulant is one of the causes of Pseudothrombocytopenia, thus using other anticoagulant must be taken into account [7].

About (0.1%) of pseudothrombocytopenia has been reported in a normal population [5, 6]. Pseudothrombocytopenia can also be seen in following viral infection including; hepatitis, cytomegalovirus, influenza. Besides, Various diseases like sepsis, multiple myeloma, acute myocardial infarction, breastcancer, and neuroendocrine carcinoma has been reported to associate with EDTA-dependent pseudothrombocytopenia [6]. The immune system can distinguish between body cells and foreign bodies. Any changes in the shape, composition and structures make immune system attacks these cells. Low temperature and calcium ions in the presence of EDTA lead to the alteration of the surface shape of platelets which composed from glycoprotein complex IIb/ IIIa and will appear as a foreign body. Thus, the hidden Ag theory leads to activation of autoantibodies and aggregation of platelets [3, 7]. When the shape of thrombocytes alternate immunoglobulins such as IgG, IgM and Ig Aattack them and resulting in accumulation of platelets. Typically, the agglutination of platelets that occur in the presence of EDTA at room temperature or at 37^{c} [6, 7].

This means that pseudothrombocytopenia may indicate the presence of other medical problems such as bone marrow biopsy, surgery, spleenectomy, steroid therapy, and platelet transfusion. Thus, other investigations to find any related causes seriously must be performed. To avoid the wrong result of platelet count and in the case of severe thrombocytopenia, meanwhile, in order to obtain the actual count of platelets, peripheral blood smear must be done for all samples with very low values of thrombocytes. In the case of finding agglutinated platelets, the following measures should be taken in order to obtain correct interpretation of laboratory results: To warm blood sample at 37° C and re-test, in order to test the blood sample on the another anticoagulant. Accumulation of platelets in the presence of EDTA as anticoagulant is due to adhesion of platelets to the leukocytes (platelet satellitism) which is a phenomenon of unknown etiology of aggregating platelets around polymorphonuclear

neutrophils and other blood cells which causes pseudothrombocytopenia [3]. In case 1; when EDTA uses for CBC test, number of leukocytes elevated while sever thrombocytopenia observed. Therefore, my explanation is that because the platelets attach leukocytes, especially polymorphonuclear leukocytes and it leads to increase the number of leukocytes. This is because blood counter instruments depend on the size and shape of cells to measure blood cells and the accumulations of platelets leads to the false leukocyte account.

Conclusion:

The current case study concluded that thrombocytopenia has several causes and it is very important to differentiate the real low platelet count with pseudothrombocytopenia. Thus, in the case of severe thrombocytopenia, re-test of blood cell count with other anticoagulant (Sodium citrate) and blood smear must be performed to avoid unnecessary blood investigations or platelet transfers. The underlying cause of the case must be taken into account.

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