Pre-operative pseudothrombocytopenia: terrifying but innocuous

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Summary

An isolated thrombocytopenia was found in a 47-year old man during pre-operative work-up for his closed radial bone fracture on left forearm after a fall. His platelet count was as low as $14 \times 10^3/\mu L$, but there was no active bleeding and past history of bleeding disorder. The clue to true diagnosis started from careful blood film examination - platelet clumps in blood film. Repeat full blood count tests were requested not only with the usual anticoagulant EDTA (Ethylene diamine tetra-acetic acid) but also with heparin as well as with citrate. EDTA-dependent pseudothrombocytopenia was diagnosed which can be confused with other life-threatening platelet disorders. The operation was successfully done without unusual bleeding. Careful blood film examination can avoid unnecessary worries about the patient and can also avoid unnecessary investigations and treatments.

Background

Thrombocytopenia accidentally seen on pre-operative work-up is not uncommon and usually lead to anxiety not only in attending doctors but also in patients. Ethylene diamine tetra-acetic acid (EDTA) - dependent pseudothrombocytopenia, although a rare phenomenon (0.1% in the general population), can lead to unnecessary worries, unnecessary investigations and unnecessary treatments.

Case presentation

A 47-year old gentleman got injury to left forearm due to a slip and fall, and was admitted to the Emergency Department of Yangon General Hospital in April 2016. Plain X-ray of left forearm antero-posteriorly and laterally revealed closed radial bone fracture on left forearm with dislocation of distal radio-ulnar joint (Galeazzi fracture). A long posterior slab of POP (Plaster of Paris) was applied to the injured forearm and the patient was admitted two weeks later to the Department of Orthopaedics, Yangon General Hospital for open reduction and internal fixation.

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He was then referred to the Department of Clinical Haematology, Yangon General Hospital for thrombocytopenia (Platelet count 14 x 10^3/μL) which was accidentally found during pre-operative assessment. He did not have constitutional symptoms like weight loss, bone pain and night sweat. He showed no active cutaneous and mucosal bleeding at the time of referral. There were no stigmata of congenital conditions. Organomegaly and lymphadenopathy were not found in this patient. He never had past abnormal bleeding and was not taking any regular medication that can lead to thrombocytopenia or bone marrow suppression. Family history of bleeding disorders was not detected.

Investigations

On reviewing the tests already done at the time of referral, his haemoglobin (Hb) was 13.9 g/dl with normal red cell parameters. White blood cell count was 5.41x10^3/μL with normal differential counts. But the platelet count only was as low as 14x10^3/μL. HBs antigen, HCV antibody, HIV antibody and Helicobacter pylori antibody were all negative. Antinuclear factor was also negative. Liver function, renal function and urinalysis were normal. Bone marrow aspiration and trephine biopsy both showed cellular marrow with active haematopoiesis. Platelet clumps were seen in the background of bone marrow aspirate.

Differential diagnosis

Isolated thrombocytopenia may be due to an underlying serious cause but before proceeding further workup, one should always confirm that whether it is a true thrombocytopenia or pseudothrombocytopenia.

Treatment

Reviewing the patient's blood film confirmed the presence of platelet clumps (Figure 1). Recheck full blood count was requested not only with the usual anticoagulant EDTA (Ethylene diamine tetra-acetic acid) but also with heparin as well as with citrate.

Outcome and follow up

Using the automated blood counter, the patient's platelet count on EDTA was still 49 x 10^3/μL but that on heparin and citrate were 108 x 10^3/μL and 142 x 10^3/μL respectively. Re-examination blood film of blood sample in heparin tube (Figure 2) and citrate tube did not show much platelet clumps.
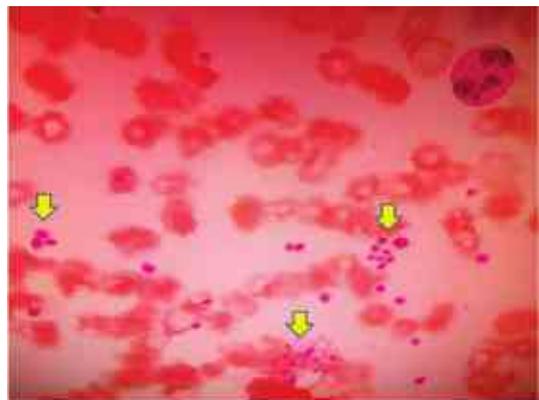

Figure (1) Peripheral blood film showing platelet clumps (EDTA sample)

The patient was referred back to the Orthopaedic Hospital but he went to a different one. Again on pre-anaesthesia work up, he was postponed for operation due to thrombocytopenia. Orthopaedic surgeon and anaesthetist requested haematologist's opinion again for operation safety and fitness. So full blood counts were repeated again. This time, the patient's platelet count on EDTA was 60 x 10³/μL but that on heparin and citrate were 142 x 10³/μL and 145 x 10³/μL respectively. The

Figure (2) Peripheral blood film showing no platelet clumps (Heparin sample)

operation was agreed then by the haematologist and it was a successful operation without unusual bleeding.

Discussion

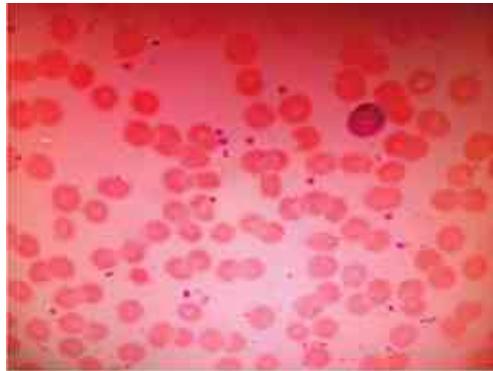Pseudothrombocytopenia is an in vitro phenomenon of platelet aggregation that results in spurious reporting of a low platelet count by automatic cell counters, which are typically EDTA dependent.[1]

EDTA is a most widely used anticoagulant in most of the laboratories. EDTA-dependent pseudothrombocytopenia can be seen rarely in about 0.1% in the general population. The presence of EDTA-dependent antiplatelet antibodies is the underlying cause of this rare phenomenon.[2]

These autoantibodies can recognize the cytoadhesive receptors gp IIb - IIIa located on the cell membrane of platelets, and can also stimulate the expression of activation antigens, and can trigger activation of tyrosine kinase, platelet agglutination and clumping in vitro. These processes finally lead to a spuriously low platelet count with the automated cell counters.[2]

It is suggested that five basic criteria should be fulfilled to raise the clinical suspicion of EDTA-dependent pseudothrombocytopenia, i.e., **(i)** abnormal platelet count, typically < 100 × 10³/μL; **(ii)** occurrence of thrombocytopenia in EDTA-anticoagulated samples at room temperature, but to a much lesser extent in samples collected with other anti-coagulants and/or kept warmed at ~37°C; **(iii)** time-dependent fall of platelet count in the EDTA specimen; **(iv)** evidence of platelet aggregates and clumps in EDTA-anticoagulated samples with either automated cell counting or microscopic analysis; **(v)** lack of signs or symptoms of platelet disorders.[2]

EDTA-dependent pseudothrombocytopenia can be more common in people with malignancy, chronic liver disease, infection, pregnancy, autoimmune diseases, and cardiovascular diseases, although it has also been observed in disease-free patients.[3,4]

Misdiagnosis of EDTA-dependent pseudothrombocytopenia as true thrombocytopenia can lead to unnecessary diagnostic tests and treatments, such as bone marrow biopsy, with holding surgery, splenectomy, steroid therapy, and platelet transfusion.[5]

In above patient, the first clue to the correct diagnosis of pseudothrombocytopenia was the blood film report - platelet clumps. Initial misdiagnosis as life-threatening thrombocytopenia and need to operate in near future led to unnecessary investigations like bone marrow examination. To avoid such a mistake in the future, whenever one comes across with a patient with thrombocytopenia, the peripheral blood film report must be looked for or requested. If necessary, opinion from an experienced pathologist or from an experienced haematologist may be very useful to prevent anxieties in patient and worries in attending doctors.

Learning Points

Severe thrombocytopenia on pre-operative work-ups is always alarming to surgeons as well as patients. This case report emphasizes the importance of differentiating pseudothrombocytopenia from true thrombocytopenia. When pseudothrombocytopenia is suspected, re-examination of blood samples with sample tubes containing other anti-coagulants and a peripheral blood film examination by a pathologist or a haematologist should be done.

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Russell's viper Envenomation - A Hard Clinical Course

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Summary

A 21 year old pregnant woman in the 35th week of gestation was admitted to Insein General Hospital complaining of unidentified snake bite to the left toe. Due to manipulation that was done at the site of the bite, local signs of envenomation and fang marks could not be identified. The patient manifested with signs and symptoms of systemic envenomation that is consistent with Russell's viper venom toxicity following six hours after the bite. She was treated with a total of 240 ml of anti-Russell's viper venom antibody that was administered in 4 divided doses. However, the patient developed anaphylactic shock on 2 occasions immediately after anti-venom was administered but the attending physicians were able to successfully resuscitate the patient. The baby was delivered through vagina but expired due to birth asphyxia. There were no postpartum complications and acute renal failure did not develop. Team-work, anticipated management of anaphylactic shock, bleeding, sepsis and renal failure saved the patient's life. Close monitoring of the patient is essential for identifying problems related to delayed envenomation. The likelihood of anaphylactic reaction occurring after administering anti-venom should not be a contraindication for using of anti-venom to neutralize the snake venom.

Case presentation

A 21 year old pregnant woman living in Insein Township was admitted to the medical ward of Insein General Hospital complaining of being bitten by an unidentified snake at 7:30 pm. Prior to being bitten by the snake her physical condition was normal and had no health problem. Around 6 pm on the day of bite, while she was walking in the compound of West Yangon University (where her husband worked), she stepped on a snake which bit her. The site of snake bite was her left big toe. She felt a burning sensation at the site of bite. The snake escaped and as a result identification of the type of snake was not possible. A tight tourniquet was applied to the leg and multiple needle punctures were made around the site of bite with the intension to drain the venom. She was concerned that the unidentified snake could be Russell's viper as the area is well known for endemicity of Russell's viper .

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On examination, she was pregnant (G_1P_0) at 36 weeks of gestation. She was fully conscious (GCS 15/15) with no neurological deficits. There was no deterioration of vital signs. The left toe and foot were not swollen. The fang marks could not be identified due to prior manipulation. However, there were bruises at the site of the bite. The inguinal lymph nodes were not enlarged. The whole blood clotting time was within 20 minutes. Urine dipstick test was clear for albumin. Peak flow rate for PEFR (Peak Expiratory Flow Rate) was 300 L/min. Monitoring of signs of envenomation was done hourly.

At around the time of mid-night, swelling developed at the site of the bite that slowly spread up to the ankle. No bleeding was noted at the site of the bite. She complained of pain at the site of bite and groin. The inguinal lymph nodes were found to be enlarged and tender. She felt loin pain and passed bloody urine. However, there was no deterioration of vital signs. The whole blood clotting time now became non-clotted within 20 minutes. Urine albumin was also found to be positive (++). At this stage she was given 80 ml of anti-Russell viper venom intravenously. This anti-venom was produced locally by the Myanmar Pharmaceutical Factory (MPF). Half hour after administering the anti-venom she became dizzy with profuse sweating, her pulse became weak and blood pressure dropped to 80/50 mmHg. She was resuscitated with adrenaline, hydrocortisone, chlorpheniramine and crystalloid solution. She was successfully resuscitated from the anaphylactic shock.

Six hours after resuscitation, there was no further deterioration of vital signs but she still was passing bloody urine. She also developed bleeding gums and loin pain. There were bruises occurring at puncture sites. The blood did not clot 6 hours after the first dose of anti-snake venom. Urine output was 400 ml for last 6 hours. The urine albumin was positive (++). She was then infused again with another bolus of 80 ml of anti-Russell viper venom (MPF). During this second time of infusion, she again became dizzy and breathless with profuse sweating, pulse became weak and blood pressure dropped to 70/40 mmHg. In addition she developed cardiac arrest. She was resuscitated again with advanced cardiopulmonary resuscitation, adrenaline, hydrocortisone, chlorpheniramine and crystalloid. She was also given parenteral antibiotics and inotropes (noradrenaline). She was again successfully resuscitated for the second time. However, the patient remained breathless. The blood pressure was found to be unstable with systolic pressure varying from 70 to 100 mmHg. The pulse rate varied from 110 to 160/min. The urine output reduced to 200 ml for the past 6 hours. The patient also complained that the fetal kick count has reduced and the fetal heart sound was barely audible and for these reasons an obstetric opinion was obtained. The results of investigations that had been done in day one were as follows:

Hb - 11.1 g/dL

WBC - 13.3 x 10^9/L

Plt - 83 x 10^9/L

Urea - 4.4 mmol/L

Creatinine - 82.4 µmol/L

INR - 1.56

APTT - 34 sec

Six hours after the second dose of anti-snake venom was administered bleeding (hematuria and gum bleeding) persisted. The vital signs again became unstable. Blood was still non-clotting. Urine albumin was positive (+++) and it has increased. It was then decided to administer a third dose of 40 ml of anti-Russell viper venom (MPF). At the time of the third dose of anti-serum administering, there was little signs and symptoms of anaphylactic reaction to anti-venom but had rigor. Obstetric assessment revealed that there was fetal distress and that onset of labour (first stage) has occurred. Due to the risk of bleeding, 2 units of fresh frozen plasma were infused. It was then decided to try for vaginal delivery as the operative risk for uncontrolled bleeding during surgery was high.

Six hours after providing the third dose of anti-snake venom, bleeding stopped. The patient still had loin pain. Urine output was 400 ml for the past 6 hours. The whole blood clotting time for 20 minutes was found to be non-clot. Urine was still positive for albumin (++). It was then decided to administer a fourth dose of 40 ml of anti-Russell viper venom (MPF) and at that time no reaction to anti-venom was noted. The patient was in labour after induction and vaginal delivery was carried out. A live female baby was delivered. As the baby was deeply asphyxiated, she was resuscitated and treated in the special baby care unit. However, the baby expired due to birth asphyxia. The mother was provided with the standard postpartum hemorrhage treatment regimen using oxytocin, tranexamic acid, prostaglandins and carbagoline. No significant blood loss occurred during the postpartum period.

Six hours after the fourth dose of anti-snake venom was administered, no more bleeding was seen. Urine output was 600 ml for the past 6 hours. There was no deterioration of vital signs. The whole blood clotting time for 20 minutes became clotted. Urine albumin reduced to one plus (+). The results of laboratory investigations at 2 days post-admission are as follows:

Hb - 9.8 g/dL
WBC - 32 x 10^9/L
Plt - 33 x 10^9/L
Urea - 4.5 mmol/L
Creatinine - 86.5 µmol/L

After giving a total of 240 ml of anti-Russell viper venom, the features of envenomation disappeared. Swelling in the limb also subsided. The blood clotted on repeated clotting test. Urinary albumin gradually disappeared. The blood pressure remained stable throughout. Urine output ranges from 1200 mL to 2600 mL / 24 hours on subsequent days. The serum creatinine was not elevated above 100 µmol/L. The patient was discharged on the sixth day of admission. At discharge, platelet count was 44 x 10^9/L and creatinine was 60 µmol/L. At the time of follow up visits the patient was observed to be having an uneventful recovery.
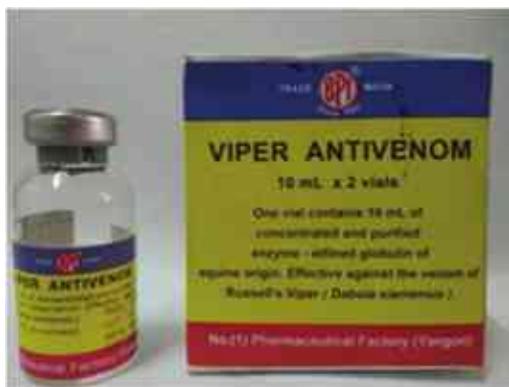
Fig 1. Anti-Russell's viper venom MPF

Discussion

Many lessons have been learnt from this snake bite case. Unidentified snake bite should never be ignored. In addition, delayed and stormy envenomation can occur later even though initially the patient presented with a dry bite. Close monitoring of the patient up to 24 hours is essential and in addition the medical team was alerted because of the patient's concern that the bite would be venomous. Unfortunately, public misconception regarding first aid measures to be carried out for snake bite victims was still very prevalent in the community. The tourniquet was tied very tightly. Thanks to the short tourniquet time (< 30 min), no serious complication occurred. The patient was very anxious about something happening to her during transportation to hospital. So she made multiple needle punctures and manipulated the site of bite as advised by her neighbors. This manipulation made the identification of fang marks difficult. In addition the manipulation might have contributed to the spread of snake venom in large amounts into the circulation. Therefore, she had a high probability of developing severe envenomation. Public awareness with regards to the "*do and don't in snake bite*" is rather low and more information is needed to increase awareness about first aid measure for snake bites.

In cases where the type of snake cannot be identified, providing appropriate treatment becomes a great challenge to clinicians. Monitoring the patient closely is a very useful approach for treatment. In this case, there was a delay in the signs and symptoms of envenomation which manifested only after 6 hours after the bite. At present, there is no recommendation for administering anti-snake venom for a dry bite, and also for the use of polyvalent anti-venom in unidentified snake bite cases (no local polyvalent anti-snake venom).

Russell's viper bite if left untreated has a high fatality rate. A quarter to one third of Russell's viper bite is reported to be a dry bite. The most common snake envenomation in Myanmar was reported to be due to Russell's viper bite. In Myanmar, the mortality rate of snake bite was reported as 3.3 cases per 100,000 population[1,2].

Russell's viper bite can cause local swelling, tenderness, bleeding manifestations, hypotension, shock, acute renal failure and disseminated intravascular coagulation. A very simple test of 20-minute whole blood clotting time using a dry glass bottle is a very helpful test to detect systemic envenomation of Russell's viper[1]. In this case, the patient developed all forms of severe manifestations of systemic envenomation. As such, the administration of anti-Russell viper venom was the only treatment of choice.

The venom composition of snake varies from species to species and also from region to region in the world. Anti-venom, although intended for Russell's viper, can also have different neutralizing capacity depending upon where it is manufactured. In this case a locally produced anti-snake venom (Anti-Russell's viper Venom, MPF) was used. As most of the vipers in Myanmar are from the eastern type of Russell's viper (*Daboia siamensis*), the anti-venom produced in India is not effective. Vipers in India are from the western type of Russell's viper (*Daboia russelli*)[2]. Therefore, the anti-snake venom used in this case was found to be the most effective therapy. The anti-snake venom that is locally produced by MPF is of equine and ovine origin, therefore, there is a high risk of anaphylactic shock and serum sickness[2]. The patient on 2 occasions developed almost fatal anaphylactic shock (both snake venom and anti-snake venom can cause anaphylaxis). But in this case, anticipation of anaphylaxis and prompt intervention with good team work saved the patient's life. The manufacturer, MPF did not mention about the incidence of anaphylaxis for their anti-snake venom in their brochures. The incidence of anaphylaxis due to anti-snake venom needs to be reduced further with more research and with the help of more advanced technology. This case has pointed out that anaphylactic shock is not a contraindication to administer anti-snake venom.

In this case, a very high dose (a total of 240 mL) of anti-snake venom was used to neutralize envenomation which is usually not the case. It could be that the snake may have injected an unusually large amount of venom or the local manipulation at the site of the bite could have resulted in promoting the spread of the venom systemically. As the patient was pregnant at the time of the snake bite and was near term, the pharmacokinetic properties of anti-snake venom could have changed as a result of a large volume of distribution (Vd) or increased protein binding capacity. The pharmacokinetic data for anti-snake venom in pregnant women is largely unknown. In addition, the effects of Russell's viper venom on the placenta and fetus are also largely unknown. In this case, fetal distress probably could have been due to sustained shock (2 bouts of anaphylactic shock) or placental bleeding and separation (as a result of generalized bleeding) or direct venom toxicity to the fetus. However, as a result of effective team-work the patient's life was saved and no postpartum hemorrhage occurred even in a state of disseminated intravascular coagulation.

Although the patient developed shock, the usual complication of Russell's viper bite that is observed during the follow-up period such as disseminated intravascular coagulation, systemic envenomation and acute renal failure did not occur. High doses of anti-snake venom, proper fluid management, control of disseminated intravascular coagulation and prompt treatment of sepsis all seems to have prevented the development of acute renal failure. Fortunately, the patient survived after 3 almost fatal episodes.

Learning points

Lessons to be learnt from this case are:

- The unidentified snake bite can be venomous.
- Close monitoring is an effective way to detect delayed envenomation.
- Anti-snake venom dose should be sufficient enough to prevent complications.
- Anaphylactic reaction to anti-snake venom is not a contraindication to provide anti-venom therapy.
- Anticipating anaphylactic reaction is important when administering anti-venom.
- Team-work is essential to ensure a good outcome.

Acknowledgement

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