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EDTA dependent pseudothrombocytopenia - cause and incidence

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Abstract

Aims and Objectives:

1. To find out the incidence of EDTA dependent pseudothrombocytopenia(PTCP)
2. To confirm EDTA dependent pseudothrombocytopaenia(PTCP) by counting the platelets using anticoagulants other than EDTA (citrate and heparin)

Introduction: Pseudothrombocytopenia (PTCP) meaning there by a spuriously low platelet count.
EDTA induced clumping of platelets is one of the important cause of pseudothrombocytopaenia.

Materials and Method

Inclusion criteria: All 3000 blood samples received during study period in hematology laboratory of BVDU Medical college sangli for complete blood count (CBC) or platelet count

Exclusion criteria:

1. Patients with known cause of thrombocytopenia were excluded.
2. Thrombocytopenia due to technical errors.

Result: The incidence of EDTA Dependent Pseudothrombocytopenia was 0.13%.

Conclusion: The incidence of EDTA dependent Pseudothrombocytopenia is quite low.
Every sample with a low platelet count with EDTA as anticoagulant and/or presence of platelet clumps on the peripheral blood smear, and after ruling out other causes of pseudothrombocytopenia, must be collected again in a vaccutainer containing different anticoagulant (heparin and citrate). If the platelet count rises therein, it should be considered as a case of EDTA dependent Pseudothrombocytopenia.

Keywords: Platelet count, Pseudothrombocytopenia, EDTA dependent Pseudothrombocytopenia

Introduction

Pseudothrombocytopaenia (PTCP) is the condition that reveals low platelet count on blood reports but the patient does not present with any manifestations of thrombocytopaenia. EDTA induced clumping of platelets is one of the important causes of pseudothrombocytopaenia. This will create fear and anxiety among patients and doctor, needless evaluation, cancellation of surgical procedure, unwarrented splenectomy, unnecessary transfusions and medicolegal problems for pathologist.

Materials and Methods

Materials

1. EDTA vacutainers (EDTA-K2)
2. Heparin vacutainers (Sodium Heparin 75 USP Units)
3. Citrate vacutainers (B.Cit, 9NC, 3.2%)
4. Automated cell counter (NIHON KOHDEN Celtacalfa Span; Model no.- MEK 6420P)
5. Blood collection kit (6ml disposable syringe and tourniquet)

Methods

The blood samples required were collected from the patients in the Central Clinical Laboratory for outpatient departments and wards of different departments in Bharati Vidyapeeth Hospital, Sangli.

Inclusion criteria: All blood samples received in haematology laboratory for complete blood count (CBC) or platelet count, until the total number has reached 3000.

Exclusion criteria: Thrombocytopenia due to technical errors. Patients with known cause of thrombocytopenia.

Observation

Out of 3000 samples 206 showed low platelet count (<1,50,000/cmm)

Repeat sample collection of all these 206 patients was done to rule out common technical errors.

Out of 206 repeat samples, fifteen repeat samples showed improvement in platelet count.

So these fifteen patients were excluded from study.

Remaining 191 patients were approached for clinical details. Of these 166 were found to have a known cause of thrombocytopenia and were excluded from study. PBS examination of blood collected in EDTA of all 25 samples was done for presence of platelet clumps. These 25 patients were re-sampled with citrate and heparin as anticoagulants. These samples were run on the same cell counter. Results of EDTA, heparin and citrate vaccutainers were compared. Out of 25 samples 5 samples showed higher platelet counts with heparin and citrate. These 5 cases were diagnosed as EDTA dependent PTCP. Small platelet clumps were seen in PBS prepared from EDTA sample of these 5

patients. In remaining 20 patients no clumps were seen on PBS examination and platelet count did not rise on using heparin and citrate as anticoagulant. So these 20 patients were not considered as cases of EDTA dependent pseudothrombocytopenia and were advised to get investigated further so as to find out cause of thrombocytopenia.

Results

Incidence of EDTA dependent PTCP among the 3000 samples is **0.13%**.

In vitro clumping of platelets was the only cause of EDTA induced PTCP in our study.

Platelet satellitism and platelet phagocytosis was not observed in our study

Discussion

Gowland et al (1969) and others were first to demonstrate the presence of a serum factor which agglutinated the platelets in presence of EDTA.[1]

They proposed three possibilities.

1. EDTA brings about activation of the anti-platelet antibody.
2. EDTA destroys the inhibitor of the anti-platelet antibody.
3. The antigenic determinants of the platelets are modified by EDTA.

According to Pegels et al(1981) these antibody are immunoglobulins that could be IgG, IgM or IgA, binding to platelet strictly in presence EDTA in a concentration as low as 0.3mM.[2]

In 1994, a study was done by Casonato et al to clarify the mechanism involved in the development of EDTA dependent pseudothrombocytopaenia.[3]

It was concluded that EDTA dependent pseudothrombocytopaenia is caused by agglutinating antibodies that recognize cytoadhesive receptors on platelet glycoprotein IIb-IIIa (alpha 2b beta 3) in presence of EDTA and that an efficient platelet metabolism is required for this to occur.

According to Bartels et al (1997) these EDTA dependent antibodies are naturally ocurring antibodies which cross react with a concealed epitome on platelet membrane glycoprotein IIb/IIIa that become exposed by EDTA induced conformantional change due to chelation of calcium.[4]

According to Sakurai et al (1997) citrate or heparin when used as anticoagulant very rarely can induce platelet clumping and a spurious low platelet count. They also stated that addition of aminoglycosides to the sample prevents in vitro clumping of platelets in EDTA induced pseudothrombocytopaenia.

Incidence of EDTA Dependent pseudothrombocytopaenia in previous studies

Documented by	Incidence of EDTA dependent PTCP in other studied
Gracia Saurez(1991)	0.07 to 0.2%
Federico(1999)[6]	0.1%
N.J. Wilkes(2000)[8]	0.21%
Guiseppe Banfi (2008)[7]	0.2%
Sema Akinci(2014)[9]	0.13%
Mao Wy(2014)[10]	0.12%
Present study	0.13%

Our incidence of EDTA dependent pseudothrombocytopaenia matched with incidence of other studied.

Conclusion

PTCP must be ruled out before diagnosing thrombocytopenia.

Pathologists should be aware of EDTA induced PTCP

In absence of obvious cause of thrombocytopenia, the count should be repeated using anticoagulant other than EDTA.

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