A STUDY OF EDTA DEPENDENT PSEUDOTHROMBOCYTOPENIA

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Article Info: Received 25 March 2019; Accepted 21 April. 2019
Cite this article as: Meena, P., & Gupta, A. (2019). A STUDY OF EDTA DEPENDENT PSEUDOTHROMBOCYTOPENIA. International Journal of Medical and Biomedical Studies, 3(4).
DOI: https://doi.org/10.32553/ijmbs.v3i4.208
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Conflict of interest: No conflict of interest.

Abstract

Objective: To find out the frequency of Ethylenediaminetetraacetic acid (EDTA) Dependent Pseudothrombocytopenia (EDTA-PTCP) in all blood counts

Material and Methods: 130 cases of suspected EDTA-PTCP were assessed for its correctness using manual platelet count as the gold standard, and compared the platelet counts in two different anticoagulants (EDTA and CPT) at different times from the time of collection of blood sample. The collected data was analysed and statistics were made accordingly.

Result: Total 151750 haemograms were done during the study period of which 2428 cases showed thrombocytopenia, accounting for 1.6%. Amongst these, the pseudothrombocytopenia was suspected in 130 cases and was found to be correct in 119 cases, thus accounting for the incidence of EDTA-PTCP in 0.07% cases of total haemograms and 4.9% cases of total thrombocytopenia. In 11 cases, the suspicion of Pseudothrombocytopenia was found to be incorrect. Of the total 130 cases included in the study, with M: F ratio of 1:1.3. The age of the patients varied from 3-85 years with the mean age of 36.78 ± 20.33 years

Conclusion: In all the cases of thrombocytopenia showed by automated blood cell counters, the examination of peripheral blood film is must for the presence of platelet clumps to detect the cases of EDTA- dependent Pseudothrombocytopenia. To get correct platelet count in these cases, the manual platelet count is the ‘gold standard’. CPT is the better alternative anticoagulant for EDTA in cases of EDTA–PTCP for automated platelet counts.

Keywords: EDTA-PTCP, haemogram, CPT, exvivo agglutination

Introduction

Spurious thrombocytopenia, also called Pseudothrombocytopenia (PTCP), is a relatively uncommon phenomenon caused by ex vivo agglutination of platelets (Shreiner and Bell, 1973; Lichtman et al, 2007). As a result of platelet clumping, platelet counts reported by automated blood cell counters may be much lower than the actual count in the blood because these devices cannot differentiate platelet clumps from individual cells (Shreiner and Bell, 1973; Pegels et al, 1982; Lichtman et al, 2007).

Mant et al, in 1975 and Manthorpe et al, in 1981 first studied the frequency of the phenomenon and reported figures of 0.3% and 1.2% respectively, which referred however to a small case series. This type of alteration is now
familiar to the clinical pathologist; and consequently it has been evaluated more accurately, and at present its frequency is considered to be within range of 0.09 – 0.11%. EDTA- dependent Pseudo thrombocytopenia (EDTA-PTCP) occurs in 0.2% of asymptomatic individuals, but the incidence may be as great as 1.9% in hospitalized patients (Berkman, et al 1991).

The agglutination persisted without disaggregation for more than 24 hours (Onder et al, 1980). The size of the aggregates approximates to that of the lymphocytes; often giving rise to suspect flag “platelet clumping” and/or flagging of the platelet parameters (Lombarts et al, 1999).

The intensity of platelet clumping varied with the length of time that the blood sample was kept in tubes containing EDTA (Hsieh et al, 2003).

Lippi et al, (1990) used a new anticoagulant-antiaggregant mixture, containing trisodium citrate 17 mmol/l, pyridoxal 5'-phosphate 11.3 mmol/l and Tris 24.76 mmol/l (called as CPT mixture), which prevented EDTA induced platelet clumping and was found to be most suitable for automated complete blood count in routine hematological practice, as an alternative anticoagulant to K3-EDTA. In CPT-anticoagulated specimens the signals and instrumental flags of platelet clumping were absent, and the platelet number correlated very well with a microscopic count from a finger stick drawn into Unopette (Lippi et al, 1990). The manual platelet counts, performed at the microscope using counting chambers, still remains as a ‘gold standard’ for confirmation of platelet count (Hyun-Sook Chi, 2010, Wu Wei et al, 2011).

MATERIALS AND METHODS

In all cases included in the study, 2 ml of blood samples were collected in K3-EDTA and CPT vials separately.

The following tests were carried out with these samples:

1) Peripheral blood smears

The smears are examined with light microscope

2) Manual platelet counts using improved Neubauer’s chamber

3) The automated platelet count was obtained by doing complete blood count on automated 3 part differential haematology analyser (Beckman coulter make Ac T diff 2 model at 30 minutes and between 3-4 hours of blood collection.

Platelet counts obtained by manual method; by automated counter at 30 minutes and at 3-4 hours time using two different anticoagulants were compared. These platelet counts were also compared with the initial platelet counts on which pseudo thrombocytopenia was suspected.

Inclusion criteria:

the cases in which the automated counter report showed thrombocytopenia with platelet counts less than 130 x 10^9/litre and simultaneous peripheral blood film examination showed platelets in fair number, either diffusely distributed or in clumps or aggregates and appeared to be within normal limits; these cases were considered as Pseudo thrombocytopenia and were included in the study.

Exclusion criteria:

1) The cases showing marginal decrease in platelet counts between 130-150 x 10^9 /litre were excluded.

2) The cases with known cause for thrombocytopenia as obtained from history, clinical examination and medical records, were excluded.

RESULTS

The findings in the present study are summarized as under:

1) Total 103 cases were suspected to have EDTA – dependent Pseudo thrombocytopenia, out of which 94 cases were found to be correct for pseudo thrombocytopenia.

2) The incidence of EDTA-PTCP was found to be 0.07% of all the haemograms and 4.9% of total cases of thrombocytopenia.
3) Study included 43 males and 60 females with M: F ratio of 1:1.3.

4) The mean age of the patients were 36.78 years with range of 3 to 85 years.

5) EDTA-PTCP was also found in normal healthy individuals in 3.9% cases.

6) The mean initial platelet count in EDTA anticoagulated blood on suspicion of pseudothrombocytopenia was found to be 103.67 x 10^9/l.

7) The mean manual platelet count with fresh blood sample in same cases was 222.63 x 10^9/l.

8) The mean automated platelet count in EDTA and CPT anticoagulated blood were found to be 171.40 x 10^9/l and 226.63 x 10^9/l respectively at 0-30 minutes and 171.63 x 10^9/l and 230.25 x 10^9/l at 3-4 hours respectively.

9) In 74 cases, the mean platelet count in EDTA and CPT anticoagulated blood were compared at 24 hours after sample collection and were found to be 183.70 x 10^9/l and 266.04 x 10^9/l respectively.

11) Thus, the mean platelet count in EDTA anticoagulated blood was found to be significantly lower than that in manual platelet count and also, in parallel platelet count in CPT anticoagulated blood at different time intervals.

12) The difference of mean initial platelet count in EDTA with manual platelet count was found to be 55% and the difference of mean platelet count in EDTA and CPT anticoagulated blood was found to be about 25% at 0-30 minutes and at 3-4 hours, and of 31% at 24 hours.

13) The mean platelet counts in CPT anticoagulated blood were found to be comparable with mean manual platelet count.

14) Counting of platelet clumps in the peripheral blood films according to their size in relation to the platelet count showed gradual increase in number of cases as well as number of small, medium and large sized clumps with gradual increase in platelet count. In each range of platelet counts, the number of small clumps predominated followed by medium and then large sized clumps except, in the range of 71.90 x 10^9/l where the number of medium sized clumps was lowest.

15) There was no significant correlation between platelet clump counts and the initial platelet count in cases of EDTA-PTCP.

16) Comparison of platelet count in EDTA anticoagulated blood at different time intervals showed significant difference between initial platelet count and that at 0-30 minutes and between initial platelet count and that at 3-4 hours. But, no statistical change was found in platelet count from 0-30 minutes to 3-4 hours in EDTA anticoagulated blood.

17) Comparison of initial platelet count in EDTA with platelet counts in CPT anticoagulated blood at different time intervals showed significant difference between initial platelet count in EDTA anticoagulated blood with platelet count in CPT anticoagulated blood at 0-30 minutes and that at 3-4 hours. But no statistical change was found in platelet count from 0-30 minutes to 3-4 hours in CPT anticoagulated blood.

DISCUSSION

Of the total 103 cases included in the study, our suspicion of Pseudothrombocytopenia was found to be correct in 94 cases. The present study observed incidence of thrombocytopenia in 1.5% cases and that of EDTA-dependent Pseudothrombocytopenia in 0.07% cases of total haemograms and 4.9% of total thrombocytopenia.

Hyun-Sook Chi (2010)\textsuperscript{11} and Lichtman et al (2007)\textsuperscript{2} reported its frequency in the range of 0.07 to 0.11% and 0.9 to 0.21% respectively. The incidences of EDTA-PTCP reported by other workers are: Bizarro (1995)\textsuperscript{13} – 0.11%, Bartels et al (1997)\textsuperscript{14} – 0.1%, Mori et al (2000)\textsuperscript{15} – 0.1%, Mant et al (1975)\textsuperscript{4} – 0.3%, Manthorpe et al (1981)\textsuperscript{5} – 1.2%, Lippi et al (1990)\textsuperscript{10} – 2% and Sakurai et al (1997)\textsuperscript{16} in the range of 0.03 to 1.9%. The present finding of 0.07% incidence of EDTA-PTCP is comparable with that of Hyun-Sook Chi (2010)\textsuperscript{11} and Sakurai et al (1997)\textsuperscript{16}.
CONCLUSION

The following conclusions are drawn from these findings:

1) In all the cases of thrombocytopenia showed by automated blood cell counters, the examination of peripheral blood film is must for the presence of platelet clumps to detect the cases of EDTA-dependent pseudothrombocytopenia.

2) To get correct platelet count in these cases, the manual platelet count is the ‘gold standard’. However, CPT anticoagulated blood also shows comparable platelet counts similar to the manual platelet counts. Therefore, CPT is the better alternative anticoagulant for EDTA in cases of EDTA-dependent pseudothrombocytopenia for automated platelet counts.

3) Awareness of the phenomenon of EDTA-dependent pseudothrombocytopenia is important because it will save the patient from misdiagnosis, unnecessary investigations and inappropriate treatment. It will prevent needless evaluations of thrombocytopenia, cancelled surgical procedures, avoidance of conduction anaesthesia, unwarranted splenectomies, undue patient expense and anxiety and unnecessary platelet transfusions.

REFERENCES

13. Nicola Bizzaro: EDTA Dependent Pseudothrombocytopenia: A Clinical and Epidemiological Study of 112 Cases, With 10-
