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Original Article

Pseudothrombocytopenia in Asymptomatic Outdoor Patients Presenting with Low Platelet Counts

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ABSTRACT

Background: Pseudothrombocytopenia is spuriously low platelet count seen in complete blood count performed on an automated hematology analyzer. It may lead to unnecessary investigations& management. It has been postulated to be caused by an anticoagulant namely Ethylene-Diamine-Tetra Acetic-Acid (EDTA) and has been reported in physiological as well as disease conditions with variable frequency. No significant research has been done on this important clinical entity in our country.

Objective: To see the prevalence of pseudothrombocytopenia in our settings.

Methods: EDTA based hematology analyzer method was used as the initial test for blood counts. Patients showing thrombocytopenia were screened for pseudothrombocytopenia by Giemsa stained blood film examination. Those with platelet aggregates were labeled as pseudothrombocytopenia and were studied further by doing manual platelet count (ammonium oxalate method) and repeat automated platelet count on a citrate based fresh blood sample. Manual platelet count was also performed on blood samples not showing platelet aggregates, as a second security check, after which, those with low platelet count were labeled as true asymptomatic thrombocytopenia.

Results: Out of 385 patients, 30 revealed pseudothrombocytopenia, eight patients were male and 22 females, female to male ratio was 2.75:1. Ten patients were less than twenty five years old; 15 patients were 25 to 50 years and five patients aged more than 50 years. Mean age of the patients was 32.7±16.5 years. Machine generated blood report using EDTA blood, revealed platelet count <50 $\times 10^3/\mu l$ in 16(53.4%), 40-100$\times 10^3/\mu l$ in 12(40%) & 100-149$\times 10^3/\mu l$ in 2 (6.7%) patients respectively. Mean platelet count was 50±25$\times 10^3/\mu l$. No patient had platelet count of >150$\times 10^3$ /µl by this method. Platelet count using sodium citrate revealed platelets >150 $\times 10^3$ /µl in 25(83.4%) and 100-149$\times 10^3/\mu l$ in 5(16.6%) patients. By manual method none of patients had platelets lower than 150 $\times 10^3/\mu l$.

Conclusion: Pseudothrombocytopenia is more common in females, when EDTA is used as anticoagulant and is seen in all age groups.

Key words: Thrombocytopenia, Pseudothrombocytopenia, Complete blood count, Manual platelet count

Introduction

Thrombocytopenia is defined as platelets count of <150$\times 10^3/\mu l$ in peripheral blood. It may result from decreased production, increased destruction or abnormal distribution of platelets. Pseudo-thrombocytopenia is a term used for a spuriously low platelet count when complete blood count is performed on an automated hematology analyzer, while in fact the platelet count is within the normal range[1].

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It has no actual clinical significance but the uncertainty created by the abnormal report may lead to unnecessary investigations, wastage of time and financial resource.

Pseudo-thrombocytopenia, an in-vitro phenomenon has been studied previously as case reports and case series[2].It has been postulated that the abnormal platelet clumping in pseudo-thrombocytopenia is caused by Ethylene-Diamine-Tetra acetic-Acid (EDTA), which when reacts with GPIIb IIIa receptor complex on platelets membrane, exposes some hidden protein part of this receptor. Preformed auto-antibodies readily attach this protein and lead to formation of platelets clumps[3]. It has been reported in pregnancy, malignancy, autoimmune diseases, liver

disease, infections, heart disease and in normal people[4-10]. The prevalence of pseudothrombocytopenia has been reported 0.1to 2 % in hospitalized and 15 to17% in outpatients[11,12]. In Pakistan, pseudothrombocytopenia has not been studied previously.

Methodology

The present study, a descriptive cross-sectional study, was conducted on 385consecutive outdoor patients, selected by purposive sampling, with a view to evaluate the low platelet count seen on their complete blood count examination. The test was performed on an automated hematology analyzer (Nihan Kohden MEK-6420), working on impedance method for blood counting. The patients attended outdoor clinics at Ayub Teaching Hospital (ATH) for non specific symptoms like headache, low vision, and body aches or had to undergo some minor procedure like removal of sebaceous cyst, in-growing nail or dental extraction. Their blood counts performed at ATH revealed isolated thrombocytopenia. They were referred to the department of Pathology Ayub Medical College for further workup including bone marrow examination. Moreover, asymptomatic patients whose complete blood count were done on their request, and were found with low platelet count, also participated in the study. The participants did not have any symptom related to thrombocytopenia. The study was conducted from May 2014 to May 2018, over a period of four years. An informed written consent was obtained from every patient at the time of enrolment in the study and approval of institutional ethical review committee was also obtained. The patients with reports showing isolated thrombocytopenia on EDTA blood sample were screened initially by examining giemsa stained blood films. Those with platelets clumps on blood film examination were enrolled in the study as pseudothrombocytopenia and were evaluated further on the same day by; a) repeat blood count on citrated blood sample and b) manual platelet count by ammonium oxalate method. This is because citrate-based machine blood counting may also give (although less common than EDTA blood) spuriously low platelets count and actual platelet count may not be possible by this method in all the patients. Therefore, manual counting of platelets by using ammonium oxalate on both the EDTA based and citrate based blood samples was carried out to find out the actual platelet count which was a requirement of the study. Manual platelet counting, repeat blood counting by citrate method and blood film examination was done by three different pathologists to avoid bias. Blood samples were kept at 37C^{o} while performing platelet count by different methods. A brief clinical examination of the patients for pallor, fever, organomegaly, sub-mucosal or subcutaneous bleeding was also performed to exclude the common manifestations of other conditions presenting with true thrombocytopenia. Present or past history of any bleeding episodes, or using any medicines affecting platelets in the past two months, was also recorded from each patient. The patients with true thrombocytopenia were investigated further (bone marrow examination) as already requested by the referring clinician. They were excluded from the study. The results were recorded on a proforma designed for this purpose.

Results

A total of 385patientsrevealed thrombocytopenia by EDTA based hematology analyzer method. After initial screening by blood film method, 30/385 (7.79%) had pseudothrombocytopenia while the remaining patients 355/300 (92.2%) had asymptomatic true thrombocytopenia. Gender wise, eight patients were male and 22 females, female to male ratio being 2.75:1. Age wise, 10 patients were less than 25-years old, 15 patients were 25 to 50 years and five patients aged more than 50 years. Mean age of the patients was 32.7±16.5 years. Machine generated blood report using EDTA blood, revealed platelet count <50 $\times 10^3/\mu l$ in 16(53.4%), 40-100$\times 10^3/\mu l$ in 12(40%) & 100-149$\times 10^3/\mu l$ in 2 (6.7%) patients. Mean platelet count of the 30 patients by this method was 50±25$\times 10^3/\mu l$. Platelet count using sodium citrate-based blood count revealed platelets >150 $\times 10^3$ /µl in 25(83.4%) and 100-149$\times 10^3/\mu l$ in 5(16.6%) patients. Mean platelet count by this method was 205±48$\times 10^3/\mu l$. By manual method all the patients had platelet count within normal range, mean platelet count being 221±35$\times 10^3/\mu l$ (Table 1). Pseudothrombocytopenia was arbitrarily classified as severe, moderate and mild, on the basis of EDTA based machine generated platelets count as <50$\times 10^3\mu l$,50-100$\times 10^3\mu l$ & 100-149$\times 10^3\mu l$ respectively.

Table 1. Severity of pseudothrombocytopenia based upon platelet count by different methods (n=30)

Thrombocytopenia ($10^3/\mu l$)	Patients no & %		
	Analyzer (EDTA)	Analyzer (Citrate)	Manual (ammonium oxalate)
Severe (< 50)	16 (53.4%)	00	00
Moderate (50-100)	12(40%)	00	00
Mild (100-149)	02 (06.7%)	05(16.6%)	00
Normal (>150-450)	00	25(83.4%)	30(100%)

EDTA, Ethylene-Diamine-Tetra Acetic-Acid

Discussion

Platelet count, like other blood cells may not remain constant and keep on varying within normal limits in different physiological conditions[13-15]. In pathological conditions platelets may fall below the reference range. If platelets fall below $50\times10^3/\mu l$, the chances of spontaneous bleeding increase. At times the actual platelet count is within the normal limit but the report of blood complete picture by hematology analyzer shows low platelet count. This condition (also called pseudothrombocytopenia), may cause anxiety and lead to unnecessary investigations or therapeutic interventions. This condition is recognized by the laboratory scientists and has been investigated and reported in literature since 1969[16]. Spuriously decreased or increased platelets was discussed in a large review in which EDTA induced platelet aggregation has been mentioned as one of the most common causes of pseudothrombocytopenia[11].Time dependent changes in platelet counts by using different anticoagulants revealed increase in platelet count with EDTA, decreased platelet count with sodium citrate and unchanged platelet count with magnesium based anticoagulant on 180 minutes storage. It was observed that, greater the time of contact, greater is the severity of pseudothrombocytopenia when sodium citrate is used as anticoagulant[17].Other researchers reported the importance of typical histograms generated by hematology analyzer as an important clue for recognizing pseudothrombocytopenia[18,19]. Magnesium based anticoagulant and the hematology analyzers showing histograms tagged for abnormal platelet clumping was not available in our laboratory. These tests were, therefore not done in the present study.

Low incidence of pseudothrombocytopenia was reported in an earlier study. Platelet count repeated in a different anticoagulant and giemsa stained blood film examination was suggested to confirm platelet clumping, which is an evidence of pseudothrombocytopenia[1].Pseudothrombocytopenia was more common in females in a study conducted on 50 patients, with M:F of 1.2:1.[20]This is in accordance with the findings of present study

In the present, study pseudothrombocytopenia was severe in16/30 (53.4%), moderate in12/30 (40%) and mild in 2/30 (6.7%) patients respectively. This is contrary to the results of a previous study in which 2% patients had severe thrombocytopenia and 98% with mild thrombocytopenia. More studies on larger sample size using different anticoagulants are much needed to see the effects of storage conditions, storage time and the different anticoagulants on platelet aggregation in our patients so that uniform recommendations may be generated to guide and educate our physicians about this important condition. We suggest that when pseudothrombocytopenia is suspected, the report should not be issued before doing initial screening by blood film examination for platelet clumps, followed by manual platelet count and/or performing repeat blood count by citrate-based anticoagulant.

Conclusion

Pseudothrombocytopenia is more common in females, when EDTA is used as anticoagulant and is seen in all age groups.

Conflict of Interest: Authors declare no conflict of interest.

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Authors' Contribution:

JF: Conception, study designing & conduction, analysis, manuscript writing, & facilitating procurement

MI: Conception, study designing & conduction, analysis & manuscript writing

NG: Conception, planning, analysis & material procurement

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