COMPARISON OF ROLE OF ALTERNATIVE ANTICOAGULANT (MgSO₄) IN EDTA-INDUCED PSEUDOTHROMBOCYTOPENIA

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ABSTRACT

BACKGROUND

Pseudothrombocytopenia is a condition in which there is falsely low platelet in patients who have thrombocytopenia but no petechiae or ecchymoses or bleeding history. Prevalence of EDTA-dependent pseudothrombocytopenia ranging from 0.1-2% in hospitalised patients to 15-17% in outpatients is evaluated. Pseudothrombocytopenia is also an artifactual thrombocytopenia caused by anticoagulant dependent agglutinins. The reason for thrombocytopenia was EDTA-induced platelet aggregation. The aim of this study was to compare the platelet count in pseudothrombocytopenia in EDTA anticoagulated sample with other alternative anticoagulant (MgSO₄).

MATERIALS AND METHODS

Blood sample was collected in a sterile EDTA containing tube and processed, our established laboratory then generated report of each patient. Informed consent was taken of all patients that took part in our study during repeat blood samples collected in MgSO₄ anticoagulant for use of their samples for medical research, after physician's request, investigated and generated the report. EDTA (Ethylenediaminetetraacetate) anti-coagulated whole blood samples for routine haematological analysis were selected for this study when suspicious for low platelet count. Both samples are run through the automated haematology analyser (three part differential, Mindray) and peripheral blood smear made for microscopic examination.

RESULTS

Study included 110 patients that shows EDTA-induced pseudothrombocytopenia, out of which 51 patients found raised platelet count $>150 \times 10^3/\mu\text{L}$ in MgSO₄ anticoagulated blood sample. Mean platelet count in 110 patients in EDTA anticoagulated blood sample was 71.67 (67.08-76.26) $\times 10^3/\mu\text{L}$ and in MgSO₄ anticoagulated blood sample was 153.63(44.96-169.84) $\times 10^3/\mu\text{L}$. Mean platelet volume in 110 patients in EDTA blood sample was 11.45 fl (Range 11.09- 11.8) and in MgSO₄ blood sample was 10.32 fl (range 9.3-11.3). Platelet count is low in EDTA blood sample in contrast to the sample anticoagulated with MgSO₄. No significant correlation was found in other parameters of CBC, in blood samples anticoagulated with EDTA and MgSO₄. In peripheral smear examination of EDTA-PTCP patients, 80% of patients showed platelet aggregation and clumping.

CONCLUSION

This study illustrates low platelet count in automated haematology analysers (Mindray) in EDTA blood sample. Peripheral blood smear should be examined for platelet clumping or aggregates in cases of low platelet count not correlating with clinical presentation or thrombocytopenia flagged in haematology analyser. Awareness of these patterns may alert to the presence of EDTA-PTCP in routine clinical practice. Alternative anticoagulant MgSO₄ can be recommended for correct estimation of platelet count and to exclude EDTA-induced PTCP in order to prevent unnecessary surgical trouble and unwanted expenditure to the patient.

KEYWORDS

Anticoagulant (MgSO₄), EDTA-induced Pseudothrombocytopenia (EDTA-PTCP).

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BACKGROUND

Ethylenediaminetetraacetic acid (EDTA) is a well-known anticoagulant since early 1950s and it has certain advantages over other anticoagulants.¹ EDTA dependent pseudothrombocytopenia is a rare phenomenon (i.e., around 0.1% in general population) which is mostly due to the presence of EDTA-dependent antiplatelet antibodies that react optimally between 0°C to 4°C, recognise the cytoadhesive receptors gplIb/IIIa, stimulate the expression of activation antigen, trigger activation of trypsin kinase, platelet agglutination and clumping in vitro. This finally leads to a spuriously decreased platelet count. Five basic criteria should be fulfilled to raise the clinical suspicion of EDTA

dependent pseudothrombocytopenia, (i) abnormal platelet count typically $<100 \times 10^9/L$. (ii) Occurrence of thrombocytopenia in EDTA-anticoagulated sample at room temperature. (iii) Time dependent fall of platelet count in EDTA specimen. (iv) Evidence of platelet aggregates and clumps in EDTA-anticoagulated samples with either automated cell counting or microscope analysis. (v) Lack of sign or symptoms of platelet disorders. Spurious thrombocytopenia also called Pseudothrombocytopenia (PTCP), results from low platelet counts due to in vitro platelet clumping.²⁻⁸ Platelet clumping in PTCP (Pseudothrombocytopenia) results in inaccurate platelet concentration which leads to misdiagnosis of thrombocytopenia when analysed with haematology analyser.^{8,9} Pseudothrombocytopenia remains a challenge in haematological laboratory. Problem is that platelet tends to easily aggregate in vitro, giving rise to lower platelet counts. In automated electronic cell counting of blood samples anticoagulated with EDTA, PTCP was observed due to in vitro platelet clumping induced by EDTA. It is caused by activation of an abnormal protein identified as an immunoglobulin/agglutinin.¹⁰ Magnesium was used in the beginning of the last century as anticoagulant for microscopic platelet counts.

MATERIALS AND METHODS

This study was conducted in the Department of Pathology in the Institute of Shyam Shah Medical College Rewa and Sanjay Gandhi Memorial Hospital, Rewa (M.P.). Approximate 110 cases during study period April 2015 to March 2016 were evaluated by EDTA (Ethylenediaminetetraacetate) blood samples and $MgSO_4$ blood samples.

Inclusion Criteria

1. Patients having platelet count less than 100000/cu. mm. in EDTA (Ethylenediaminetetraacetate), anticoagulated sample.
2. Samples showing flags in automated haematological analyser for low platelet count.
3. Only indoor patients included in study.
4. Patient age > 15 years.

Exclusion Criteria

1. Patients with normal platelet count.
2. Patients with higher platelet count.
3. OPD (Outpatient department) patients.
4. Patient Age < 15 years.
5. Patient with h/o of bleeding disorder.

Blood samples were collected in a sterile EDTA containing tube and processed following our established laboratory procedure, then report of each patient generated. Informed consent was taken from all patients that took part in our study during repeat blood samples, collected in $MgSO_4$ anticoagulant, for use of their samples for medical research, after doing physician request, investigated and generated the report. EDTA (Ethylenediaminetetraacetate) anti-coagulated whole blood samples for routine haematological analysis selected for this study when suspicious for low platelet count. Both samples were run through the automated haematology analyser (three-part differential, Mindray) and made peripheral blood smear for microscopic examination.

RESULTS

This study included a total of 110 patients, out of which Females-84 and Males-36 aged between 15 to 70 years showing EDTA-induced pseudothrombocytopenia due to platelet aggregation. These patients were not associated with symptoms and history of bleeding disorder. Patients included in study were having diseases like Upper respiratory tract infection (36 patients), Coronary heart disease (11 patients), Diabetes mellitus (10 patients), Acute trauma (08 patients), Hypertension (24 patients), Tuberculosis (15 patients), others (07 patients).

Out of which 51 patients found raised platelet count $>150 \times 10^3/\mu L$ in $MgSO_4$ anticoagulated blood samples. Mean platelet count in EDTA anticoagulated blood sample of 110 patients was $71.67 (67.08-76.26) \times 10^3/\mu L$ and in $MgSO_4$ was $153.63 (44.96-169.84) \times 10^3/\mu L$. (Table 1). Comparison of two anticoagulants was performed using paired t-test. $p < 0.0001$ (software GraphPad for statics), which shows statistically extremely significant. Mean platelet volume in EDTA was $11.45 (11.09-11.08) fL$ and in $MgSO_4$ was $10.32 (9.35-11.3) fL$ (Table 2). In peripheral smear examination of EDTA-PTCP patients, 80% of patients showed platelet aggregation and clumping (Table 3). We also compared other parameter of complete blood count in EDTA and $MgSO_4$ anticoagulated blood sample. On comparison of Red blood cell count and Total leucocyte count in both anticoagulants that is EDTA and $MgSO_4$ in 110 blood samples, mean $\pm$ SD of EDTA was $3.68 (\pm 4.04)/pL$ and of $MgSO_4$ was $3.49 (\pm 1.00)/pL$, EDTA was $8.66 (\pm 8.58) \times 10^3/\mu L$ (Table-4) and of $MgSO_4$ was $8.43 (\pm 8.44) \times 10^3/\mu L$ (Table-5) respectively. Table-6 Shows Mean $\pm$ SD in EDTA and $MgSO_4$ blood samples of Neutrophil was $67.58 (\pm 15.16) \%$ and $67.09 (\pm 15.16) \%$, Lymphocyte was $24.88 (\pm 14.17) \%$ and $25.72 (\pm 15.55) \%$, Mid was $7.61 (\pm 3.77) \%$ and $7.90 (\pm 4.17) \%$ respectively. No significant difference was found in mean of RBC count, Total leucocyte count and Differential leucocyte count in both anticoagulated blood samples.

Parameter	EDTA	$MgSO_4$
Mean Platelet Count $\times 10^3/\mu L$	71.67	153.63
Standard Deviation ($\pm$ SD)	24.27	65.79
Standard Error of Mean (SEM)	2.31	6.27
Range	67.08-76.26	44.96-169.84

Table 1. Comparisons of Mean Platelet Counts in Blood Sample Anticoagulated with EDTA and $MgSO_4$

Graph 1. Comparison of Mean Platelet Counts in Blood Samples Anticoagulated with EDTA and $MgSO_4$

Sl. No.	Parameter	EDTA	MgSO ₄
1.	Mean in	11.45	10.32
2.	±SD	1.893	5.179
3.	Standard error of mean	0.1805	0.493
4.	Range(lower-upper) limit	11.092-11.80	9.350-11.30

Table 2. Comparison of Mean Platelet Volume in Blood Samples Anticoagulated with EDTA and MgSO₄

Graph 2. Comparison of Mean Platelet Volume in Blood Samples Anticoagulated with EDTA and MgSO₄

Sl. No.	Causes	Total Patient	No. of Patient	%
1.	EDTA- platelet aggregation and clumping	110	88	80%
2.	Partial clotting of specimen	110	10	9.09%
3.	Platelet satellitism around WBC	110	05	5.54%
4.	Giant platelet	110	07	6.36%

Table 3. Causes of Pseudothrombocytopenia

Graph 3. Causes of Pseudothrombocytopenia

Sl. No.	Parameter	EDTA	MgSO ₄
1.	Mean of RBC /pL	3.68	3.49
2.	Standard Deviation ±	4.04	1.00
3.	Standard Error of Mean (SEM)	0.38	0.09
4.	Range (lower-upper limit)	1-44	1.2-6.8

Table 4. Comparison of RBC Count in Blood Samples Anticoagulated with EDTA and MgSO₄

Sl. No.	Parameter	EDTA	MgSO ₄
1.	Mean of TLC ×10 ³ /μL	8.66	8.43
2.	Standard Deviation	8.58	8.44
3.	SEM	0.81	0.80
4.	Range (lower- upper limit)	2.2-66.8	2.2-89

Table 5. Comparison of Total Leucocyte Count in Blood Samples Anticoagulated with EDTA and MgSO₄

Sl. No.	Parameter	Neutrophil		Lymphocyte		MID	
		EDTA	MgSO ₄	EDTA	MgSO ₄	EDTA	MgSO ₄
1.	MEAN	67.58	67.096	24.88	25.72	7.61	7.90
2.	SD	15.16	15.34	14.17	15.55	3.77	4.17
3.	SEM	1.445	1.46	1.352	1.48	0.3602	0.3977
4.	MINI LIMIT	64.7	64.19	22.20	22.78	6.90	7.11
5.	MAX LIMIT	70.5	69.99	25.6	28.66	8.33	8.69

Table 6. Comparison of Differential Leucocyte Count in Blood Samples Anticoagulated with EDTA and MgSO₄

Figure 1. 100X Peripheral Blood Smear of EDTA Blood Samples Showing Platelet Aggregation

Figure 2. 100x Peripheral Smear of MgSO₄ Blood Sample

DISCUSSION

Ethylenediaminetetraacetic acid (EDTA) is commonly used as an anticoagulant for estimation of blood cell counts. EDTA-PTCP is an in vitro phenomenon due to antiplatelet antibodies that cause platelet clumping in blood that had been anticoagulated with EDTA.¹¹

Aim of this study is to demonstrate that Magnesium sulphate can effectively prevent platelet aggregation in whole blood samples from patients showing PTCP in the presence of EDTA. Platelets are not stabilised in EDTA anticoagulated blood samples. They undergo changes in shape and size, thus leading to time dependent changes of the mean platelet volume (MPV).^{12,13}

In this study, EDTA-PTCP was confirmed by microscopic examination of peripheral blood smear for platelet clumping and aggregates that shows low platelet count on Mindray three-part differential automated analyser.

Peter Schuff Werner et al, estimated correct platelet count in EDTA-induced pseudothrombocytopenic patients by using MgSO₄ anticoagulant, found higher platelet count in MgSO₄ anticoagulated sample. Nakamoto K et al also found raised platelet count in MgSO₄ anticoagulated blood sample than sample anticoagulated with EDTA. Peter Kohlschein et al also found magnesium sulphate anticoagulated samples prevent platelet aggregation in individuals with EDTA associated pseudothrombocytopenia.

In this study, we found that sample anticoagulated with magnesium sulphate do not show platelet aggregation in peripheral blood smear when examined in light microscope. It shows significantly higher mean platelet count in MgSO₄ anticoagulated blood sample than EDTA anticoagulated sample.

In this study, we also compared other parameter of complete blood count (CBC) in EDTA blood sample and MgSO₄ blood sample in patients with PTCP. Magnesium Sulphate normalised platelet counts without interfering with other blood cell counts; shows no significant correlation in mean of Red blood cells, Total leucocyte count and Differential leucocyte count in EDTA anticoagulated blood sample and MgSO₄ blood sample.

If PTCP in EDTA sample is not identified in laboratory for clinical purpose, then falsely low platelet count may suggest unnecessary, expensive and even invasive diagnostic or therapeutic procedure. To avoid this, a safe and practical alternative should be used. Magnesium sulphate anticoagulated blood samples can be used for platelet counting, when platelet count in EDTA blood sample do not match with clinically, or flagging in haematology analyser, as suggestive of platelet aggregates. This can also be confirmed by examination of peripheral blood smear.

CONCLUSION

Before EDTA was introduced as the anticoagulant of choice for haematology testing, magnesium salts were used as anticoagulants for sampling capillary blood for platelet enumeration.¹⁴ Present study has been attempted to determine the role of MgSO₄ as anticoagulant in EDTA-induced pseudothrombocytopenic patients. Our observation confirms MgSO₄ as an anticoagulant inhibits platelet aggregation in blood sample. No aggregation seen in

peripheral smear. Magnesium sulphate normalised the platelet counts without interfering with other blood cell count or differentiation result. To avoid this clinical misinterpretation of platelet count, we suggest the use of MgSO₄ as an anticoagulant if blood count does not match with clinical expectation and flagging in automated analyser.

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