Estimation of Platelet Counts and Other Hematological Parameters in Pseudothrombocytopenia Using Alternative Anticoagulant: Magnesium Sulfate

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ABSTRACT: The platelet count, mean platelet volume, and other hematological parameters were compared in blood samples anticoagulated with $MgSO_4$ and EDTA. A total of 15 samples were taken, and the platelet counts were observed to be significantly high in $MgSO_4$-anticoagulated blood samples ranging from 53×10^3 to $499 \times 10^3/\mu L$, whereas in EDTA-anticoagulated blood samples, the counts ranged from 10×10^3 to $353 \times 10^3/\mu L$. This increased platelet count was also statistically significant with the *P* value being .005. The morphology of red blood cells and white blood cells in Leishman-stained smears from $MgSO_4$-anticoagulated blood was below average. In conclusion, $MgSO_4$ can be used as an alternative anticoagulant only to estimate the platelet counts in EDTA-induced pseudothrombocytopenia.

KEYWORDS: Low platelet count, $MgSo_4$, sodium citrate, antiaggregation

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Introduction

Salts of EDTA are routinely used as anticoagulants for routine hematological analysis. EDTA has been recommended as the anticoagulant of choice for hematological testing because it allows the best preservation of cellular components and morphology of blood cells.[1] One rare drawback of EDTA as anticoagulant is spuriously low platelet count or pseudothrombocytopenia. In pseudothrombocytopenia, a laboratory disease, platelets tend to easily aggregate in vitro owing to anticoagulant-dependent agglutinins giving rise to spuriously low platelet values.[2]

This EDTA-induced pseudothrombocytopenia can be recognized by the presence of platelet clumps in the peripheral smear of blood anticoagulated with EDTA.[3]

Hematology analyzers count the resulting platelet clumps as single giant platelets or as small lymphocytes in the white blood cell gate and indicate thrombocytopenia.[4] Despite its harmlessness, EDTA-induced pseudothrombocytopenia if undiagnosed, may lead to anxiety, unwanted platelet transfusions, and unnecessary delay in surgeries and medical management in subjects.[4]

Different approaches to avoid the time and temperature dependent in vitro aggregation of platelets in the presence of EDTA were tested, but none of them proved optimal for routine use.[5] Similarly, although platelet aggregation is reversed with sodium citrate (Na citrate) as anticoagulant, few cases are unaffected with the use of Na citrate.[5,6] Although the practical and best suited approach to overcome EDTA-induced pseudothrombocytopenia is recollection and analysis of samples using Na citrate as alternative anticoagulant, there are quite a number of cases unresolved by this method and other approaches such as addition of additives, such as sodium fluoride, ammonium oxalate, kanamycin, amikacin, to EDTA-anticoagulated blood.[1]

Historically, magnesium sulfate ($MgSO_4$) was used as anticoagulant to estimate manual platelet count and also as a systemic anticoagulant in patients with cardiac disease.[4,7] $MgSO_4$ was found to be an effective alternate anticoagulant to resolve EDTA-induced pseudothrombocytopenia by Schuff-Werner et al.[4]

The objectives of our study were as follows:

1. To compare total platelet count, mean platelet volume as analyzed in automated hematology analyzer with $MgSO_4$ and EDTA as anticoagulants in subjects with EDTA-induced pseudothrombocytopenia.
2. To compare the other estimated parameters in hematology analyzer with EDTA-anticoagulated and $MgSO_4$-anticoagulated samples.

Methods

This is a systematic review which was conducted at the Pathology Department of Chettinad Hospital and Research Institute, Chennai, Tamil Nadu for a span of 5 months involving 15 subjects. This study was approved by the Institutional Human Ethics Committee of Chettinad Academy of Research and Education.

Sample collection

Tripotassium EDTA (K3EDTA)–anticoagulated (Greiner Bio-One Vacutainer System, India) whole blood samples for

Table 1. Summary of platelet counts and MPV when anticoagulated with EDTA and $MgSO_4$.

PARAMETER	MEAN	STANDARD DEVIATION	RANGE	
			MINIMUM	MAXIMUM
Platelet count in EDTA-anticoagulated blood	$17 \times 10^3/\mu L$	9.89	$10 \times 10^3/\mu L$	$353 \times 10^3/\mu L$
Corrected platelet count in $MgSO_4$-anticoagulated blood	$110 \times 10^3/\mu L$	10.88	$53.9 \times 10^3/\mu L$	$499.4 \times 10^3/\mu L$
MPV in EDTA-anticoagulated blood	9.19 fL	1.53	6.9 fL	12 fL
MPV in $MgSO_4$-anticoagulated blood	7.86 fL	1.95	5.7 fL	15.2 fL

Abbreviation: MPV, mean platelet volume.

routine hematological analysis were selected for this study when flagged for platelet aggregates (even in the case of platelet counts still appearing to be within the normal range). If platelet aggregates were confirmed by microscopic examination of blood smears, the patient was asked for informed consent to obtain additional blood samples using collecting tubes. Blood is collected from the antecubital vein by venipuncture and was quickly added to test tube containing $MgSO_4$. About 2.7 mL whole blood was collected and was added to a tube containing 0.3 mL $MgSO_4$ at a concentration of 4.060 mOsmol/mL; 0.3 mL $MgSO_4$ was aliquoted from commercially available $MgSO_4$ injection (Magneon; Neon Laboratories Ltd, Mumbai, India). If patient consented, another standard 2.7 mL blood fill, in 3.2% Na citrate Vacutainer tube (Becton Dickinson Vacutainer System, India), with 0.3 mL of trisodium citrate anticoagulant was collected.

Laboratory analysis

Platelet count, platelet volume, and other estimated hematological parameters were estimated by automated routine hematological analyzer: Coulter LH 750 system (Beckman Coulter, Chennai, India). Blood smears were prepared, Leishman stained according to standard operating procedure, and examined. Platelet count, red blood cell (RBC) count, white blood cell (WBC) count, hemoglobin (Hb), and hematocrit obtained in blood collected with $MgSO_4$ and sodium citrate as anticoagulant are multiplied by 1.1 to account for the different blood-to-anticoagulant ratio in the $MgSO_4$ and sodium citrate–anticoagulated tube.[8,9]

Statistical analysis

SPSS version 17.00 was used for statistical analysis. Descriptive statistics including mean, standard deviation and standard error of mean were calculated to characterize the study population. The normal distribution of the complete blood count parameters was checked using the Kolmogorov-Smirnov test. All comparisons for statistical significance between 2 anticoagulant parameters were performed using the paired t test. Statistical significance was achieved if $P < .05$.

Results

The criteria for selecting EDTA-induced pseudothrombocytopenic subjects was the presence of platelet aggregates in Leishman-stained smears and flagging for platelet aggregates in the routine hematology analyzer run. A total of 15 patients were included in this study.

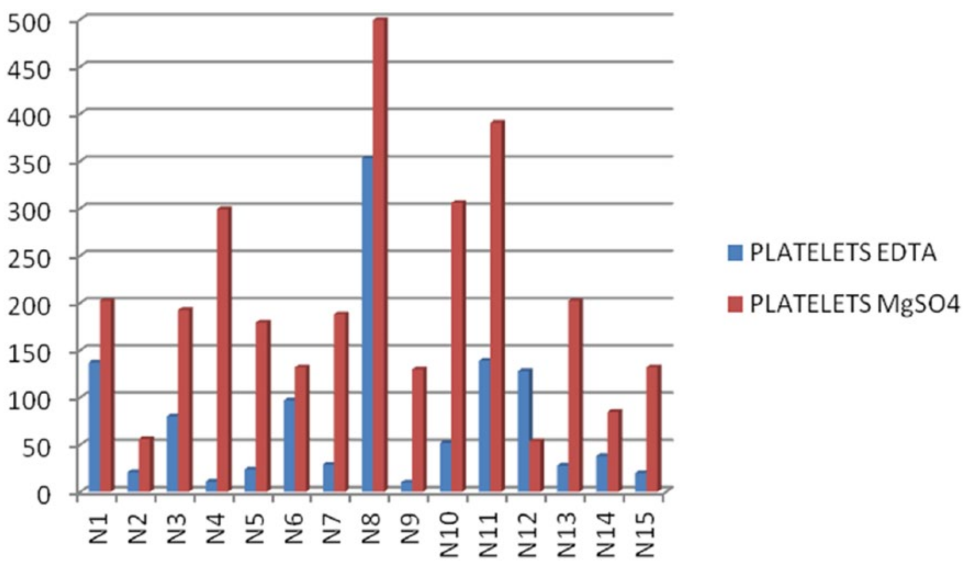
Platelet counts ranged from 10×10^3 to $353 \times 10^3/\mu L$, with a mean platelet count of $17 \times 10^3/\mu L$ in samples anticoagulated with EDTA, whereas in samples anticoagulated with $MgSO_4$, the mean platelet count was $110 \times 10^3/\mu L$ and the platelet counts ranged from 53×10^3 to $499 \times 10^3/\mu L$. However, the mean platelet volumes varied from 6.9 to 12 fL in EDTA-anticoagulated samples and from 5 to 15 fL in $MgSO_4$-anticoagulated samples (Table 1) (Graphs 1 and 2).

The mean difference in the platelet count between EDTA-anticoagulated and $MgSO_4$-anticoagulated blood samples was $93 \times 10^9/L$ with a 95% confidence interval (84.1-101.8). The mean difference in the MPV between the EDTA-anticoagulated and $MgSO_4$-anticoagulated blood samples was 1.33 fL with a 95% confidence interval (2.14-0.52). The difference in platelet count and MPV ascertained with EDTA-anticoagulated and $MgSO_4$-anticoagulated blood samples was statistically significant (Table 2).

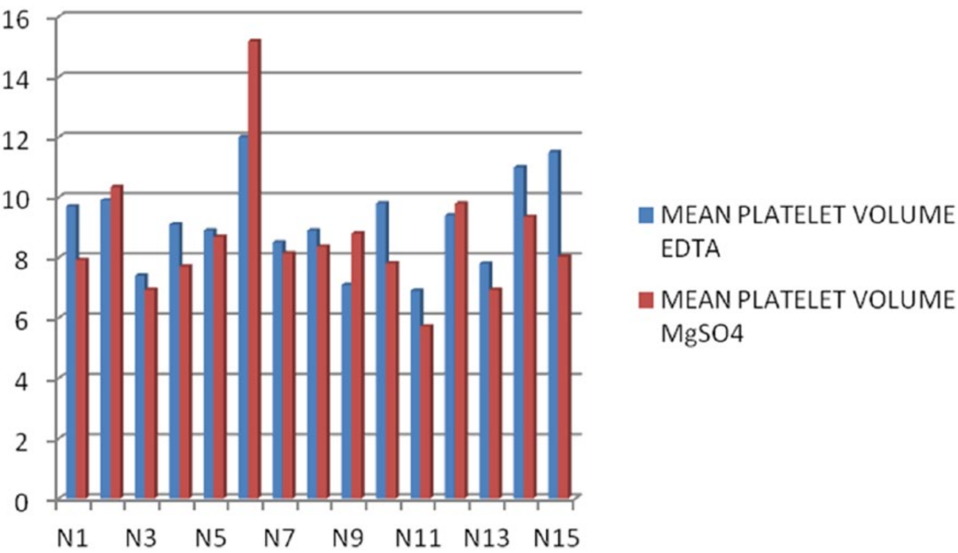
The other estimated parameters such as RBC count, Hb, mean corpuscular volume (MCV), WBC count in automated hematology analyzer and morphology of blood smears were compared in EDTA-anticoagulated and $MgSO_4$-anticoagulated blood. Red blood cell count, WBC count, Hb, and the differential count were comparable between the EDTA-anticoagulated and $MgSO_4$-anticoagulated blood. However, MCV showed a statistical difference between EDTA-anticoagulated and $MgSO_4$-anticoagulated blood (Table 3).

Of the 15 subjects, blood samples from 2 subjects were also collected in Na citrate–anticoagulated tube. In these subjects, the platelet counts were higher when compared with EDTA-anticoagulated blood; however, it was lesser than the platelet count estimated in $MgSO_4$-anticoagulated blood (Table 4). Because there were only 2 subjects, statistical analysis was not performed.

The quality of the Leishman-stained smears collected in $MgSO_4$-anticoagulated tube was below average when compared with EDTA smears. The morphology of WBC and



Graph 1. A graphical comparison between the platelet counts when samples are anticoagulated with EDTA and $MgSO_4$.



Graph 2. A graphical comparison of mean platelet volume when samples are anticoagulated with EDTA and $MgSO_4$.

RBC was below par in $MgSO_4$ smears; however, the platelet morphology was of average quality (Figures 1 and 2).

Discussion

This study aims at using an alternative anticoagulant to estimate the platelet count in spuriously low platelet counts due to EDTA-dependent platelet aggregation.

EDTA, though, is a good anticoagulant for routine hematology analysis; overall, 0% to 10% prevalence of EDTA-induced pseudothrombocytopenia is reported across the globe.[4] EDTA-induced pseudothrombocytopenia may be attributed to preformed antiplatelet antibodies being able to interact with hidden epitopes of platelet GPIIa/GPIII receptor complex made accessible by conformational changes induced by calcium complexing effect of EDTA.[4,10]

Magnesium has a well-known antiaggregatory effect on platelets and it was used for platelet enumeration in capillary blood before the use of EDTA as an anticoagulant.[4] In vivo, magnesium inhibits the action of thromboxane A_2, prostaglandin I_2, and 12-hydroxyeicosatetraenoic acid which are important platelet aggregatory agents.[11] It also inhibits the normal clotting action of factors VIIa, IXa, and the proteins C and S. Magnesium is a natural calcium antagonist. It competes with calcium for binding sites on prothrombin, hence inhibiting coagulation.[12–15]

Inhibition of fibrinogen binding to the platelet membrane glycoprotein IIb/IIIa by altering membrane fluidity of platelets and inhibition of intracellular calcium mobilization are the main mechanisms of the antiaggregatory action of magnesium in vitro.[4,7,16,17]

Table 2. Summary of differences in platelet counts and mean platelet volumes in samples anticoagulated with EDTA and $MgSO_4$.

PAIRED SAMPLE	MEAN DIFFERENCE	95% CONFIDENCE INTERVAL		*P* VALUE
		LOWER	UPPER	
Platelet count in $MgSO_4$-anticoagulated and EDTA-anticoagulated blood	$93 \times 10^3/\mu L$	84.1	101.8	.005
MPV in $MgSO_4$-anticoagulated and EDTA-anticoagulated blood	1.33 fL	2.14	0.52	.003

Abbreviation: MPV, mean platelet volume.

Table 3. Comparison of blood samples anticoagulated with $MgSO_4$ and EDTA.

SUBJECT	RBC COUNT $\times 10^6/\mu L$		HEMOGLOBIN, G/DL		MCV, FL		WBC COUNT $\times 10^6/\mu L$		PLATELETS $\times 10^3/\mu L$		MPV, FL	
	$MgSO_4$	*EDTA*	*$MgSO_4$*	*EDTA*	*$MgSO_4$*	*EDTA*	*$MgSO_4$*	*EDTA*	*$MgSO_4$*	*EDTA*	*$MgSO_4$*	*EDTA*
N1	4.103	4.05	13.09	13.00	104.61	95.20	5.50	6.80	202.40	137.00	7.92	9.70
N2	3.531	3.56	11.00	11.20	102.19	94.50	9.46	6.30	56.10	21.00	10.34	9.90
N3	3.201	3.60	8.36	9.10	85.36	79.10	5.72	8.00	192.50	80.00	6.93	7.40
N4	2.816	2.58	8.80	7.90	101.09	92.90	9.46	8.20	299.20	11.00	7.70	9.10
N5	4.499	4.74	13.09	13.70	97.68	89.50	18.04	19.70	179.30	24.00	8.69	8.90
N6	5.379	4.93	10.56	9.80	70.95	64.20	9.02	6.90	132.00	97.00	15.18	12.00
N7	3.047	2.91	8.25	7.90	89.65	80.80	5.72	8.20	188.10	29.00	8.14	8.50
N8	3.685	3.47	11.11	10.70	107.03	99.50	9.57	10.60	499.40	353.00	8.36	8.90
N9	3.806	4.07	9.68	10.30	85.69	79.60	8.03	12.10	129.80	10.00	8.80	7.10
N10	4.686	4.82	11.33	11.50	82.28	74.90	10.56	18.10	305.80	52.00	7.81	9.80
N11	3.179	3.85	9.79	11.70	106.59	96.10	13.86	19.70	390.50	139.00	5.72	6.90
N12	5.456	4.11	10.89	8.20	74.58	65.10	9.24	5.70	53.90	128.00	9.79	9.40
N13	2.706	2.59	8.14	7.70	99.22	89.30	8.47	7.70	202.40	28.00	6.93	7.80
N14	3.421	3.92	8.69	9.70	84.92	78.40	5.39	4.70	84.70	38.00	9.35	11.00
N15	4.004	4.43	11.55	12.80	98.67	90.50	12.21	17.90	132.00	20.00	8.03	11.50
P value	0.954		0.840		<0.05		0.141		<0.05		0.03	

Abbreviation: MPV, mean platelet volume; RBC, red blood cell; WBC, white blood cell.

Table 4. Comparison of blood samples anticoagulated with $MgSO_4$, EDTA, and Na citrate.

SUBJECT	RBC COUNT $\times 10^6/\mu L$			HB, G/DL			MCV, FL		
	MgSO4	*EDTA*	*Na CITRATE*	*MgSO4*	*EDTA*	*Na CITRATE*	*MgSO4*	*EDTA*	*Na CITRATE*
N5	4.499	4.74	4.488	13.09	13.70	12.87	97.68	89.50	96.36
N9	3.806	4.07	4.059	9.68	10.30	10.23	85.69	79.60	86.24
SUBJECT	**WBC COUNT $\times 10^6/\mu L$**			**PLATELETS $\times 10^3/\mu L$**			**MPV, FL**		
	MgSO4	*EDTA*	*Na CITRATE*	*MgSO4*	*EDTA*	*Na CITRATE*	*MgSO4*	*EDTA*	*Na CITRATE*
N5	18.04	19.70	18.04	179.3	24.00	117.7	8.69	8.90	9.13
N9	8.03	12.10	8.58	129.8	10.00	102.3	8.80	7.10	9.35

Abbreviation: Hb, hemoglobin; MPV, mean platelet volume; RBC, red blood cell; WBC, white blood cell.

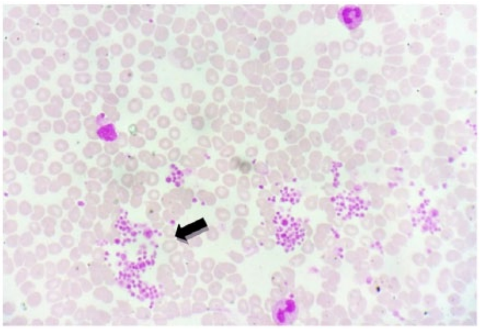


Figure 1. Leishman-stained smear of EDTA-anticoagulated sample showing platelet aggregates (indicated by arrow) (×40).

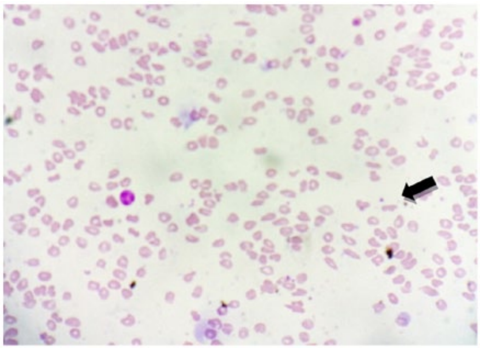


Figure 2. Leishman-stained smear of $MgSO_4$-anticoagulated sample showing dispersed platelets (indicated by arrow) (×40).

In this study, we investigated the usefulness of $MgSO_4$ as an alternative anticoagulant to estimate the platelet count in subjects with EDTA-induced pseudothrombocytopenia.

$MgSO_4$-anticoagulated samples gave a significantly higher platelet count in subjects with EDTA-induced pseudothrombocytopenia. Out of 15 subjects, only 4 (26.67%) of them showed a flag for platelet aggregates in the routine hematology analyzer run. The smears from the subjects with a platelet aggregate flag showed platelet aggregation. However, the platelet aggregation in $MgSO_4$ samples was smaller in size as compared with platelet aggregates found in smears made from EDTA-anticoagulated blood in the same subjects. The concentration of 4.060 mOsmol/mL $MgSO_4$ used as an anticoagulant is much lesser than the concentration of 33.8 µmol used as an alternative anticoagulant by Schuff-Werner et al.[4]

The lesser concentration may have attributed to the platelet clumps observed in 26.67% of $MgSO_4$-anticoagulated blood samples. Further studies on different concentrations of $MgSO_4$ have to be tried to determine the effective concentration of $MgSO_4$ to be used as an ideal alternative anticoagulant in EDTA-induced pseudothrombocytopenia.

Mean platelet volume was significantly higher in EDTA-anticoagulated samples when compared with $MgSO_4$-anticoagulated samples. This phenomenon is possibly due to aggregation of platelets giving a false high platelet volume and EDTA-induced swelling of platelets.[4] Also, the difference in platelet volume can be attributed to the fact that automated hematology analyzers are calibrated with EDTA blood samples.[4] However, the actual effect of $MgSO_4$ on platelet volume can be ascertained by comparing EDTA-anticoagulated and $MgSO_4$-anticoagulated blood samples from subjects without EDTA-induced pseudothrombocytopenia.

The other parameters such as RBC count, WBC count, hemoglobin, and differential WBC count showed comparable results between EDTA-anticoagulated and $MgSO_4$-anticoagulated blood samples. However, the MCV showed a significant difference which can again be attributed to the fact that automated hematology analyzers are calibrated with EDTA blood samples.[4]

However, the morphology of RBC and WBC in Leishman-stained $MgSO_4$-anticoagulated blood samples was below average quality. The RBCs had a dull pink hue with elongated morphology, and WBCs had an inadequate staining of cytoplasm and nuclear enlargement.

Among the 2 subjects with platelet count estimated by 3 anticoagulants, EDTA, $MgSO_4$, and Na citrate, $MgSO_4$-anticoagulated sample had the higher platelet count compared with Na citrate–anticoagulated sample. Moreover, both the smears from Na citrate–anticoagulated blood showed platelet aggregates, whereas the smears from $MgSO_4$-anticoagulated blood of the same subjects showed no platelet aggregates.

Conclusions

Based on the results, $MgSO_4$ significantly increased the platelet counts when used as an anticoagulant in subjects with EDTA-induced pseudothrombocytopenia. However, as the morphology of RBC and WBC in Leishman-stained smears is below average quality and there is a significant difference in MCV, MPV estimation in $MgSO_4$-anticoagulated samples, $MgSO_4$ can be used as an alternative anticoagulant only to estimate the platelet counts in EDTA-induced pseudothrombocytopenia. It can be concluded that $MgSO_4$ can be used as an alternate anticoagulant to estimate platelet count when platelet aggregates or spuriously low platelet counts are observed in EDTA-anticoagulated blood.

Further studies on different concentrations of $MgSO_4$ have to be tried to determine the effective concentration of $MgSO_4$ to be used as an ideal anticoagulant in EDTA-induced pseudothrombocytopenia.

Author Contributions

CC: Conception, design, acquisition of data, analysis, interpretation, drafting, revising and final approval.
RKNR: Design, acquisition of data, drafting, revising.
NS: Design, acquisition of data, drafting.

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