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Research Article

Comparative study of estimation of Platelet Counts and Other Hematological Parameters in Pseudothrombocytopenia Using Alternative Anticoagulant: Magnesium Sulfate

Dr CH Pawan Pratap Singh[1], Dr Manoj Kumar Yadav[2]

[1] MD, Assistant Professor, Department of Pathology, Dr KNS Memorial Institute of Medical Sciences, Barabanki, U.P. India.
[2] Assistant professor, Autonomous State medical college Sonebhadra, U.P., India.

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Corresponding Author:

Dr CH Pawan Pratap Singh
MD, Assistant Professor, Department of Pathology, Dr KNS Memorial Institute of Medical Sciences, Barabanki, U.P. India.

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ABSTRACT

The platelet counts other hematological parameters were compared in blood samples anticoagulated with MgSO4 and EDTA. A total of 15 samples were taken, and the platelet counts were observed to be significantly high in MgSO4- anticoagulated blood samples ranging from 50 × 103 to 499 × 103/µL, whereas in EDTA-anticoagulated blood samples, the counts ranged from 15 × 103 to 345 × 103/µL. 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 MgSO4-anticoagulated blood was below average. In conclusion, MgSO4 can be used as an alternative anticoagulant only to estimate the platelet counts in EDTA-induced pseudothrombocytopenia.

Keywords*: Low platelet count, MgSo4, sodium citrate, antiaggregation.*

INTRODUCTION

EDTA has been recommended as the anticoagulant of choice for hematological testing because it allows the best preservation of cellular compo- nents 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 l2ow platelet values.[2]

EDTA-induced pseudothrombocytopenia can be rec- ognized 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 transfu- sions, and unnecessary delay in surgeries and medical manage-ment in subjects.[4]

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 pseudo-thrombocytopenia 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 (MgSO4) was used as anticoagulant to estimate manual platelet count and also as a systemic anticoagulant in patients with cardiac disease.[4,7] MgSO4 was found to be an effective alternate anticoagulant to resolve EDTA-induced pseudothrombocytopenia.

Objectives:

1) To compare total platelet count, mean platelet volume as analyzed in automated hematology analyzer with MgSO4 and EDTA as anticoagulants in subjects with EDTA-induced pseudothrombocytopenia.
2) To compare the other estimated parameters in hematol- ogy analyzer with EDTA-anticoagulated and MgSO4-anticoagulated samples.

METHODS

This is a systematic review which was conducted at the Pathology Department of Dr KNS Memorial Institute of Medical Sciences, Barabanki, U.P. India.

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	$7.45\times10^3/\mu L$	9.895	$10\times10^3/\mu L$	$353\times10^3/\mu L$
Corrected platelet count in $MgSO_4$-anticoagulated blood	$^1 10\times10^3/\mu L$ 1	10.789	$53.9\times10^3/\mu L$	$499.4\times10^3/\mu L$
MPV in EDTA-anticoagulated blood	9.190 fL	1.539	6.934 fL	12.43 fL
MPV in $MgSO_4$-anticoagulated blood	7.860fL	1.958	5.732 fL	15.234 fL

Abbreviation: MPV, mean platelet volume.

If there were 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 contain- ing MgSO4.

2.50 ML whole blood was collected and was added to a tube containing 0.3 mL MgSO4 at a concen- tration of 4.098 mOsmol/mL; 0.3 mL MgSO4 was aliquoted from commercially available MgSO4 injection (Magneon; Neon Laboratories Ltd, Mumbai, India). If patient con- sented, 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

Hemato- logical parameters were estimated and run 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 hema- tocrit obtained in blood collected with MgSO4 and sodium citrate as anticoagulant are multiplied by 1.1 to account for the different blood-to-anticoagulant ratio in the MgSO4 and sodium citrate–anticoagulated tube.[8,9]

Statistical analysis

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

RESULTS

The criteria for selecting EDTA-induced pseudothrombocyto- penic 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.

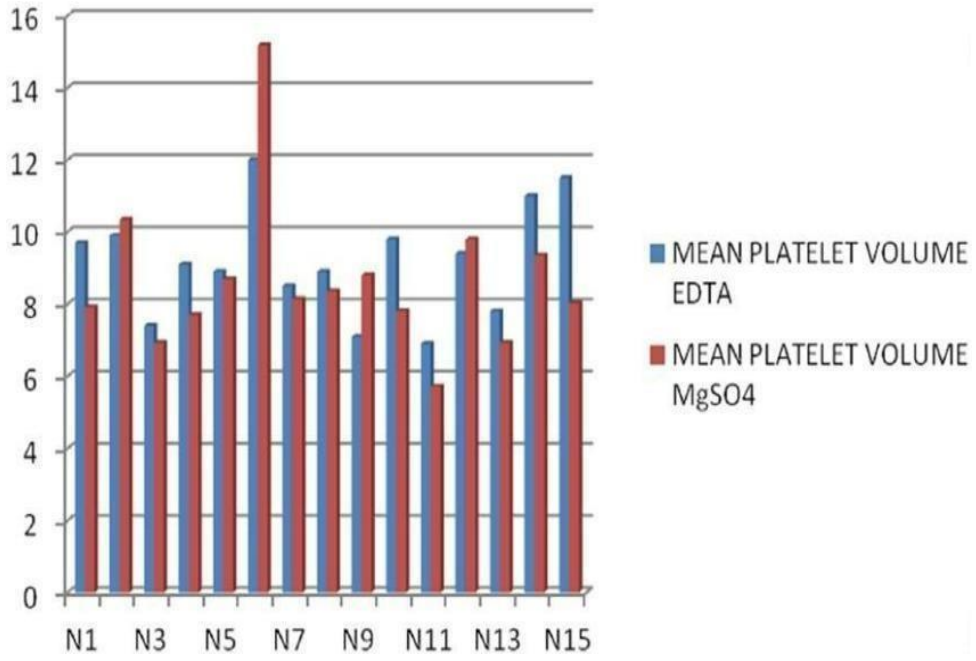
Platelet counts ranged from 15×10^3 to 345×10^3 /µL, with a mean platelet count of 17×10^3 /µL in samples anticoagulated with EDTA, whereas in samples anticoagulated with $MgSO_4$, the mean platelet count was 110×10^3/µL and the platelet counts ranged from 50×10^3 to 499×10^3/µL. However, the mean plate- let volumes varied from 6.9 to 12 fL in EDTA- anticoagulated samples and from 5 to 15 fL in MgSO4-anticoagulated samples.

The mean difference in the platelet count between EDTA- anticoagulated and MgSO4-anticoagulated blood samples was $9^3 \times 10^9$/L with a 95% confidence interval (84.1-101.8). The mean difference in the MPV between the EDTA-anticoagulated and MgSO4-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 MgSO4-anticoagulated blood samples was statistically significant.

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 MgSO4-anticoagulated blood. Red blood cell count, WBC count, Hb, and the differen- tial count were comparable between the EDTA- anticoagulated and MgSO4-anticoagulated blood. However, MCV showed a statistical difference between EDTA- anticoagulated and MgSO4-anticoagulated blood.

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 MgSO4-anticoagulated blood. Because there were only 2 subjects, statisti- cal analysis was not performed.

The quality of the Leishman-stained smears collected in MgSO4-anticoagulated tube was below average when com- pared with EDTA smears. The morphology of WBC and A graphical comparison of mean platelet volume when samples are anticoagulated with EDTA and MgSO4.



RBC was below par in MgSO4 smears; however, the platelet morphology was of average quality.

DISCUSSION

This study aims at using an alternative anticoagulant to esti- mate the platelet count in spuriously low platelet counts due to EDTA-dependent platelet aggregation. EDTA, though, is a good anticoagulant for routine hema- tology analysis;[4] EDTA-induced pseudothrombocytopenia is 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 A2, prosta- glandin I2, 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 com- petes with calcium for binding sites on prothrombin, hence inhibiting coagulation.

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]

Abbreviation: MPV, mean platelet volume.

Comparison of blood samples anticoagulated with MgSO4 and EDTA.

SUBJECT	RBC COUNT×10⁶/µL		HEMOGLOBIN, G/DL		MCV, FL			WBC COUNT×10⁶/µL		PLATELETS×10³/µL		MPV, FL
	$MgSO_4$	EDTA	$MgSO_4$	EDTA	$MgSO_4$	EDTA		$MgSO_4$	EDTA	$MgSO_4$	EDTA	$MgSO_4$
Np1	4.1034	4.054	13.094	13.004	104.61	95.20	5.50	6.80	202.40	137.00	7.92	9.705
Npp2	3.5313	3.563	11.003	11.2044	102.19	94.50	9.46	6.30	56.10	21.00	10.34	9.904
Np3	3.2013	3.605	8.3678	9.1034	85.36	79.10	5.72	8.00	192.50	80.00	6.93	7.403
Np4	2.8162	2.586	8.808	7.9035	101.09	92.90	9.46	8.20	299.20	11.00	7.70	9.103
Np5	4.4991	4.7488	13.097	13.705	97.68	89.50	18.04	19.70	179.30	24.00	8.69	8.900
Np6	5.3790	4.937	10.5653	9.808	70.95	64.20	9.02	6.90	132.00	97.00	15.18	12.004
Npp7	3.0457	2.9163	8.254	7.906	89.65	80.80	5.72	8.20	188.10	29.00	8.14	8.504
Np8	3.6854	3.473	11.117	10.704	107.03	99.50	9.57	10.60	499.40	353.00	8.36	8.905
Np9	3.8063	4.072	9.6854	10.303	85.69	79.60	8.03	12.10	129.80	10.00	8.80	7.104
Np10	4.6868	4.829	11.336	11.503	82.28	74.90	10.56	18.10	305.80	52.00	7.81	9.803
Np11	3.1799	3.850	9.794	11.708	106.59	96.10	13.86	19.70	390.50	139.00	5.72	6.905
Np12	5.4566	4.110	10.896	8.205	74.58	65.10	9.24	5.70	53.90	128.00	9.79	9.404
Np13	2.7063	2.5900	8.145	7.706	99.22	89.30	8.47	7.70	202.40	28.00	6.93	7.803
Np14	3.4212	3.9287	8.6944	9.703	84.92	78.40	5.39	4.70	84.70	38.00	9.35	11.004
Np15	4.004	4.436	11.555	12.804	98.67	90.50	12.21	17.90	132.00	20.00	8.03	11.505
P value	0.9540		0.8405		<0.05		0.141		<0.05		0.03	

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

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

SUBJECT	RBC COUNT × 10^6/ L			HB, G/DL			MCV, FL		
	$MgSO_4$	EDTA	Na CITRATE	$MgSO_4$	EDTA	Na CITRATE	$MgSO_4$	EDTA	Na CITRATE
Np5	4.489	4.745	4.488	13.094	13.7034	12.874	97.6855	89.50	96.3644
Np9	3.999	4.075	4.059	9.684	10.3210	10.234	85.645	79.606	86.243
SUBJECT	WBC COUNT × 10^6/ L			PLATELETS × 10^3/ L			MPV, FL		
	$MgSO_4$	EDTA	Na CITRATE	$MgSO_4$	EDTA	Na CITRATE	$MgSO_4$	EDTA	Na CITRATE
Np5	18.0444	19.704	18.04	179.334	24.0034	117.733	8.6967	8.906	9.1355
Np9	8.034	12.103	8.584	129.845	10.0045	102.334	8.807	7.104	9.3565

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

In this study, we studied the usefulness of MgSO4 as an alternative anticoagulant to estimate the platelet count in subjects with EDTA-induced pseudothrombocytopenia.

MgSO4-anticoagulated samples gave a significantly higher platelet count in subjects with EDTA-induced pseudothrombocytopenia. Out of 15 subjects, only 4 (26.674%) 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 plate- let aggregation in MgSO4 samples was smaller in size as com- pared with platelet aggregates found in smears made from EDTA-anticoagulated blood in the same subjects.
The lesser concentration may have attributed to the platelet clumps observed in 26.67% of MgSO4-anticoagulated blood samples.

MPV (Mean platelet volume) was significantly higher in EDTA- anticoagulated samples when compared with MgSO4-anticoagulated samples. This phenomeno$_4$n is possibly due to aggregation of platelets giving a false high platelet volume and EDTA-induced swelling of platelets. Also, the difference in platelet volume can be attributed to the fact that automated can be ascertained by comparing EDTA-anticoagulated and MgSO4-anticoagulated blood samples from subjects without
EDTA-induced pseudothrombocytopenia.
Parameters such as RBC count, WBC count, hemoglobin, and differential WBC count showed comparable results between EDTA-anticoagulated and MgSO4- 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 MgSO4-anticoagulated blood samples was below aver- age quality. The RBCs had a dull pink hue with elongated morphology, and WBCs had an inadequate staining of cyto- plasm and nuclear enlargement.

Among the 2 subjects with platelet count estimated by 3 anticoagulants, EDTA, MgSO4, and Na citrate, MgSO4-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 MgSO4-anticoagulated blood of the same subjects showed no platelet aggregates.
hematology analyzers are calibrated with EDTA blood sam- ples. However, the actual effect of MgSO4 on platelet volume.

CONCLUSIONS

On the basis of the results, MgSO4 significantly increased the plate- let 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 MgSO4-anticoagulated samples, MgSO4 can be used as an alternative anticoagulant only to estimate the platelet counts in EDTA-induced pseudothrom- bocytopenia. Lastly It can be concluded that MgSO4 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.

REFERENCES

1. Choccalingam R, Radha R. *Estimation of Platelet Counts and Other Hematological Parameters in Pseudothrombocytopenia Using Alternative Anticoagulant — Magnesium Sulfate.* J Clin Lab Anal. 2017. — Direct comparison of EDTA vs $MgSO_4$ for platelet counts in PTCP. PMC
2. Männuss S, Schuff-Werner P, Dreißiger K, Burstein C. *Effective estimation of correct platelet counts in pseudothrombocytopenia using a magnesium-containing anticoagulant.* Br J Haematol. 2013. — Case series of 44 patients where Mg-anticoagulated samples restored correct platelet counts vs EDTA. PMC+1
3. Lippi G, Salvagno GL, Franchini M, Guidi GC. *Measurement of platelet count with different anticoagulants in thrombocytopenic patients.* Haematologica. 2019. — Comparison of K3EDTA, CTAD, $MgSO_4$ and CPT; recommends $MgSO_4$ for platelet counting in some settings. Haematologica
4. Cid J, et al. *Magnesium sulfate as an alternative in vitro anticoagulant in pseudothrombocytopenia / Inhibition of platelet aggregation by $MgSO_4$.* American Journal of Clinical Pathology / Platelets research (AJCP). 2016–2017. — Analytical study on platelet count, MPV and platelet activation using $MgSO_4$; shows method-dependent differences. Oxford Academic+1
5. Soulard M, Ketatni H, Visseaux C, Croix P, Cohen P. *Impact of Shaking EDTA, Citrate, or $MgSO_4$ Tubes on Platelet Count Results.* Journal of Clinical Medicine. 2024;13:5350. — Recent pre-analytical study: handling/transport affects platelet results in $MgSO_4$ and other tubes. MDPI
6. Zandecki A, Geneviève F,Gérard J,Godon A. *Spurious counts and spurious results on haematology analysers: Part I — Platelets.* Int J Lab Hematol. 2007. Foundational review describing EDTA-dependent PTCP and alternative anticoagulants (mentions $MgSO_4$). Haematologica
7. Cattaneo M, et al. *Pseudothrombocytopenia—A review on causes, occurrence and clinical consequences.* J Clin Med. 2020. — Contemporary review that discusses $MgSO_4$, ACD, CTAD as alternate anticoagulants and lab recommendations. MDPI
8. Soulard M, et al. *Influence of anticoagulants on platelet counts — French-speaking Cellular Hematology Group (GFHC) recommendations / study.* (Report / preprint / ResearchGate). — Multicentre/real-life data comparing stability of platelet counts across anticoagulants; $MgSO_4$ among the better options for stability. ResearchGate
9. Raval (et al.). *Effective estimation of correct platelet counts in pseudothrombocytopenia using magnesium-containing anticoagulant.* British Journal of Haematology (full text / PDF). — PDF copy and analysis confirming $MgSO_4$ efficacy in preventing PTCP artefact. air.unimi.it+1
10. Soulard M, Ketatni H, Visseaux C, et al. *Pseudothrombocytopenia case reports and handling recommendations.* J Clin Med / ResearchGate (case series and pre-analytic guidance). — Useful for lab workflows when switching to $MgSO_4$ tubes. ResearchGate+1
11. Wikipedia — *Pseudothrombocytopenia* (summary page with references). — Good quick summary and pointer list of primary literature (use only as index, not as primary citation). Wikipedia
12. Additional AJCP / OUP PDF on $MgSO_4$ vs EDTA platelet measurement (detailed methods and analyzer comparisons). — Useful for understanding analyzer-dependent differences (impedance vs light scatter). Oxford Academic+1
13. Gaseous / transport effect study: *Impact of Shaking EDTA, Citrate, or $MgSO_4$ Tubes on Platelet Count and Indices.* J Clin Med (MDPI). 2024 — Shows handling (agitation/transport) affects platelet counts in $MgSO_4$ and citrate tubes; practical implications for sample handling when using $MgSO_4$. MDPI