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Research Article

Effect of magnesium on rotational thromboelastometry (ROTEM) and total blood products requirement in patients undergoing liver transplantation

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KEYWORDS

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Abstract *Aim:* This study aimed to assess the effect of Mg^{2+} therapy in improving ROTEM and total blood products requirement during liver transplantation.
Patients and method: The study includes 30 patients suffering from end-stage liver disease with MELD (model for end-stage liver disease) classification ranging from 15 to 24. The patients were divided into two equal groups: Group I: The patients were given placebo (100 mL) normal saline (NS). Group II: The patients were given 2 g Mg in 100 mL NS.
Results: In group II significant decrease in CT (clotting time) in EXTEM (which evaluates the extrinsic pathway after addition of tissue factor), INTEM (which evaluates the intrinsic pathway after contact activation), APTEM (assess fibrinolytic pathway) and FIBTEM (assess fibrinogen level after tissue factor activation), decrease in clot formation time (CFT) in EXTEM, increase α-angel (the angle determined from the reaction time to the inflection point of the amplitude of the forming clot), in INTEM and FIBTEM and increase A10 (clot strength after 10 min) EXTEM, FIBTEM and APTEM in comparison with group I.

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Conclusions: (1) The ROTEM analysis shows the effect of Mg ions in coagulation that can be assessed by laboratories not clinically. (2) Mg improved coagulation in patients about to undergo liver transplantation who showed ROTEM evidence of hypocoagulability.

1. Introduction

The significance of magnesium and its relationship to the origin of life has been traced from the composition of the earth's crust (rich in iron–magnesium) and the primeval ocean rich in magnesium. The central role of magnesium within the chlorophyll molecule and as a co-factor for the enzymes in the 12 trans-phosphorylation reaction in photosynthesis make it probably the most important inorganic element in the production of food and fossil fuels [1]. Calcium ions are the essential constituents of the coagulation cascade. However, participation of Mg ions in this mechanism has been ignored, recently folding of the native tertiary structure of factor IX requires not only Ca but also Mg ions, as factor IX has a specific binding site(s) for Mg ions (Fig. 1) that does not interact with Ca ions, and, moreover, that the binding of Mg ions promotes additional changes in the Ca bound conformation of the molecule [2]. The Ca-dependent activation of factor IX by the intrinsic pathway activator factor XIa was accelerated by Mg ions. Thus, the Mg-induced additional conformational change appears to enhance the protein's ability to function. The activation by factor XIa is, however, not essential for physiological coagulation. Therefore, the effect of Mg ions on the function of factor IX in the coagulation pathway initiated by VIIa {TF} [4].

The liver plays a major role in hemostasis, as most of the coagulation factors, anticoagulant proteins and components of the fibrinolytic system are synthesized by hepatic parenchymal cells. Additionally, the reticuloendothelial system of the liver helps to regulate coagulation and fibrinolysis by clearing these coagulation factors from the circulation [3]. Patients with end-stage liver disease usually show hypomagnesaemia before liver transplantation due to decreased intake and gastrointestinal absorption of magnesium, increased excretion of magnesium due to laxatives used to prevent hepatic coma and diuretics used to treat ascites and is accompanied by hypokalemia and hypocalcaemia. Hypomagnesaemia worsens during liver transplantation, because of massive blood transfusion since the anticoagulant citrate absorbs magnesium as well as calcium ions and causes cardiovascular instability such as the development of perioperative cardiac arrhythmias [5]. The etiology of impaired hemostasis resulting from abnormal liver function is often multifactorial and may include impaired coagulation factors synthesis, synthesis of dysfunctional coagulation factors, increased consumption of coagulation factors, altered clearance of activated coagulation factors and quantitative and qualitative platelet disorders [3].

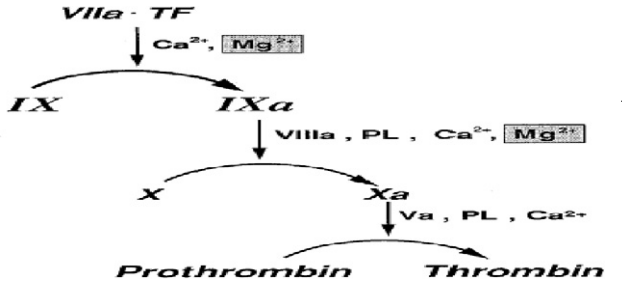

Figure 1 The main stream of the blood coagulation cascade.

Point of care (POC) monitoring devices assessing the viscoelastic properties of whole blood, i.e. thromboelastography (TEG), rotational thromboelastometry (ROTEM) and Sonoclot analysis, may overcome several limitations of routine coagulation tests in the perioperative setting [6].

The term "TEG" was introduced by Hartert in his first publication on thromboelastography in 1948. Surprisingly, in 1993, an American company obtained a trade mark on this term in the USA, after 45 years of its use as a generic medical term. In order to achieve a global uniformity of the name, the manufacturer of the ROTEM® system (Pentapharm GmbH, Munich) has renamed its instrument from "ROTEG" into "ROTEM" and the tests accordingly from "EXTEG" into "EXTEM", "INTEG" into "INTEM" etc. in 2003. "TEM" thereby stands for "thromboelastometry" (analogous to the term thromboelastography), thus the plotting of the clot firmness [7].

2. Patients and methods

After approval of local ethical committee and patients informed consent was obtained, the study was done on 26 male and 4 female patients suffering from end-stage liver disease with MELD classification ranging from 15 to 24.The patients were randomly allocated by closed envelopes into two equal groups: group (I) (control group): the patients were given placebo 100 mL of normal saline (NS) infused (IV) over 5 min and group II (study group): the patients were given 2 g Mg in 100 mL NS infused IV over 5 min. Patients scheduled for liver re-transplantation, patients with portal vein thrombosis, patients with previous major abdominal surgery and patient with platelet count $< 50{,}000 \times 10^3/\mu L$ were excluded from the study. The surgical team and personnel inside the operating room were blinded to the assigned technique; the same surgical team operated on all cases.

Rotational thromboelastometry (ROTEG 05) generated data were obtained from a computer controlled (software version linux S/W 1.0.6). ROTEM system (Pentapharm, Munich, Germany). This ROTEG includes four channel instrument computer, activators and disposable cups and pins. For quality assurance the ROTEM system was assessed with ROTROL N (lyophilized normal plasma) without activator (NATEM) with partial thromboplastin phosopholipids made from rabbit brain (INTEM) and with thromboplastin from rabbit brain (EXTEM) The ROTEM analyses were performed by electronic pipette that mixes blood into plastic cup with the specific reagent the reduction in the pin's movement caused by the clot was mathematically transformed into firmness amplitude and plotted against time, resulting into thromboelastomeric traces.

Table 1 Demographic data of the studied groups.

	Group (I)	Group (II)	*P*
Age (mean)	48.7 ± 5.5	47.8 ± 5.8	>0.05
Weight (mean)	63.4 ± 5.94	61.7 ± 6.51	>0.05

Table 2 Causes of liver cell failure.

	Group I	Group II	Total
	No.	No.	No.
HCV	8/15	8/15	16/30
HCV and HBV	3/15	4/15	7/30
HCV and BIL	1/15	1/15	2/30
BIL	1/15	1/15	2/30
HCV and HCC	2/15	0/15	2/30
HCC	0/15	1/15	1/30

Parameter of clot initiation, propagation and strength were studied using ROTEM hemostasis systems. Clot initiation was denoted by (CT), which the time required to obtain 2 mm amplitude following the initiation of data collection, clot propagation was designated by the alpha (α) angle, (CFT) is the time from initiation of clotting until a clot firmness of 20 mm is detected and clot strength after 10 min was designated by (A10).We performed the following ROTEM assays, EXTEM, INTEM, APTEM and FIBTEM and platelet antagonism.

For every patient and Prior to skin incision, a central venous catheter introduced through the right internal jugular vein was used to draw a 4 mL blood sample using the two syringe technique were immediately mixed with 0.5 mL of 3.2% citrated sodium solution then underwent ROTEM analysis to obtain a base line assessment. Half the patients in the trial were infused with 2 g $MgSO_4$ mixed with 100 mL normal saline over 5 min. The other half were infused with placebo. After a 10 min interval another blood sample was withdrawn using the same technique to determine ROTEM changes. No other drugs were given during the 10 min interval. Other blood samples withdrawn before and after infusion were sent to the laboratory to measure the serum Mg concentration, PT, PTT, INR and PC.

3. Statistical analysis

The collected numerical data were presented as mean, standard deviation. Categorical data were presented as number. Data were analyzed using Statistical Package of Social Science (SPSS) version 16. Student *t* test was used to analyze the data. Changes between means *P* values of <0.005 were considered significant.

4. Results

4.1. Demographic features of patients

The demographic data of the patients had no significant correlation (Table 1).

4.1.1. Causes of liver cell failure

The patients of group (I) were; 8/15 patients with HCV, 3/15 patients with HCV and HBV, 1/15 patient with HCV and bilharizial cirrhosis, 1/15 patient with bilharizial cirrhosis, 2/15 patients with HCV and HCC and 0/15 patient with HCC. The patients of group (II) were; 8/15 patients with HCV, 4/15 patients with HCV and HBV, 1/15 patient with HCV and bilharizial cirrhosis, 1/15 patient with bilharizial cirrhosis, 0/15 patient with HCV and HCC and 1/15 patient with HCC (Table 2).

4.1.2. Hemodynamic and laboratory data

In group II, heart rate and systolic blood pressure were significantly but central venous pressure and diastolic blood pressure showed no significant changes (Table 3). PT and PTT were highly significantly decreased but PC and INR were not significantly changed (Table 4).

Table 3 Hemodynamic data of the patients.

	HR		SBP		DBP		CVP	
	Group (I)	Group (II)	Group (I)	Group (II)	Group (I)	Group (II)	Group (I)	Group (II)
Before Mg	81.8 ± 11.4	81.8 ± 11.4	130.0 ± 14.2	130.3 ± 14.1	82.7 ± 12.5	83.5 ± 11.9	12.47 ± 2.5	12.47 ± 2.4
After Mg	80.4 ± 13.1	76 ± 7.1	130.1 ± 12.1	121.4 ± 14.2	82.5 ± 11.1	78 ± 11.3	12.9 ± 2.2	12.9 ± 2.3
T	1.19	2.35	0.05	4.15	0.07	1.6	1.29	1.28
P	>0.05	<0.05*	>0.05	<0.001**	>0.05	>0.05	>0.05	>0.05

Table 4 Laboratory data of the patient.

	PT		PC		INR		PTT	
	Group (I)	Group (II)	Group (I)	Group (II)	Group (I)	Group (II)	Group (I)	Group (II)
Before Mg	17.6 ± 2.2	18.5 ± 1.2	48.6 ± 10.6	49.8 ± 7	1.71 ± 0.18	1.6 ± 0.15	39.6 ± .3.6	42.94 ± 3.2
After Mg	17.86 ± 2.1	17.3 ± 1.3	49.6 ± 13.1	50 ± 7.5	1.72 ± 0.2	1.58 ± 0.16	40.8 ± 4.4	38.48 ± 3.9
T	1.5	11.61	0.7	0.77	0.97	0.01	0.63	4.54
P	>0.05	<0.001**	>0.05	>0.05	>0.05	>0.05	>0.05	<0.001**

Table 5 Comparison between the two groups as regards blood products requirement.

	Group I		Group II		*t*	*P*
	No.	Mean ± SD	No.	Mean ± SD		
Blood	15	10.93 ± 2.865	15	10.93 ± 2.685	0.01	> 0.05
Plasma	15	17.47 ± 1.807	15	17.73 ± 2.404	0.3	> 0.05
Platelet	15	13.60 ± 8.007	15	13.20 ± 7.243	0.1	> 0.05
Cryoprepecitate	15	3.00 ± 0.845	15	3.00 ± 0.926	0.01	> 0.05

Table 6 ROTEM data.

	Before		After		*T*	*P*
	No.	Mean ± SD	No.	Mean ± SD		
CT in EXTEM	15	85.93 ± 14.429	15	57.60 ± 16.172	6.4	< 0.001
CT in INTEM	15	207.87 ± 32.240	15	171.40 ± 35.936	5.8	< 0.001
CT in FIBTEM	15	66.73 ± 22.899	15	47.13 ± 12.861	3.7	< 0.05
CT in APTEM	15	160.00 ± 313.786	15	65.00 ± 13.638	4.26	< 0.001
CFT in EXTEM	15	234.53 ± 68.039	15	193.60 ± 68.517	5.6	< 0.001
CFT in INTEM	15	237.87 ± 97.798	14	240.79 ± 94.190	1.1	> 0.05
CFT in FIBTEM	12	631.67 ± 405.372	11	741.17 ± 139.943	0.9	> 0.05
CFT in APTEM	12	249.67 ± 119.114	14	192.43 ± 85.208	1.9	> 0.05
α-angel in EXTEM	15	60.93 ± 10.573	15	66.87 ± 10.426	1.8	> 0.05
α-angel in INTEM	15	67.60 ± 13.700	15	71.00 ± 13.099	2.3	< 0.05
α-angel in FIBTEM	12	65.33 ± 11.023	10	69.20 ± 10.830	2.3	< 0.05
α-angel in APTEM	15	60.07 ± 12.702	15	64.87 ± 15.914	1.2	> 0.05
A10 in EXTEM	15	35.40 ± 7.510	15	38.00 ± 5.237	2.6	< 0.05
A10 in INTEM	15	37.40 ± 11.274	15	38.13 ± 11.319	0.6	> 0.05
A10 FIBTEM	13	7.38 ± 3.280	12	10.00 ± 2.594	4.2	< 0.01
A10 APTEM	13	39.54 ± 14.931	14	44.14 ± 12.322	3.2	< 0.05

4.1.3. Blood products requirement

Comparison between two groups with and without Mg in blood, plasma, platelet and cryoprepcitate requirement showed non significant differences (Table 5).

4.1.4. ROTEM data

There were highly significant decreases in CT in EXTEM, INTEM, APTEM and significant decrease CT in FIBTEM. There were also highly significant decreases in CFT in EXTEM and significant increases in α-angel in INTEM, FIBTEM finally highly significant increase in A10 in FIBTEM and significant increase EXTEM and APTEM (Table 6).

5. Discussion

In accordance with our hypothesis and based on previous data, we found that magnesium therapy improved coagulation in patients about to undergo liver transplantation who showed ROTEM evidence of hypocoagulability. The fact that end-stage liver disease patients show ROTEM findings of general hypocoagulability had already been reported [6,8].

Our findings as decreases in CT in EXTEM, INTEM, APTEM and FIBTEM after Mg infusion were considered as means to decrease the time of beginning of clot formation, to help in activation extrinsic and intrinsic pathways, to help to increase fibrin formation and to improve speed of cross linking. Increased α-angel in INTEM and FIBTEM after Mg infusion was considered the result of increased propagation of the clot. Increased A10 in EXTEM, FIBTEM and APTEM after Mg infusion was considered the result of increased strength of the clot.

Most commonly, routine laboratory-based coagulation tests (e.g., PT, INR, APTT, and fibrinogen) and platelet count are being used to assess the patient's current coagulation status. However, the value of these tests has been questioned in the acute perioperative setting because there are delays from blood sampling to obtaining results (45–60 min), coagulation tests are determined in plasma rather than whole blood, no information is available on platelet function (PF) and the assays are performed at a standard temperature of 37 °C rather than the patient's temperature [9].

TEG/ROTEM both measure and graphically display the changes in viscoelasticity at all stages of the developing and resolving clot, i.e., the time until initial fibrin formation (TEG reaction time; ROTEM clotting time [CT]), the kinetics of fibrin formation and clot development (TEG kinetics, α angle; ROTEM clot formation time, α angle, the ultimate strength and stability of the fibrin clot (TEG maximum amplitude [MA]; ROTEM maximum clot firmness [MCF]), and clot lyses (fibrinolysis) [10].

Jong et al. denoted decreased potassium (K) and coagulation time ($r + k$) after magnesium therapy as the result of increased early fibrin formation and improved speed of cross linking. Increased maximum amplitude (MA) was thought to be a result of improved clot strength secondary to improved function of platelets. The increased TEG index suggests an overall improvement in coagulation [5].

On the other hand; Ames et al., reported no change in coagulation following the administration of therapeutic doses of Mg. The only significant change was in α-angel which actually suggested an increase in coagulant activity [11]. No significant change was seen on the TEG when healthy volunteers were treated with magnesium. The claim that the effect of magnesium on the coagulation system is insignificant is based on an *in vitro* study [12]. Briel et al. also investigated the effect of magnesium on thromboelastography and demonstrated inhibitory influences on all measured parameters. These studies were, however, *in vitro* and although both showed significant changes in thromboelastographic parameters, neither claimed clinical significance [13]. Wall et al., compared normal women in labor with women in premature labor receiving magnesium, again using thromboelastography, and concluded no differences [14]. Fuentes et al. demonstrated, in vivo, that magnesium appears to prolong the bleeding time in pregnancy [15].

Jong et al., recommended that a study is needed to investigate the effect of magnesium therapy and the improvement of TEG variables on blood product requirements during liver transplantation to confirm the findings when using ROTEM [5].

5.1. Our results must be interpreted with caution

First, the number of patients were 30 because of limited donors and the high cost. *Second*, the device gives multiple Code error, according to the representative of the manufacturer (Pentapharm Gmbh) in Egypt, 4033 Firmness cannot be determined due to mechanical failure, 3023 Cuvette or pin not correctly placed, 5043 Axis heavily disturbed during placing of pin OR Damaged Ball Bearing on axis, 10003 and 6033 Running measurement influenced by drying of sample. Errors 4033, 3023, 6033 and 10003 are Operation/User Errors; that can be overcome by correct operation. If error 5043 appears only once or twice, can be a random error; but; if appeared frequently then instrument needs some maintenance service. *Third*, the cause of human error and drying sample may be due to use of multiple reagents and seven steps of the pipetting. The number of all results from ROTEM with and without Mg, before and after Mg infusion were 960 and the number of error of sample 58 which represented 6.04% was excluded from the results.

6. Conclusion

1. The ROTEM analysis shows the effect of Mg ions in coagulation that can be assessed laboratories not clinically.
2. Mg improves coagulation in patients about to undergo liver transplantation who showed ROTEM evidence of hypocoagulability.

7. Recommendations

First, to increase the number of patients. *Second,* to use single shot reagents. *Third*, to decrease the number of steps and decrease reagents waste decreasing the cost.

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