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Research Article / Araştırma Makalesi

The Relationship Between Thromboelastography and Clinical Outcome in Acute Stroke Patients Receiving Thrombolytic Therapy

Trombolitik Tedavi Alan Akut İnmeli Hastalarda Tromboleastografi ile Klinik Sonlanım Arasındaki İlişkinin Araştırılması

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Abstract: Thromboelastography (TEG) is a hemostatic test that measures the shear elasticity and the dynamics of clot formation and the strength and stability of formed clot. There are limited data about TEG in acute ischemic stroke who receives thrombolytic therapies. This study aimed to investigate the impact of coagulation parameters obtained by rotational thrombelastography (ROTEM) method on clinical outcome and intracerebral hemoorage in acute stroke patients receiving thrombolytic treatment. The study included 29 patients with acute stroke who received rtPA treatment between June 2013 and March 2014. Blood samples were taken from the patients before starting thrombolytic therapy. By ROTEM®; INTEM and EXTEM analysis, the parameters of CT (clotting time=sec), CFT (clot formation time=sec) and MCF (maximum clot firmness=mm) were tested. The demographic information of patients, NIHSS scores at the time and 24 hours after the admission and brain tomography results were recorded. In addition, the data obtained by ROTEM method were compared with the normal group. Compared to healthy group, ischemic stroke patients had lower intemCT ($p<0.05$), extemCT ($p=0.01$) and extemCFT ($p<0.05$) and higher extemMCF ($p<0.05$). These results were consistent with hypercoagulability. TEG parameters were not correlated with symptomatic hemorrhage, mortality and poor outcome in patients who receive trombolytic treatment. Thrombelastography shows that patients with ischemic stroke are in hypercoagulable state. Further studies are needed to examine this observation and its relationship with clinical outcome.

Keywords: Thrombelastography, acute stroke, hypercoagulable, thrombolytic therapy

Özet: Tromboelastografi (TEG), pıhtı oluşumunun elastikiyetini ve dinamiklerini ve oluşan pıhtıların gücünü ve stabilitesini ölçen hemostatik bir testtir. Trombolitik tedavi alan akut iskemik inmede TEG hakkında sınırlı veri vardır. Bu çalışmada, trombolitik tedavi alan akut inmeli hastalarda rotasyonel trombelastografi (ROTEM) yöntemi ile elde edilen pıhtılaşma parametrelerinin klinik sonuç ve intraserebral kanama üzerine etkisi araştırıldı. Çalışma, Haziran 2013-Mart 2014 tarihleri arasında rtPA tedavisi alan akut inmeli 29 hasta içermiştir. Hastalardan trombolitik tedaviye başlamadan önce kan örnekleri alındı. ROTEM® ile; INTEM ve EXTEM analizi, CT (pıhtılaşma süresi = sn), CFT (pıhtı oluşum süresi = sn) ve MCF (maksimum pıhtı sertliği = mm) parametreleri test edildi. Hastaların demografik bilgileri, başvuru sırasındaki ve 24 saat sonra NIHSS skorları, 3.ay mRs ve beyin tomografi sonuçları kaydedildi. Ayrıca ROTEM yöntemiyle elde edilen veriler kontrol grup ile karşılaştırıldı.Sağlıklı gruba göre iskemik inme hastalarında düşük intemCT ($p <0.05$), extemCT ($p = 0.01$) ve extemCFT ($p <0.05$) ve daha yüksek extemMCF ($p <0.05$) vardı. Bu sonuçlar hiper pıhtılaşabilirlik ile tutarlıydı. TEG parametreleri, trombolitik tedavi alan hastalarda semptomatik kanama, mortalite ve 3 ay sonraki kötü klinik ile korele değildi. Trombelastografi, iskemik inmeli hastaların hiper pıhtılaşma durumunda olduğunu gösterir. Bu gözlemi ve klinik sonlanım ile ilişkisinin incelenmesi için daha ileri çalışmalar gereklidir.

Anahtar Kelimeler: Trombelastografi, akut inme, hiperkoagulasyon, trombolitik tedavi

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1. Introduction

Thromboelastography (TEG) is a hemostatic test that measures the shear elasticity and the dynamics of clot formation and the strength and stability of formed clot [1]. It is an alternative method to conventional coagulation tests used for an overall evaluation of the hemostatic system. Compared to the standard test, TEG, ROTEM® yields faster and more reliable results. It is possible to evaluate the intrinsic and extrinsic coagulation system (INTEM and EXTEM) in a short time by ROTEM® [2].

Stroke is a clinical condition characterized by sudden neurologic dysfunction caused by blockage or bleeding in vessels to the central nervous system. Stroke occurs due to blockage of cerebral vessel in 87% of the cases and bleeding in 13% of the cases [3]. The use of thrombolytic treatment in acute ischemic stroke patients presented within 3 hours of the symptom onset as has been recommended since 1995 [4]. Moreover tPA is indicated in acute stroke patients who presented within 3-4.5 horus of symptom onset

Few studies refer to TEG for analyzing the formation of clotting in patients of acute stroke who received thrombolytic treatment. In our study, we investigated the potential usefulness of blood clotting and clot formation in ischemic stroke patients who received rtPA treatment by TEG.

Aim and hypotheses

1. To obtain the data on clot formation by TEG parameters in acute stroke patients
2. To compare the TEG parameters of acute stroke patients between a healthy control group
3. To compare the TEG parameters between patients with symptomatic hemorrhage, 3.rd months poor outcome and mortality.

2. Methods

Ethics and governance and Written consent procedure

This study was approved by the Committee for the Protection of Human Subjects at Osmangazi University Medical Center, Eskişehir, Turkey. Written consent was collected from the patients or their first degree relatives.

Patients inclusion and exclusion criteria

Patients who presented with the symptoms and signs of acute stroke to the ED and who started their symptoms within 4.5 hours according to the published acute stroke guideline were included in the study. Patients who were not treated with thrombolytic therapy and/or underwent interventional acute stroke treatment were excluded from the analysis.

Data collection and patient management

The following data were recorded for each patient: age, gender, vital signs, comorbidities, laboratory tests, bleeding parameters, NIHSS scores at the time of admission. In addition to that, baseline CT was performed before thrombolysis and control CT was obtained after 24 hours. The data were recorded by the ED team and neurologists (emergency medicine residents and stroke neurologist). All patients were assessed by stroke neurologists regarding the decision making in administration of IV tPA. All patients were seen at three months follow up by stroke neurologist and neurological outcome was determined by modified Rankin scale.

Control group

Blood samples were taken from 37 healthy and volunteering individuals in our medical centre and tested by the same healthcare personnel and with the use of the same device.

Blood Samples

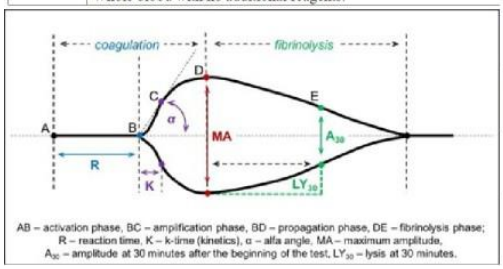
Blood was collected under minimum stasis using a 19-gauge needle. First, 2-ml blood was discarded to prevent tissue thromboplastin contamination before drawing samples for TEG analysis into 4.5 ml

vacutainers (Becton Dickinson) containing 3.2% trisodium citrate with a citrate/blood ratio of 1:9.

ROTEM® Coagulation Analyzer (Tem International, Munich, Germany) was utilized to measure TEG parameters, and the samples were analyzed within 30-90 min of blood collection by the same investigator. 300 µl citrated blood was re-calcified with 20µl 0.2 mol/l $CaCl_2$ (star-TEM; Tem International, Munich, Germany) after incubating the test solution at 37 ‘C for 2 min, and both INTEM and EXTEM analyses were performed according to the standard procedure recommended by the manufacturer (Figure 1). In INTEM analysis, the coagulation is activated with 20µl of contact activator (partial thromboplastin-phospholipid from rabbit brain extract and ellagic acid, in-TEM; Tem International, Munich, Germany). In EXTEM analysis, the coagulation is activated by 20µl of tissue factor (TF, tissue thromboplastin from rabbit brain extract, ex-TEM; Tem International, Munich, Germany). The parameters obtained from ROTEM analysis were clotting time (CT) reflecting the initiation of the coagulation, clot formation time (CFT) reflecting the rate of clot formation once the formation is initiated and maximum clot formation (MCF) representing the firmness of the clot. The method and the parameters of ROTEM® have been described in detail previously. A ‘‘hypercoagulable profile’’ was defined as a shorter CT, shorter CFT and/or higher MCF than the corresponding values in healthy controls [5].

Table 1. RoTEM subtypes.

Test	Description
INTEM	Reagents: phospholipids and ellagic acid. Ellagic acid is a natural phenol that is able to activate Factor XII (intrinsic coagulation pathway).
EXTEM	Reagent: tissue factor. Activates extrinsic coagulation pathway.
HEPTEM	Reagent: phospholipids, ellagic acid, heparinase. Heparinase is an enzyme that neutralizes heparin. Serves as an adjunct to INTEM to determine the impact of heparin on coagulopathy to guide protamine sulfate therapy.
APTEM	Reagents: tissue factor, aprotinin. Aprotinin is a bovine pancreatic trypsin inhibitor that also inhibits plasmin. Serves as an adjunct to EXTEM to predict the clinical effect of fibrinolysis inhibitors in case of hyperfibrinolysis. Mimics treatment with tranexamic acid.
FIBTEM	Reagent: cytochalasin D. Cytochalasin D is a fungus produced alkaloid that is able to inhibit platelet activity. Helps to differentiate between hypofibrinogenemia and platelet deficiency.
NATEM	Reagent: none. Whole blood with no additional reagents.



AB – activation phase, BC – amplification phase, BD – propagation phase, DE – fibrinolysis phase; R – reaction time, K – k-time (kinetics), α – alfa angle, MA – maximum amplitude, A_{30} – amplitude at 30 minutes after the beginning of the test, LY_{30} – lysis at 30 minutes.

Table 2. TEG parameters with normal values.

Parameter	Description	Reference values	Biological meaning
Reaction time (R)	Time from the beginning of the test to the first detectable clot formation (amplitude of 2 mm)	5-10 min	**Activation phase.** Time that is needed to activate the intrinsic pathway and initiate fibrin deposition. Depends on the concentration and function of the coagulation factors (single or multiple), reflects the ability of blood to generate thrombin. May be affected by congenital or acquired coagulation factors deficiency and anticoagulation therapy.
Kinetics (K)	Time from the beginning of clotting to the formation of the clot with a certain level of strength, corresponding to the amplitude of 20mm	1-3 min	**Amplification phase.** The speed of initial fibrin deposition and cross-linking. Depends on the concentration of fibrinogen and its activation (the abundance of thrombin). To a lesser extent dependent on platelets.
Alpha angle (A)	Angle between R and imaginary line form the time of clotting initiation to the point of the maximal clot formation speed. Closely related to K time.	53-72 degrees	**Propagation phase.** Characterizes the maximal speed of thrombin generation, fibrin deposition, and cross-linking (clot growth and strengthening). Depends on the concentration of fibrinogen and to a lesser extent on platelets.
Maximum amplitude (MA)	Maximal amplitude of the TEG curve	50-70 mm	**Termination phase.** The maximal mechanical strength of the clot. Depends on the platelet abundance, GPIIb/IIIa interactions, fibrin cross-linking, and clot contraction.
			May be affected by thrombocytopenia, thrombocytopathy, and antiplatelet agents.
Lysis at 30 minutes (A30 or LY30)	Percentage of the amplitude reduction 30 minutes after reaching the maximal amplitude	0-8%	**Fibrinolysis phase.** The speed of endogenous fibrinolysis. Depends on the presence of plasmin, plasminogen, and its activators in the blood sample.

Figure 1. INTEM and EXTEM analyses
(Theombelastography Tables. Contributed by Maxim Shaydakov)

Outcome measures

The primary purpose of this study is to investigate the potenital predictive value of TEG parameters on clinical outcome and the intracranial symptomatic hemorrhage rate after thrombolytic treatment.

Statistical analysis

This study includes patients who presented to the ED due to ischemic stroke and fulfilled thrombolytic treatment criteria and who thus underwent IV rtPA treatment. The continuous

data were presented in the form of Mean ± Standard Deviation and Median (Q1 – Q3). Shapiro Wilk's test was used to find out whether the data showed normal distribution. Independent samples t-test, a parametric test, was used for the analysis of data with normal distribution in comparisons between groups. Mann-Whitney U test, a non-parametric test, was used for the analysis of data without normal distribution in comparisons between groups. Spearman's rank correlation coefficient was used to define the correlations between continuous variables. IBM SPSS Statistics version 21.0 was used for the analysis of data. The value of $p<0.05$ was considered to determine statistical significance.

3. Results

Forty nine patients acute stroke patients underwent IV thrombolysis between June 2013 and March 2014 were included, as a candidate in the current study.

However, twenty of the forty-nine patients were excluded from the study due to the inadequate blood samples. The demographic data, risk factors and the laboratory values of to patients and control group were given in Table 1.

There was no difference between acute stroke patients and controls for age (64.8±13 vs 57.2±14,8-p=0.17).

Table 1.Demographic Characteristics of Patients and Control Group and Laboratory Results

	Ischemic Stroke Group (n=29)	Control Group (n=37)	Statistacally Difference (p)
Age (year)	64.8±13	57.2±14,8	0.17
Gender			
Male	16 (55.2%)	22 (59.5%)	
Female	13 (44.8%)	15 (40.5%)	
Comorbidities			
DM	14 (48.3%9)	3 (8.1%)	
HT	19 (65.5%)	5 (13.5%)	
Hyperlipidemia	11 (37.9%)	8 (21.6%)	
Atrial fibrillation	10 (34.5%)	-	
Kidney failure	1 (3.4%)	-	
Laboratory Results (Mean values)			
Hb (gr/dl)	12.68 (±2.12)	14.47 (±1.71)	0.2
Platelet ($x10^9$/L)	261.82 (±83.66)	239 (±55.68)	0.496
Leucocyte ($x10^9$/L)	9.6 (±3.15)	7.15 (±1.6)	***0.02***
Triglyceride (mg/dl)	134.10 (±61.09)	126.89 (±55.9)	0.69
LDL (mg/dl)	112.28 (±34.79)	108.21 (±34.43)	0.89
HDL (mg/dl)	44.33 (±15.08)	43.48 (±14.68)	0.98

LDL:Low Density Lipoprotein HDL: High Density Lipoprotein

In labaoratory analysis only leukocytes levels were found to be statisticaly different between acute ischemic patients and control group (p=0.02).

NIHSS score in initial admission to the ED was determined to be less than 10 in 6 (20.7%) patients. Thirteen patients had right and 16 had left hemispheric involvement. In the NIHSS score evaluation of the patients at 24 hours of post rtPA treatment, 12 patients (%41.37) showed improvement (score of 8 and over). Intracerebral bleeding was detected in 2 patients.

The comparison of ischemic stroke patients with the healthy control group suggested statistically significance difference with regard to the parameters of intemCT ($p<0.05$), extemCT ($p=0.01$), extemCFT ($p<0.05$) and extemMCF ($p<0.05$) (Table 2).

Table 2.The comparison of baseline of TEG parameters between ischemic stroke patients and a healthy control group

	Group Comparisons Mean±STD.DEV Median (Q1-Q3)		
	Patients (n=29)	**Control (n=37)**	p
Intem CT[a] (seconds)	158.24 ± 50.43 163.00 (131.00 – 187.00)	175.00 ± 26.93 177.00 (162.00 – 193.00)	**0.011**
Intem CFT[a] (seconds)	135.55 ± 232.188 69.00 (62.00 – 99.00)	89.59 ± 20.55 87.00 (75.00 – 99.50)	0.679
Intem MCF[a] (seconds)	57.93 ± 13.85 63.00 (50.00 – 69.00)	57.54 ± 5.56 57.00 (53.50 – 62.00)	0.064
Extem CT[a] (seconds)	102.07 ± 43.22 93.00 (78.00 – 114.00)	69.70 ± 12.69 73.00 (57.50 – 77.00)	**<0.001**
Extem CFT[a] (seconds)	124.28 ± 151.33 71.00 (66.00 – 95.00)	108.62 ± 25.26 103.00 (89.50 – 129.50)	**0.012**
Extem MCF[a] (seconds)	59.41 ± 16.46 64.00 (50.00 – 72.00)	58.76 ± 5.32 60.00 (55.00 – 61.50)	**0.033**

[a]*Mann-Whitney U Test*

In our study, intrinsic and extrinsic CF, CFT and MCF parameters could not be correlated with intracerebral symptomatic and asymptomatic hemorrhage, NIHSS, mRs (3-6) and mortality (Table 3). In patients that developed intracerebral hemorage after rtPA treatment, the value of intemCT was lower than the patients that did not have bleeding (Table 3).

Table 3. Comparison of TEG parameters with clinical outcome and mortality

TEG parameters	NIHSS ≥10 (n:6)	Symptomatic hemorrhage (n:2)	Asymptomatic hemorrhage (n:7)	mRS: 3-6 3 months (n:14)	Mortality (n:5)
Intem CT	0.69	0.31	0.82	**0.03**	0.63
Intem CFT	0.89	0.39	0.23	0.65	0.29
Intem MCF	0.41	0.54	0.56	0.12	0.84
Extem CT	0.77	0.31	0.74	0.18	0.67
Extem CFT	0.89	0.54	0.82	0.62	0.63
Extem MCF	0.93	0.31	0.82	0.18	0.35

4. Discussion

Thromboelastography (TEG), has been used since the 1940s, is the only stand alone test that can provide integrated information on the balance between the two separate but simultaneously occuring components of coagulation, thrombosis and lysis. It measures the coagulation process from initial clotting cascade to clot strength.

This is the first study to examine coagulation status as reflected by TEG method in stroke patients with intracerebral hemorrhage after rtPA treatment. Our study demonstrated that, compared to controls, acute ischemic stroke patients are hypercoagulable on various TEG parameters before being treated with tPA, but not may predict intracerebral hemorrhage, clinical outcome and mortality

In stroke patients, rtPA treatment, as well as endovascular treatment, is one of the options. If rtPA is administered in 4.5 hours following the onset of symptoms, it results in remarkable recovery in three months [6]. However, the rtPA treatment also causes several complications. These complications include life-threatening conditions suh as bleeding (intracerebral and systemic),

reperfusion damage and angioedema. Particularly symptomatic intracranial hemorrhage may develop in 7 to 14% of the cases [7-9]. Intracranial hemorrhage is associated with older age, glucose level at the time of presentation and severity of stroke. Furthermore, the risk increases in patients using antiplatelet or anticoagulant drugs.

Evaluation of coagulation with TEG method has been previously used for various diseases and conditions [10-12]. However, literature data on the use of TEG parameters in stroke patients is limited. In our study, TEG data measured by ROTEM ® method; when compared with normal healthy subjects, stroke patients have significant hypercoagulability. This hypercoagulability was diagnosed by the presence of an accelerated clot formation, as evidenced by shortening of CFT and/or CT and an increase of the clot strength, as evidenced by the rise in MCF.

In our study, IntemTC, ExtemCT, and Extem CT were shorter and extem MCF ($p<0.05$) was higher in stroke patients compared to the healthy control group. These results show us the increase in coagulation in stroke patients by TEG method. Similar to our study, Elliott et al. showed the same results when comparing stroke patients to the control group. Although the data obtained by the TEG method show a disorder in the coagulation parameters in stroke patients, there was no relation between these data and the NIHSS score at the first admission. There was no relation between these results and clinical outcome. However, these parameters taken before rtPA treatment did not show any benefit in terms of clinical outcome. In future studies, we think that results may change if more patients are included.

5. Conclusion

In conclusion, our study demonstrates that many acute ischemic stroke patients are hypercoaguable.

Although thromboelastographic measurement does not inform us about the outcome, further studies are needed to investigate this observation and its relationship with clinical outcome.

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