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The relationship between serum bilirubin levels and early neurological improvement in patients with acute ischemic stroke treated with intravenous tissue plasminogen activator

[1]Aysegul Demir *MD*, [2]Fettah Eren *MD*

Department of Neurology, [1]Konya City Hospital, [2]Selcuk University, School of Medicine, Konya, Turkey

Abstract

Background & Objective: Acute ischemic stroke is defined as the development of sudden neurological deficits resulting from a focal injury due to vascular occlusion in the central nervous system. Intravenous tissue plasminogen activator (t-PA) therapy in these patients is an internationally accepted, evidence-based effective treatment. Previous data suggest that increased serum bilirubin may reflect the intensity of oxidative stress associated with stroke severity. This study aimed to investigate the effect of bilirubin levels on early neurological improvement after intravenous t-PA treatment and whether bilirubin is a determinant in in-hospital mortality. Methods: The determinant role of admission bilirubin levels in the severity of stroke, in-hospital mortality and early neurological improvement after intravenous t-PA was investigated. An independent association of bilirubin with mortality and neurological improvement were identified by multivariate logistic regression analysis. Results: A total of 184 ischemic stroke patients who received intravenous t-PA were included in the study. Serum direct bilirubin, indirect bilirubin, and total bilirubin of patients in the group without early improvement were significantly higher than the group with improvement (p= 0.041, p= 0.030, p= 0.026, respectively). Also, in binary logistic regression analysis, total bilirubin was found to be an independent predictor for early neurological improvement (OR 6.257, 95%CI 1.482-26.413, p=0.013).
Conclusions: There was a significant relationship between the severity of the stroke and in-hospital mortality and increased serum bilirubin levels in our study. Again, there was a significant relationship between the lack of early neurological improvement after intravenous t-PA and increased serum bilirubin levels, and serum direct bilirubin levels were independent predictor of neurological improvement.

Keywords: Acute ischemic stroke, tissue plasminogen activator, oxidative stress, bilirubin, National Institutes of Health Stroke Scale, mortality, disability

INTRODUCTION

Acute ischemic stroke (AIS) is one of the leading causes of mortality and permanent disability worldwide. A sudden decrease in cerebral blood flow due to AIS can cause deaths along with temporary and permanent loss of brain functions.[1] In AIS, intravenous tissue plasminogen activator (t-PA) is administered within the first 4.5 hours from the onset of symptoms, is the first preferred treatment option, although it has limitations such as the development of hemorrhage and brain edema.[2-4] However, significant neurological improvement can be seen in approximately 50% of patients.[5,6]

During the acute phase of any type of ischemic stroke, excessive oxidative stress increase may play an essential role in the pathophysiology of structural and functional damage to the brain.[7] Bilirubin is the end product of heme metabolism, and it has two forms as direct bilirubin (DB) and indirect bilirubin (IDB). Although bilirubin is known to have antioxidant properties, it has been reported in previous studies that it may cause neural apoptosis by affecting mitochondrial membrane permeability at high concentrations, damaging mitochondrial function, and reducing the activity of astrocytes.[8,9] Previous data suggest that increased serum bilirubin may reflect the

Address correspondence to: Aysegul Demir, MD. Department of Neurology, Konya City Hospital, Konya, Turkey. Tel: +90 0 332 310 50 00, email: D_Raysegul@hotmail.com

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intensity of oxidative stress associated with stroke severity.[7,10]

Previous studies have shown the relationship between increased serum bilirubin levels and the severity of stroke and mortality.[10-12] Our study aimed to compare the bilirubin levels of patients hospitalized with a diagnosis of AIS and with and without early neurological improvement at the 24th hour after intravenous t-PA treatment and investigate whether bilirubin levels were a determinant for in-hospital mortality.

METHODS

This was a retrospective study involving 184 patients aged 18-95 (88 women, 96 men) who were admitted to our clinic with AIS (showing acute ischemic lesions on brain magnetic resonance imaging), hospitalized (presenting within 4.5 hours of symptom onset) and received thrombolytic therapy with intravenous t-PA between June 2018 and June 2020. The flow chart of the patients included in the analysis is shown in Figure 1. The study was approved by the Health Sciences University Medical Specialty Education Board (Approval no: 39-18). Informed consent was obtained from each patient.

The file records were evaluated, and it was checked whether the thrombolytic therapy contraindications were followed in line with the stroke guideline recommendations and whether the appropriate dose of t-PA was administered to the patients.[13] Demographic characteristics, stroke risk factors, National Institutes of Health Stroke Scale (NIHSS) scores calculated before and 24 hours after thrombolytic therapy were recorded from the patients file reviews. Also, blood glucose, creatinine, alanine aminotransferase (ALT), low-density lipoprotein cholesterol (LDL-C), DB, IDB, total bilirubin (TB), troponin, creatine kinase-MB, C-reactive protein levels, and hematological parameters measured at the time of admission emergency department were recorded. Patients who developed intracranial hemorrhage after thrombolytic therapy and those who died during hospitalization were determined.

Patients with contraindications for thrombolytic therapy, patients who died within the first 24 hours after thrombolytic therapy, patients with hepatitis, cirrhosis, bile duct obstruction, cancer patients, hemolytic and chronic inflammatory diseases were excluded from the study.

The patients were divided into two groups according to the NIHSS score calculated at the time of hospitalization (NIHSS >14: severe disability group, NIHSS <15: moderate and mild disability group).[14] According to the NIHSS score calculated during the patients' hospitalization, a decrease of 4 units in the calculated NIHSS score at the 24th hour after the administration of thrombolytic therapy or the NIHSS score of 0-1 at the 24th hour was considered as early neurological improvement.[15] Patients who developed intracranial hemorrhage within the first 24 hours after thrombolytic therapy and had changes in NIHSS values due to hemorrhage were excluded from early neurological improvement evaluation according to the NIHSS score checked at the 24th hour.

Statistical analysis

Statistical analysis was performed by using SPSS for Windows version 21.0 (SPSS Inc., Chicago, Illinois). Normal distribution of continuous variables was tested using Kolmogorov-Smirnov

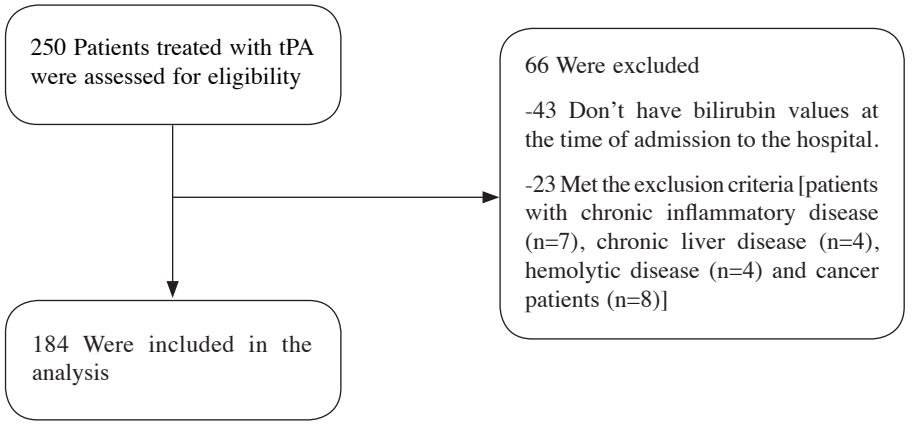

Figure 1. Flow chart of patients included in the analysis

test. Continuous variables with normal distribution were compared using Student's-t test and those without normal distribution were compared using Mann-Whitney's U test. The chi-square test was used for comparing categorical variables. Continuous variables were defined as means ± standard deviation or median (min-max) and categorical variables were given in percentages. Changes of arterial stiffness parameters according to clinical status were compared within groups by using paired sample t-test or Wilcoxon Signed Ranks Test. Changes of arterial stiffness parameters according to clinical status were compared between groups by using repeated measures mixed ANOVA. Binary logistic regression analysis was used to determine the independent associates of in-hospital mortality and neurological improvement. Possible confounding factors were tested in the univariate regression model and confounders with a p value <0.2 were included in the multivariate logistic regression model. P < 0.05 was considered as statistically significant for all tests.

RESULTS

The mean age of the patients included in the study was 72.6 years, and 48% were female. According to the NIHSS score calculated before thrombolytic therapy, the number of patients with severe disability was 49 (26.6%). According to the NIHSS score calculated at the post-thrombolytic therapy 24th hour (164 patients), the number of patients who had early neurological improvement was 78 (47.6%). Forty-six (25%) patients died during the hospitalization period. The causes for in hospital mortality detected by clinicians were respiratory diseases 21 (45.5%) (aspiration pneumonia, respiratory failure and pulmonary embolism), intracranial bleeding 12 (26.0%), stroke itself 5 (11.0%) and ischemic heart disease 8 (17.5%).

Baseline demographic characteristics and laboratory findings of patients with and without a severe disability are presented in Table 1. Patients with severe disability were older and the number of female patients was higher (76.1±12.3 vs 71.3±12.2; p=0.023, 30 (61.2%) vs 58 (43%); p= 0.028, respectively). Patients with a severe disability had a significantly higher intracranial hemorrhage rate than patients without severe disability (p=0.012). Also, serum glucose, DB, IDB, and TB levels were significantly higher in patients with a severe disability than patients without severe disability (p=0.020, p=0.004, p=0.018, p=0.008, respectively). TB values measured at the time of hospitalization as a result of binary logistic regression analysis were found to be a determinant in determining the severity of stroke (OR 3.37, 95% CI 1.05-10.79, p=0.041) (Table 2).

Baseline demographic characteristics and laboratory findings of patients with early neurological improvement and patients without neurological improvement are summarized in Table 3. The baseline demographic characteristics and stroke risk factors of these two groups were similar. Troponin and CRP values were higher in the group without early neurological improvement than in the group with improvement but did not reach statistical significance (p=0.697, p=0.636, respectively). On the other hand, DB, IDB, and TB values of patients in the group without early improvement were significantly higher than the group with improvement (p= 0.041, p= 0.030, p= 0.026, respectively). Also, in binary logistic regression analysis, TB values were found to be an independent predictor for early neurological improvement (OR 6.257, 95% CI 1.482-26.413, p=0.013) (Table 4).

The baseline demographic characteristics and laboratory findings of the patients who died in hospital during the hospitalization period, and those who were discharged are shown in Table 5. Patients who died in hospital were older (77.3±10.5 vs 71±12.6; p= 0.001, respectively), NIHSS scores were higher at admission (16 (2-24) vs 8 (2-24); p <0.001, respectively) and intracranial hemorrhage rates were higher than in discharged patients (12 (26.1%) vs 8 (5.8%); p <0.001, respectively). Serum glucose levels of patients who died in-hospital (136 (89-381) vs. 121 (57-427); p= 0.005, respectively) and CRP values (5.88 (3.02-183) vs. 3.53 (3.02-237); p= 0.041, respectively) were higher than the patients who were discharged and were statistically significant. Besides, troponin values of patients who died in-hospital were higher but did not reach statistical significance (p=0.079). Also, DB, IDB, and TB values of the patients who died in-hospital were significantly higher than those discharged from the hospital (p <0.001, p=0.004, p=0.001, respectively). In binary logistic regression analysis, diabetes mellitus (DM) (OR 0.322, 95% CI 0.118-0.883, p=0.028), NIHSS score at admission to hospital (OR 1.307, 95% CI 1.184-1.443, p <0.001) and DB levels (OR 11.597, 95% CI 2.020-66.595, p=0.006) were determined to be an independent predictor for in-hospital mortality (Table 6).

Table 1: Clinical and characteristic features of the patients according to the admission NIHSS scores.

	Severe disability (NIHSS>14) (n=49)	No severe disability (NIHSS<15) (n=135)	p value
Age (years)	76.1±12.3	71.3±12.2	**0.023**
Female, n (%)	30 (61.2)	58 (43)	**0.028**
Hypertension, n (%)	32 (65.3)	92 (68.1)	0.716
Diabetes Mellitus, n (%)	14 (28.6)	39 (28.9)	0.966
Hypercholesterolemia, n (%)	5 (10.2)	19 (14.1)	0.491
NIHSS on admission	17 (15-24)	7 (2-14)	**<0.001**
t-PA treatment according to the time of onset of symptoms			
0-1 hours, n (%)	1 (2)	2 (1.5)	0.058
1-2 hours, n (%)	2 (4.1)	24 (17.8)	
2-3 hours, n (%)	17 (34.7)	52 (38.5)	
3-4.5 hours, n (%)	29 (59.2)	57 (42)	
Intracranial hemorrhage, n(%)	10 (20.4)	10 (7.4)	**0.012**
Serum glucose (mg/dl)	143 (80-381)	123 (57-427)	**0.020**
LDL-C (mg/dl)	102±31	125±35	**0.002**
Creatinine (mg/dl)	1.06±0.36	0.99±0.26	0.209
ALT (u/L))	13 (5-58)	14 (4-130)	0.666
Haemoglobin (g/dl)	13.5±1.9	13.7±1.8	0.465
White blood cell ($x10^9$/L)	9.31±3.83	8.92±2.54	0.427
Platelet ($x10^9$/L)	227±77	257±78	**0.025**
CRP (mg/dl)	4.12 (3.02-71.50)	3.78 (3.02-237)	0.127
Troponin, ng/ml	0.030 (0.001-0.33)	0.014 (0.001-3.37)	0.122
CK-MB (IU)	1.43 (0.10-12.62)	1.31 (0.18-7.70)	0.992
DB (mg/dl)	0.14 (0.06-0.70)	0.10 (0.04-0.30)	**0.004**
IDB (mg/dl)	0.45 (0.08-1.69)	0.34 (0.01-1.82)	**0.018**
TB (mg/dl)	0.6 (0.14-2.39)	0.43 (0.09-2.12)	**0.008**

ALT, Alanin aminotransferaz; CK-MB, creatine kinase isoenzyme MB; CRP, C-reactive protein; DB, Direct bilirubin; IDB, Indirect bilirubin; LDL-C, Low-density lipoprotein cholesterol; NIHSS, National Institutes of Health Stroke Scale; NOACs, The novel oral anticoagulants; TB, Total bilirubin; t-PA, tissue plasminogen activator.
*a p value<0.05 denotes statistical significance.

DISCUSSION

Three significant results were found in this study. First, among patients hospitalized due to AIS, patients with severe stroke had significantly higher bilirubin levels than patients without severe stroke. Second, patients' bilirubin values without early neurological improvement at 24 hours after intravenous t-PA were significantly higher than patients with early neurological improvement, and DB levels were independent predictors for early neurological improvement. Finally, bilirubin levels of patients who died in hospital during their hospitalization were significantly higher than those discharged from the hospital, and DB levels, NIHSS scores at admission, and DM presence were independent predictors for in-hospital mortality.

AIS is defined as the development of sudden neurological deficits resulting from a focal injury due to vascular occlusion in the central nervous system. Etiological factors include atherosclerosis of the large arteries and cardioembolic causes. The two most important factors that play a role in the development and progression of atherosclerosis are inflammation and oxidative stress.[16] The annual incidence of stroke worldwide is approximately 17 million, and it is the second cause of death after coronary artery disease.[17] The treatment of AIS

Table 2: Binary logistic regression analyzes to determine independent associates of stroke severity based on NIHSS scoring

Variables	Univariate		Multivariate	
	OR (95% CI)	p	OR (95% CI)	p
Gender	0.47 (0.24-0.93)	0.030	0.42 (0.20-0.85)	**0.017**
Age	1.03 (1.005-1.067)	0.024		
Hypertension	1.13 (0.57-2.26)	0.716		
Hyperlipidemia	1.41 (0.50-4.09)	0.493		
Serum glucose	1.005 (1.000-1.010)	0.069	1.005 (1.000-1.011)	0.071
Creatinine	2.18 (0.76-6.2)	0.147		
Haemoglobin	0.93 (0.78-1.15)	0.450		
White blood cell	1.04 (0.93-1.16)	0.426		
Platelet	0.994 (0.990-0.999)	0.028	0.995 (0.990-1.000)	**0.041**
CRP	0.99 (0.98-1.01)	0.863		
Troponin	0.40 (0.05-3.01)	0.380		
ALT	1.006 (0.98-1.03)	0.625		
TB	3.5 (1.16-10.93)	0.026	3.37 (1.05-10.79)	**0.041**

ALT, Alanine aminotransferase; CRP, C-reactive protein; TB, Total bilirubin.
CI, confidence interval; OR, Odds ratio
*a p value <0.05 denotes statistical significance.

aims to reduce deaths and disability by re-opening the occluded cerebral arteries and reperfusion of ischemic areas. Intravenous t-PA therapy in these patients is an internationally accepted, evidence-based effective treatment. However, it is reported that only 7% of all ischemic stroke patients refer to the hospital at the appropriate time for intravenous t-PA administration.[18]

Excessive oxidative stress that occurs in the acute phase of ischemic stroke causes structural and functional damage in the brain and plays a role in the pathogenesis of ischemic brain damage. The human brain has fewer endogenous sources of antioxidants compared to other organs. Bilirubin is an end product of the heme catabolic pathway, but also an endogenous antioxidant, and shows anti-inflammatory and platelet activation inhibitory effect.[19] Bilirubin, known as a powerful antioxidant under physiological and stable conditions, may increase serum levels as a compensatory mechanism in oxidative stress conditions.[20] Increased serum total bilirubin in patients with stable coronary artery has been associated with a better prognosis by improving ischemic myocardial function.[21] Increased bilirubin levels in patients with acute myocardial infarction (AMI) can be explained by the increase in both oxygenase-1 activities due to the stress occurring after MI.[20] Sahin *et al*. found an independent relationship between the severity of coronary artery disease and increased serum TB levels in MI patients with ST elevation, and Kaya *et al*. found this in MI patients with non-ST elevation.[22,23] In another study conducted by Okhura *et al*. where serum bilirubin was measured at three-hour intervals in AMI patients, the bilirubin levels reached the peak values at the mean twenty-first hour of their hospitalization. The authors attributed this with the increase in both oxygenase-1 activities due to stress during AMI.[24]

In a survey conducted by Peristein *et al*., including 13,214 patients, they found that patients with high serum bilirubin levels had a significantly lower history of ischemic stroke. The authors attributed these results to the antioxidant and anti-inflammatory effects of bilirubin.[25] In a prospective cohort study conducted by Kimm *et al*. in Korea, low serum bilirubin levels were found to be an independent predictor of stroke development in males.[26] However, previous studies have shown that stimulation of free radicals and pro-inflammatory cytokines resulting from hypoxia in tissues results in an increase in bilirubin production as the compensatory mechanism.[27] Pineda *et al*. found a relationship between increased DB values at admission and stroke severity in AIS patients, but they did not find a significant relationship between functional improvement and DB levels at discharge.[10] Luo

Table 3: Baseline demographic, clinical, and laboratory parameters of the study population according to early neurological improvement

	Early Neurological Improvement (n=78)	No Early Neurological Improvement (n=86)	p value
Age (years)	71±13	73±12	0.222
Female, n (%)	38 (48.7)	37 (43)	0.465
Hypertension, n (%)	49 (62.8)	61 (70.9)	0.270
Diabetes mellitus, n (%)	18 (23.1)	29 (33.7)	0.132
Hypercholesterolemia, n (%)	9 (11.5)	10 (11.6)	0.986
NIHSS on admission	9 (2-20)	9 (2-24)	0.519
t-PA treatment according to the time of onset of symptoms			
0-1 hours, n (%)	1 (1.3)	2 (2.3)	0.311
1-2 hours, n (%)	14 (17.9)	9 (10.5)	
2-3 hours, n (%)	28 (35.9)	33 (38.4)	
3-4.5 hours, n (%)	35 (44.9)	42 (48.8)	
Serum glucose (mg/dl)	121 (80-427)	132 (57-381)	0.316
LDL-C (mg/dl)	122±26	116±44	0.375
Creatinine (mg/dl)	1.01±0.27	1.0±0.29	0.751
ALT (u/L))	13 (4-130)	14 (4-58)	0.414
Haemoglobin (g/dl)	13.8±1.7	13.6±1.9	0.484
White blood cell ($x10^9$/L)	9.15±2.47	8.97±3.28	0.683
Platelet ($x10^9$/L)	257±84	252±73	0.696
CRP (mg/dl)	3.48 (3.02-237)	4.02 (3.02-183)	0.636
Troponin, ng/ml	0.02 (0.01-0.32)	0.15 (0.01-3.37)	0.697
CK-MB (IU)	1.15 (0.10-12.62)	1.40 (0.18-6.50)	0.373
DB (mg/dl)	0.10 (0.04-0.20)	0.11 (0.05-0.53)	**0.041**
IDB (mg/dl)	0.33 (0.01-0.70)	0.37 (0.01-1.08)	**0.030**
TB (mg/dl)	0.43 (0.09-0.86)	0.48 (010-1.20)	**0.026**
Medications at discharge			
Antiplatelet therapy, n (%)	58 (74.4)	69 (80.2)	0.369
Warfarin therapy, n (%)	13 (16.7)	10 (11.6)	0.353
NOACs therapy, n (%)	7 (9)	7 (8.1)	0.848

ALT, Alanin aminotransferaz; CK-MB, creatine kinase isoenzyme MB; CRP, C-reactive protein; DB, Direct bilirubin; IDB, Indirect bilirubin; LDL-C, Low-density lipoprotein cholesterol; NIHSS, National Institutes of Health Stroke Scale; NOACs, The novel oral anticoagulants; TB, Total bilirubin; t-PA, tissue plasminogen activator.
*a p value<0.05 denotes statistical significance.

et al. showed the relationship between increased serum DB and TB levels with stroke severity in AIS patients[7]. On the other hand, Xu *et al*. found a relationship between increased serum bilirubin levels and the severity of the stroke, but no relationship was found between clinical improvement and bilirubin levels at discharge.[28] Arsalan *et al*. showed that high bilirubin levels measured at admission in AIS patients were associated with the severity of the stroke, prolonged hospitalization, and poor prognosis.[12] Muskari *et al*. found a significant relationship between increased cerebral infarct volume and increased serum IDB levels in AIS patients, and the IDB at admission was significantly higher than those on the 7th day of the stroke.[29] In our study, when classified according to the NIHSS score, the serum DB, IB, and TB values of those with severe stroke were significantly higher than those without severe stroke, and also, as a result of multivariate analysis, high serum DB levels were found to be a marker in determining the severity of stroke. The previous studies showed that stroke severity and increased serum glucose

Table 4: Binary logistic regression analyses to determine the independent associates of improvement in clinical status

Variables	Univariate		Multivariate	
	OR (95% CI)	p	OR (95% CI)	p
Gender	1.258 (0.680-2.329)	0.465		
Age	1.016 (0.991-1.041)	0.20	1.015 (0.989-1.041)	0.261
Diabetes mellitus	0.590 (0.296-1.177)	0.134	0.572 (0.283-1.156)	0.120
Hypertension	0.692 (0.360-1.332)	0.271		
NIHSS	1.030 (0.973-1.090)	0.305		
Serum glucose	1.004 (0.999-1.010)	0.128	1.003 (0.997-1.009)	0.316
Creatinine	0.839 (0.283-2.481)	0.750		
Haemoglobin	0.942 (0.796-1.114)	0.484		
Platelet	0.999 (0.995-1.003)	0.692		
CRP	1.003 (0.991-1.016)	0.609		
Troponin	1.181 (0.540-2.584)	0.677		
TB	6.257 (1.482-26.413)	0.013	6.257 (1.482-26.413)	**0.013**

CRP, C-reactive protein; NIHSS, National Institutes of Health Stroke Scale; TB, Total bilirubin
CI, confidence interval; OR, Odds ratio
*a p value <0.05 denotes statistical significance.

level have a significant effect on post-intravenous t-PA therapy intracerebral hemorrhage.[30,31] Jian *et al*. reported that high admission bilirubin levels are an independent predictor of hemorrhagic transformation and symptomatic intracranial hemorrhage in AIS patients treated with mechanical thrombectomy.[32] In our study, serum glucose levels of patients with severe stroke were significantly higher than patients without severe stroke, and intracranial hemorrhage rates were higher.

Mortality rate in our study was slightly higher when compared to previous studies.[33] There are some factors for this finding. First, our study consists an older population and average age was high. Second, admission NIHSS scores of patients who died in-hospital were high. Third, the number of patients who recieved t-PA treatment within 3-4.5 hours after the onset of symptoms was 47% of the entire patient population. And finally, patients with intracerebral hemorrhage constitute 10.9% of the entire patient population.

Markaki *et al*. stated that increased serum bilirubin levels are independent predictors of mortality in patients with both AIS and transient ischemic attacks.[34] Also, Sagheb Asl *et al*. found a significant relationship between increased bilirubin levels and stroke severity and death.[11] In our study, serum DB, IB, and TB values of patients who died in-hospital were significantly higher than those who were discharged, and also, as a result of the multivariate analysis, serum DB increase, high NIHSS score and presence of DM were found to be an independent predictors for in-hospital mortality.

Liu *et al*. found the absence of atrial fibrillation as an independent predictor of early neurological improvement, based on neurological evaluations made by calculating NIHSS at 24th hours and seventh days after intravenous t-PA in a group of patients who were given intravenous t-PA with the diagnosis of AIS.[35] In our study, after the exclusion of patients with intracranial hemorrhage in the first 24 hours after intravenous t-PA administration, the DB, IB, and TB values of the patients without early neurological improvement were significantly higher than the patients with early neurological improvement according to the neurological evaluations in the first 24 hours. Besides, increased serum DB levels were independent predictors for early neurological improvement failure.

While the relationship between serum bilirubin levels and the severity of AIS and in-hospital mortality has been shown in previous studies, the evaluation of the relationship between early neurological improvement after intravenous t-PA and serum bilirubin levels was shown in our study for the first time in the literature.

There are several limitations to our study. First, it was a single-center and retrospective study. Second, the number of patients in the study group was relatively small, and third, patients only had bilirubin values at admission, so the change between neurological improvement and bilirubin

Table 5: Clinical and laboratory parameters of patients discharged and of those who died during hospitalization

	Patients who died during hospitialization (n=46)	Patients who were discharged (n=138)	p value
Age (years)	77.3±10.5	71±12.6	**0.001**
Female, n (%)	24 (52.2)	64(46.4)	0.495
Hypertension, n (%)	29 (63)	95(68.8)	0.468
Diabetes Mellitus, n (%)	17 (37)	36(26.1)	0.159
Hypercholesterolemia, n (%)	6 (13)	8 (13)	1,0
NIHSS on admission	16 (2-24)	8 (2-24)	**<0.001**
t-PA treatment according to the time of onset of symptoms			
0-1 hours, n (%)	1 (2.2)	2 (1.5)	0.154
1-2 hours, n (%)	3 (6.5)	23 (16.7)	
2-3 hours, n (%)	15 (32.6)	54 (39.1)	
3-4.5 hours, n (%)	27 (58.7)	59 (42.7)	
Intracranial hemorrhage, n(%)	12 (26.1)	8 (5.8)	**<0.001**
Serum glucose (mg/dl)	136 (89-381)	121 (57-427)	**0.005**
LDL-C (mg/dl)	98±37	124±33	**0.007**
Creatinine (mg/dl)	1.07±0.36	0.99±0.27	0.164
ALT (u/L))	15 (7-58)	15 (4-130)	0.546
Haemoglobin (g/dl)	13.3±1.8	13.8±1.9	0.105
White blood cell ($x10^9$/L)	9.25±4.05	8.95±2.47	0.640
Platelet ($x10^9$/L)	234±81	254±77	0.105
CRP (mg/dl)	5.88 (3.02-183)	3.53 (3.02-237)	**0.041**
Troponin, ng/ml	0.30 (0.01-1.82)	0.15(0.01-3.37)	0.079
CK-MB (IU)	1.42 (0.41-4.88)	1.31 (0.10-12.62)	0.795
DB (mg/dl)	0.15 (0.07-0.70)	0.10 (0.04-0.21)	**<0.001**
IDB (mg/dl)	0.46 (0.01-1.82)	0.34 (0.01-1.08)	**0.004**
TB (mg/dl)	0.61 (0.10-2.39)	0.43 (0.09-1.20)	**0.001**
Medications at discharge			
Antiplatelet therapy, n (%)		102 (73.9)	
Warfarin therapy, n (%)		21 (15.2)	
NOACs therapy, n (%)		15 (10.9)	

ALT, Alanin aminotransferaz; CK-MB, creatine kinase isoenzyme MB; CRP, C-reactive protein; DB, Direct bilirubin; IDB, Indirect bilirubin; LDL-C, Low-density lipoprotein cholesterol; NIHSS, National Institutes of Health Stroke Scale; NOACs, The novel oral anticoagulants; TB, Total bilirubin; t-PA, tissue plasminogen activator.
*a p value<0.05 denotes statistical significance.

levels could not be evaluated.

In conclusion, there was a significant relationship between the severity of the stroke and in-hospital mortality and increased serum bilirubin levels in our study, moreover, increased DB levels, high NIHSS score and presence of DM were independent predictors of in-hospital mortality. Again, there was a significant relationship between the lack of early neurological improvement after intravenous t-PA and increased serum bilirubin levels, and serum DB levels were independent predictors of neurological improvement. We think that bilirubin levels can be evaluated as a simple laboratory parameter to predict neurological improvement after treatment in patients with AIS, and these results can be supported by a larger population and prospective studies.

Table 6: Binary logistic regression analyses to determine the independent associates of in-hospital mortality

Variables	Univariate		Multivariate	
	OR (95% CI)	p	OR (95% CI)	p
Gender	0.793 (0.406-1.547)	0.496		
Age	1.050 (1.016-1.085)	0.004	1.043 (0.998-1.091)	0.062
Diabetes mellitus	0.602 (0.296-1.223)	0.161	0.322 (0.118-0.883)	**0.028**
Hypertension	1.295 (0.644-2.605)	0.468		
NIHSS	1.317 (1.207-1.436)	<0.001	1.307 (1.184-1.443)	**<0.001**
Duration of symptoms	0.130 (0.006-2.677)	0.186	0.143 (0.001-23.106)	0.454
Intracranial hemorrhage	5.735 (2.172-15.144)	<0.001	0.455 (0.124-1.665)	0.234
Serum glucose	1.007 (1.001-1.012)	0.015	1.003 (0.994-1.012)	0.537
Creatinine	2.396 (0.824-6.964)	0.109	1.888 (0.324-11.011)	0.480
Haemoglobin	0.860 (0.717-1.033)	0.106	0.903 (0.667-1.223)	0.511
White blood cell	1.034 (0.926-1.155)	0.552		
Platelet	0.997 (0.992-1.001)	0.148		
CRP	1.009 (0.997-1.021)	0.158	1.010 (0.996-1.023)	0.162
Troponin	0.899 (0.364-2.223)	0.818		
TB	10.183 (2.650-39.133)	0.001	11.597 (2.020-66.595)	**0.006**

CRP, C-reactive protein; NIHSS, National Institutes of Health Stroke Scale; TB, Total bilirubin
CI, confidence interval; OR, Odds ratio
*a p value <0.05 denotes statistical significance.

DISCLOSURE

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