# Relationship between no-reflow phenomenon and serotonin levels in patients with acute ST-elevation myocardial infarction who underwent primary percutaneous intervention

## *Akut ST yükselmeli miyokart enfarktüsü nedeniyle primer perkütan girişim uygulanan hastalarda no-reflow fenomeni ile serotonin düzeyi arasındaki ilişki*

*Ramazan Topsakal, Mehmet G. Kaya, Ekrem Karakaya, Özgür Günebakmaz, Ali Doğan, M. Tuğrul İnanç, Bahadır Şarlı, İbrahim Özdoğru, Ali Ergin*

Department of Cardiology, Erciyes University, School of Medicine, Kayseri, Turkey

## Abstract

**Objective:** Our aim was to investigate the effects of serotonin, which is a severe vasoconstrictor agent, on the occurrence of no-reflow phenomenon.

**Methods:** In this cross-sectional controlled study, 40 patients, admitted to our clinic with chest pain in the first 12 hours and underwent primary percutaneous coronary intervention because of acute myocardial infarction were enrolled. Patients with TIMI 0 grade basal flow and normal post-procedure flow were included in group 1 and patients with flow grade TIMI ≤were enrolled in group 2. To measure the serotonin levels, blood samples were collected from the coronary ostium before the procedure.

**Results:** In group 1, there were 25 patients (20 males, 5 females) and the mean age was 58±11 years; in group 2 there were 15 patients (13 males, 2 females) and the mean age was 62±8 years. The mean serotonin level in platelet in group 1 was 476±208 ng/$10^9$ platelet and in group 2-542±273 ng/$10^9$ platelet. The difference was not statistically significant (p=0.39). When we compared the serum serotonin levels, it was 41.4±40.8 ng/ml for group 1, but 66.7±45.7 ng/ml for group 2. Although the serum serotonin levels were higher in group 2, the difference was not statistically significant (p=0.07).

**Conclusion:** There was no effect of serotonin level in the development of no-reflow, in patients to whom primary coronary percutaneous intervention was applied. *(Anadolu Kardiyol Derg 2010; 10: 253-9)*

**Key words:** No-reflow phenomenon, serotonin, vasoconstriction, myocardial infarction

## Özet

**Amaç:** Çalışmamızın amacı, vazokonstriktör bir ajan olan serotoninin no-reflow fenomeni gelişimi üzerine olan etkisini araştırmaktı.

**Yöntemler:** Bu enine-kesitli kontrollü çalışmaya akut miyokart enfarktüsü nedeniyle, göğüs ağrılarının ilk 12 saati içinde kliniğimize başvuran ve primer perkütan girişim uygulanan 40 hasta alındı. İşlem öncesi akım TIMI 0 iken işlem sonrası akım normal olanlar grup 1'i, işlem sonrası akım TIMI ≤2 olanlar grup 2'yi oluşturdu. Serotonin düzeyini ölçmek için işlem öncesi koroner ostiumdan kan alındı.

**Bulgular:** Grup 1'de 25 hasta (20 erkek, 5 kadın) vardı, ortalama yaş 58±11 yıl idi; Grup 2' de 15 hasta vardı (13 erkek, 2 kadın) ve ortalama yaş 62±8 yıl idi. Grup'1 de trombosit içi ortalama serotonin düzeyi 476±208 ng/$10^9$ trombosit ve grup 2'de 542±273 ng/$10^9$ trombosit idi. Fark istatistiksel olarak anlamlı değildi (p=0.39). Serum serotonin düzeyi grup'1 de 41.4±40.8 ng/ml, grup'2 de 66.7±45.7 ng/ml olarak bulundu. Aradaki fark anlamlı değildi (p=0.07).

**Sonuç:** Primer perkütan girişim uygulananlarda serotonin düzeyinin no-reflow gelişimi üzerine etkisi yoktur. *(Anadolu Kardiyol Derg 2010; 10: 253-9)*

**Anahtar kelimeler:** No-reflow fenomeni, serotonin, vazokonstriksiyon, miyokart enfarktüsü

**Address for Correspondence/Yazışma Adresi:** Dr. Özgür Günebakmaz, Erciyes University, School of Medicine, Department of Cardiology 38039 Kayseri, Turkey
Phone: +90 352 437 49 37/27792 Fax: +90 352 437 61 98 E-mail: drgunebakmaz@yahoo.com

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## Introduction

Primary percutaneous coronary intervention (PCI) aims to ensure TIMI 3 flow in patients with acute ST-elevation myocardial infarction (1). No-reflow is defined as a flow of less than TIMI 3 without the presence of a significant stenosis, dissection or angiographically observed thrombosis distal to the part of the infarct-related artery where the intervention took place (2, 3). The number of cases with angiographic no-reflow has grown up with the increase of percutaneous interventions in recent years, and as a result, no-reflow phenomenon has become a critical healthcare issue. Elevated microvascular resistance, thrombocyte agglutination, fibrin embolisms, endothelium damage and vasospasm have been implicated for the development of no-reflow (4, 5).

Serotonin (5-hydroxytryptamine) that is synthesized from the essential amino acid L-Tryrophan is a vasoconstrictor and an effective stimulant for the smooth muscles (6). In animal studies carried out by using myocardial contrast echocardiography technique, it was observed that endothelin antagonists increased reperfusion and intracoronary epinephrine resolved refractor no-reflow. Therefore, vasoconstriction has been associated with no-reflow mechanism (7, 8).

If it can be demonstrated that serotonin, which is responsible for vasoconstriction, has a role in occlusion of the vessel causing the infarct and if the impact of serotonin on the development of no-reflow can be established, it may be possible to ensure effective reperfusion by using serotonin receptor antagonists, adjuvant to conventional therapy in patients with acute myocardial infarction.

The purpose of our study was to investigate the effect of serotonin levels on no-reflow phenomenon following a primary PCI in patients with acute ST-elevation myocardial infarction.

## Methods

The 40 patients enrolled in this cross-sectional controlled study had presented within the first 12 hours of chest pain, had been diagnosed with acute ST-elevation myocardial infarction (STEMI) and had undergone primary PCI with a pre-intervention flow of TIMI 0 between February 2007 and November 2007 at the Cardiology Department of Medical School of our university. Patients were excluded if they had cardiogenic shock, stent thrombosis, systemic infection, chronic renal or hepatic failure, hematological disorder and malignancy. Patients who were undergoing thrombolytic therapy or receiving selective serotonin reuptake inhibitors were excluded, as well.

Detailed anamnesis was taken from each patient. Then, the patients underwent physical examination. Risk factors for coronary artery disease (CAD) were also established. Results of fasting blood glucose levels, hepatic and renal function tests, blood lipid profiles and complete blood count were recorded. We also performed 12 lead ECG before the intervention and at the $60^{th}$ minute. Furthermore, creatine kinase (CK), CK-MB and troponin levels were measured at 0, 6, 12 and 24 hours. Baseline high-sensitive C-reactive protein (hs-CRP) values were measured, as well.

All patients were given a therapy of 300 mg acetylsalicylic acid, angiotensin converting enzyme inhibitor, beta-blocker, statin and heparin. They also received a loading-dose of 600 mg clopidogrel. Glycoprotein IIb/IIIa antagonists were not given to any of the patients.

The study was approved by the ethics committee of the Medical School of our university and it was performed according to the Declaration of Helsinki principles. Written informed consent was obtained from all patients before they were enrolled.

### Diagnosis of acute ST segment elevation myocardial infarction

Diagnosis of STEMI was made in the presence of the following characteristics: typical type chest pain lasting more than 30 minutes, new onset or presumably new ST segment elevation in two or more contiguous leads ≥0.2 mV in men and ≥0.15 mV in women with respect to J in V2 and V3 and ≥0.1 mV in other as well as elevation in myocardial enzymes and troponin.

### Echocardiographic examination

The patients underwent echocardiographic examination before they were discharged. Ejection fraction (EF) was determined by using Simpson's method. Transthoracic echocardiographic examinations were performed by using Vivid 7 Dimension (GE Vingmed Ultrasound AS N-190 Horten, Norway) with a 2.5 MHz probe.

### Coronary angiography

Coronary angiography was performed by using standard Judkins method through femoral artery by means of a Philips Integris H 3000 system (Netherlands). Coronary angiography examinations were saved on compact disk in DICOM format for further analysis. Angiographically, no-reflow phenomenon was defined as a flow of TIMI 2 or less without the presence of dissection, mechanical obstruction, significant residual stenosis or other plausible causes (2, 3). Stents were placed in all patients following pre-dilation.

### Biochemical parameters

Blood lipids, total cholesterol, high-density lipoprotein (HDL) cholesterol and low-density lipoprotein (LDL) cholesterol were determined by using specific Thermo kits on a Konelab 60I® system (Thermo Clinical Lab system®, Finland). Complete blood count was performed on CBC XT 2000i® system (Japan). High sensitivity C-reactive protein was measured by using hs-CRP BN2 (Dade-Boehring, Germany).

### Serotonin

Pre-procedure blood samples for serotonin measurement were collected from the coronary ostium by using catheter without side holes. Complete blood sample was put in a test tube containing 5.4 mg ethylene aminotetraacetic acid (EDTA) and centrifuged at 1,100 rpm for 10 minutes to measure the level of serotonin in thrombocytes. Then, 200 µl was taken from its plasma part to be diluted in saline solution (800 µl). The mixture was centrifuged at 5.200 rpm for 10 minutes. The resulting throm-

bocyte agglutination was combined with 200 µl of distilled water in order to obtain an enriched thrombocyte concentration. Serum and enriched thrombocyte concentration were kept at -70°C. The sample, after reaching room temperature, was centrifuged at 10,000 rpm for 2 minutes in order to measure the level of serotonin in thrombocytes. Then, 10 µL of the sample was utilized to establish the level of serotonin in thrombocytes and serum, by using BIOSOURCE® ELISA kit (BioSource International, California, USA). The serotonin value obtained was divided by thrombocyte count in order to establish the level of serotonin in thrombocytes, which was expressed as ng/$10^9$.

**Statistical analysis**

SPSS for Windows version 13.0 software (Chicago, IL, USA) was used as the statistical software program. Kolmogorov-Smirnov test was used to determine whether the parameters fit with the normal distribution. Data within the normal distribution were expressed as mean±standard deviation (Mean±SD). Mann-Whitney U test was used to compare data that did not fit with the normal distribution. Unpaired Student t test was used for comparing data within the normal distribution. Chi-square test was used for comparison of categorical variables. A p value under 0.05 was accepted as the level of significance.

## Results

A total of 25 patients with normal flow (20 men, mean age 58±11 years) and 15 patients observed with no-reflow (13 men, mean age 62±8 years) formed group 1 and 2, respectively. Age range was 30-80 years. There was no statistically significant difference between the groups in terms of age, gender, smoking, hyperlipidemia, family history of CAD, diabetes mellitus (DM), and hypertension (HT) incidence. An analysis of the blood lipid profiles of the patients demonstrated that the groups were similar with respect to total cholesterol, HDL and LDL cholesterol values. There was no significant difference between the groups in terms of body mass index. Clinical characteristics of the patients are given in Table 1.

The mean values for systolic and diastolic blood pressure were 124±20/76±16 mmHg for group 1, and 129±21/80±15 mmHg for group 2, with no significant difference between the groups (p>0.05).

The intervention in the group with normal flow had been performed earlier. The time elapse between onset of pain to intervention was 4.2±2.5 hours in group 1 and 6.3±2.9 hours in group 2 (p=0.021). The mean value for left ventricular EF of the patients with TIMI 3 flow in group 1 was higher than that of the patients with no-reflow in group 2 (50±8 vs. 44±7, p=0.049) (Table 1).

In terms of angiographic data, infarct-related coronary artery was the right coronary artery (RCA) in 11 patients, the left anterior descending (LAD) in 10 patients and the circumflex (Cx) in 4 patients in the group with normal flow. In the group with no-reflow, it was RCA for 6 patients, LAD for 7 patients and Cx for 2 patients. There was no significant relationship between the vessel responsible for myocardial infarction and no-reflow phenomenon (p=0.91). Of the patients in group 1, 64% had single-vessel, 12% had two-vessel and 24% had three-vessel disease. Of the patients in group 2, 27% had single-vessel, 60% had two-vessel and 13% had three-vessel disease. No-reflow was more common in patients with two-vessel disease (p=0.006). There was no significant difference between the groups with respect to stent size (p=0.65) and stent diameter (p=0.89) (Table 2).

While mean level of serotonin in platelets in group 1 was 476±208 ng/$10^9$platelet, it was 542±273 ng/$10^9$ platelet in group 2. Although the mean level of serotonin in thrombocytes was observed to be higher in the no-reflow group, the difference was not statistically significant (p=0.39). While mean serum serotonin level in group 1 was 41.4±40.8 ng/ml, it was noted to be 66.7±45.7 ng/ml in group 2. Although the mean level of serum serotonin was observed to be higher in the no-reflow group, the

**Table 1. Baseline characteristics of the study population**

| Clinical Feature | Group 1 (n=25) | Group 2 (n=15) | *p |
|---|---|---|---|
| Age, years | 58±11 | 62±8 | 0.70 |
| Sex, Male/Female | 20/5 | 13/2 | 0.59 |
| BMI, kg/m2 | 28±4 | 27±2 | 0.47 |
| **Coronary risk factors** | | | |
| Hypertension, n (%) | 11 (44) | 6 (40) | 0.80 |
| Diabetes mellitus, n (%) | 2 (8) | 3 (20) | 0.26 |
| Hyperlipidemia, n (%) | 16 (64) | 11 (74) | 0.54 |
| Smoking, current and ever, n (%) | 17 (68) | 7 (47) | 0.18 |
| Family history of CAD, n (%) | 10 (40) | 5 (33) | 0.67 |
| **Laboratory findings** | | | |
| Hemoglobin, g/dl | 15.0±1.4 | 14.4±1.9 | 0.28 |
| Leukocyte, $10^3$/µL | 12.1±3.2 | 12.7±4.5 | 0.62 |
| Neutrophil, $10^3$/µL | 85.6±4.2 | 10.6±4.8 | 0.27 |
| Platelets, $10^3$/µL | 268±96 | 299 ±143 | 0.41 |
| Total Cholesterol, mg/dl | 196±56 | 175±55 | 0.26 |
| LDL Cholesterol, mg/dl | 138±48 | 126±34 | 0.40 |
| HDL Cholesterol, mg/dl | 37±8 | 33±6 | 0.11 |
| Triglyceride, mg/dl | 166±65 | 158±47 | 0.37 |
| Hs CRP, mg/dl | 3.1(3.1-15.4) | 5.4 (3.1-31.0) | 0.014 |
| **Serotonin levels** | | | |
| Serum, ng/$10^9$ | 41.4±40.8 | 66.7±45.7 | 0.07 |
| In thrombocyte, ng/$10^9$ | 476±208 | 542±273 | 0.39 |
| Anterior MI | 10 (40) | 7 (47) | 0.68 |
| Predischarge LVEF, % | 50±9 | 44±7 | 0.049 |
| Pain to intervention time, hours | 4.2±2.5 | 6.3±2.9 | 0.021 |

Data are presented as the mean value ± SD, median (interquartile range) or number/percentage of patients

* Student t test, Mann-Whitney U test and Chi-square test

BMI - body mass index, CAD-coronary artery disease, HDL - high-density lipoprotein, Hs-CRP - high sensitive C-reactive protein, LDL - low-density lipoprotein, LVEF - left ventricular ejection fraction, MI - myocardial infarction

difference was not statistically significant (p=0.07) (Fig. 1).

However, the mean baseline serum hs-CRP level in the no-reflow group was established to be significantly higher [5.4 (3.1-31.0) vs. 3.1(3.1-15.4), p=0.014] (Table 1).

**Table 2. Angiographic characteristics of the study population**

| Variables | Group 2 (n=25) | Group 2 (n=15) | *p |
|---|---|---|---|
| **Myocardial infarct territory, n (%)** | | | |
| Left anterior descending artery | 10 (40) | 7 (47) | |
| Left circumflex artery | 4 (16) | 2 (13) | 0.91 |
| Right coronary artery | 11 (44) | 6 (40) | |
| **Severity of CAD, n (%)** | | | |
| Single-vessel disease | 16 (64) | 4 (27) | |
| Two-vessel disease | 3 (12) | 9 (60) | 0.006 |
| Three-vessel disease | 6 (24) | 2 (13) | |
| Stent size, mm | 19.2±3.2 | 18.8±2.9 | 0.65 |
| Stent diameter, mm | 3.4±0.3 | 3.3±0.3 | 0.89 |

Data are presented as the mean value ± SD and number/percentage values
* Student t test and Chi-square test
CAD - coronary artery disease

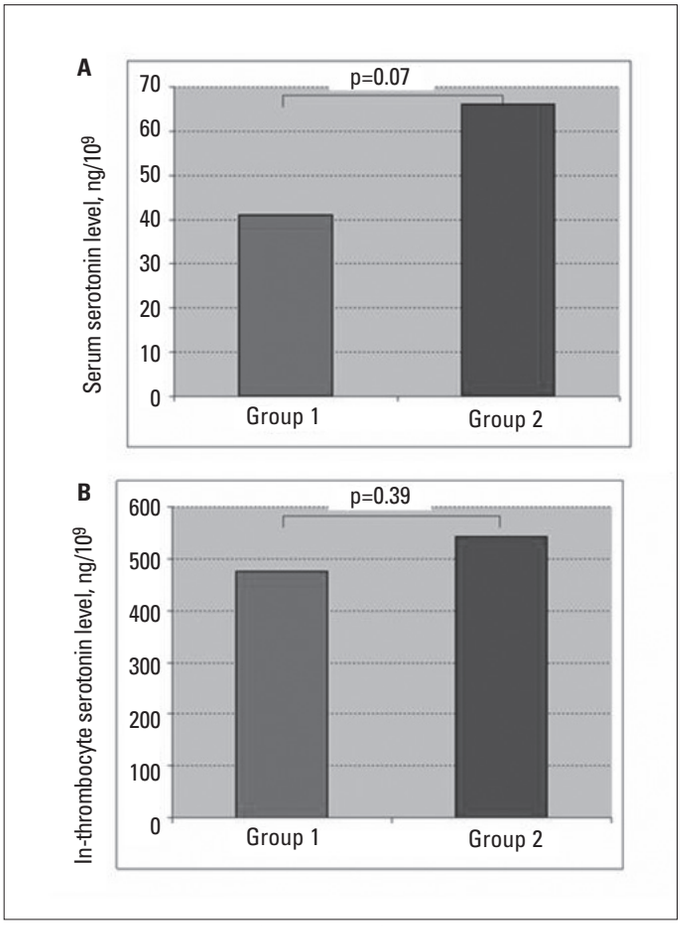


**Figure 1. Serum serotonin (upper panel) and in-thrombocyte serotonin (bottom panel) levels in patients with no-reflow and normal flow**

## Discussion

We did not find statistically significant difference in serum and in-platelet serotonin levels between patients with no-reflow and normal flow in this unique study. However, the median baseline serum hs-CRP level in the patients with no-reflow was established to be significantly higher than in the patients with normal flow.

No-reflow indicates poor perfusion to the myocardial tissue despite an absence of occlusion in the infarct-related artery (12). Although the pathophysiology of no-reflow has not been clearly elucidated, its etiology is rather complicated and this phenomenon may be attributed to a number of factors.

It is known that angiographic no-reflow development is strongly correlated with short and long-term morbidity and mortality in acute myocardial infarction (9-11). There is a relationship between the recovery of left ventricular function after an acute myocardial infarction and no-reflow phenomenon, as well. Factors with an impact on recovery are the extent of the infarction, the flow in the infarct-related artery, asynergy and elevated wall stress (11). No-reflow has an impact on all of the above listed parameters.

No-reflow can be demonstrated by means of invasive or non-invasive approaches. Angiographic TIMI grading is regarded to be a simple method to establish no-reflow (3). Myocardial contrast echocardiography can establish no-reflow as it demonstrates tissue perfusion level clearly (13, 14). However, contrast echocardiography is a complicated procedure to perform. No-reflow can also be established by utilizing methods such as scintigraphy, nuclear magnetic imaging, positron emission tomography, intracoronary pressure measurement and coronary artery flow velocity (15-19).

While no-reflow incidence is lower in elective PCI, it is higher in primary PCI in acute myocardial infarction. Higher incidence of no-reflow in acute myocardial infarction can be attributed to endothelium damage, inflammation, neutrophil plugging and higher distal microembolisation.

In a study carried out by Ko et al. (20), femoral artery blood samples and blood samples aspirated from the culprit coronary artery of 18 patients with STEMI during PCI were drawn to measure serum serotonin, hs-CRP, soluble CD 40 ligand, IL-6, tissue factor and factor VIIa levels. It was observed that the serotonin and the levels of all the other factors, except hs-CRP, were significantly elevated in the culprit artery. Samer et al. (21) conducted a study on 23 patients with acute coronary syndrome and evaluated thrombocyte reactivity through P-selectin release in blood samples drawn from the aorta and coronary ostium. They demonstrated that thrombocyte reactivity was significantly higher in coronary ostium than it was in the aorta (21). Therefore; we collected samples from the ostium of the culprit coronary artery to measure serotonin levels for this present study.

Hs-CRP is an inflammation marker with a half-life of 19 hours and it is released six hours after a coronary event, on average. Hs-CRP elevation has been demonstrated in acute coronary syndromes and it has been shown to be a predictor of potential

major cardiac events, which confirms the relationship between inflammation and acute coronary syndrome. Inflammation has also been implicated in the development of no-reflow phenomenon. However, studies investigating the relationship between no-reflow phenomenon after reperfusion and baseline hs-CRP levels have reported conflicting results. A study investigating patients with STEMI undergoing primary PCI in the first six hours of chest pain failed to show a relationship between baseline hs-CRP levels and no-reflow phenomenon (22). On the other hand, significant correlation was established between microvascular occlusion score and baseline hs-CRP levels as a result of single-variable analysis in another study investigating no-reflow phenomenon in patients with STEMI by using magnetic resonance imaging (23). In this present study, baseline hs-CRP levels were observed to be significantly higher in patients with angiographic no-reflow, indicating the effect of inflammation in no-reflow pathogenesis, as it had been suggested previously.

Distal vasoconstriction is one of the possible causes of no-reflow phenomenon. Local vasoconstrictors have been suggested to be largely responsible for the vasoconstriction occurring distal to the segment involved (24). The cause of this type of constructive response may be serotonin release from activated platelets and/or endothelin-1 release from ischemic reperfused myocardial tissue or from plaque during plaque ruptures (25). The endothelin (ET) family consists of the three distinct isopeptides ET-I, ET-2 and ET-3 (26). ET-I, the most abundant of the ET peptides in the cardiovascular system, is the most potent endogenous vasoconstrictor (26, 27). It is also important modulator of neutrophil function and a stimulator of adhesion molecules (28, 29). While increasing microvascular vasoconstriction and leukocytes adhesiveness to microvessel, ET-1 might aggravate no-reflow. Niccoli et al. (30) demonstrated that in patients admitted with STEMI, ET-1 plasma levels predicted angiographic no-reflow after primary or rescue PCI (30).

Thromboxane A2 (TxA2) is a strong platelet agonist that elicits platelet activation and aggregation and an important mediator of platelet-induced coronary artery vasoconstriction through the activation of its specific membrane receptor. It is a key mediator in the pathogenesis of thrombotic diseases (31). Niccoli et al. (31) showed that TXA2 is an independent indicator of no-reflow. In addition, Schumacher et al. (32) reported that the thromboxane receptor antagonist SQ 30,741 improved reflow during thrombolysis (32).

An animal study carried out on hypercholesterolemia rabbit model demonstrated the significance of serotonin release in early microvascular construction following atherosclerotic plaque rupture (33). The results of the study revealed that administration of ritanserin, a 5-hydroxytryptamine-2 receptor antagonist, led to a marked decrease in distal microvascular resistance, removing microvascular obstruction. Another study carried out on an animal model demonstrated that sarpogrelate, a 5-hydroxytryptamine-2 receptor antagonist, administered before creating myocardial infarct, led to a decrease in the extent of infarct and improved cardiac functions. Therefore, those animal studies established that serotonin augmented the damage in the cardiac tissue occurring as a result of ischemia by means of 5-hydroxytryptamine-2 receptor and that 5-hydroxytryptamine-2 receptor antagonists could be beneficial in decreasing the damage (34).

Management of no-reflow, which is a common complication observed following primary PCI in patients with acute STEMI, is of critical importance. Therefore, various therapeutic approaches have been used consistent with the pathophysiology of this phenomenon. Pharmacological and mechanical approaches have been tried as prophylactic therapy. Rapid and forceful administration of intracoronary saline solution may be beneficial in resolving microvascular stasis. Nitroglycerine is the pharmacological agent of choice used to resolve spasms occurred the following the procedure. Intracoronary thrombolytic papaverine, nicorandil and verapamil have been used in no-reflow management (35-38). In a study carried out by Assali et al. (39), 5.9% of the patients administered with intracoronary adenosine were observed with no-reflow, while the ratio of patients with no-reflow was 28.6% in the group not receiving adenosine. Kunichika et al. (40) reported that tirofiban, a glycoprotein receptor inhibitor, decreased the extent of infarct and no-reflow incidence. Lower incidence of no-reflow phenomenon has been reported in patients undergoing direct stenting without predilation (41). Two large trials demonstrated that a distal occlusive protection device and a rheolytic thrombectomy device did not improve myocardial reperfusion, compared with conventional primary PCI (42, 43). Hovewer, Silva-Orrego et al. (44) showed that manual thrombus aspiration before primary PCI led to lower risk of distal embolization, and no reflow compared with standard primary PCI in the DEAR-MI (Dethrombosis to Enhance Acute Reperfusion in Myocardial Infarction) study. In addition, these findings were confirmed by recent studies (45, 46). It is therefore likely to say that thrombus aspiration improves myocardial reperfusion and is associated with lower rate of no-reflow. Blocking serotonin release by using ketanserin led to a marked decrease in distal coronary vasoconstriction occurring following coronary angioplasty and stenting in a study of patients who had undergone elective PCI (47).

**Study limitations**

Small number of our patient sample and lack of certain diagnostic approaches such as magnetic resonance imaging and myocardial contrast echocardiography in addition to angiographic examination in establishing no-reflow phenomenon in our patients were the limitations of this present study. In addition, one of the most significant limitations is the fact that serotonin levels was not derived from the coronary sinus and/or distal to the culprit lesion at anytime during the course of PCI. Those measures could have been more meaningful in order to validate our study hypothesis. In addition, the TIMI flow could have been integrated with myocardial blush grade in order to better define angiographic no-reflow. The other important limitations were the lack of glycoprotein IIb/IIIa antagonists and thrombus aspiration in the management of the patients for the prevention of no-reflow phenomenon.

## Conclusion

In conclusion, baseline level of serotonin in thrombocytes and serum in patients with normal flow and in patients with no-reflow were not observed to be significantly different in STEMI patients undergoing primary PCI.

**Conflict of interest:** none declared

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