Changes in Platelets and Anticoagulant Protein Activity During Adenosine-Exercise Single-Photon Emission Computed Tomography Stress Test

Slobodan Obradović[1], Vesna Subota[2], Branislav Baškot[3], Marija Dopudja[3], Nebojša Antonijević[4], Nemanja Djenić[1], Sniježana Vukotić[1], Sonja Šalinger[5], Aleksandra Jovelić[6], Danijela Miković[7], Jovan Antović[8], Aleksandra Grdenić[9], Branko Gligić[1]

[1] Clinic of Emergency Internal Medicine, Military Medical Academy, Belgrade, Serbia;
[2] Institute of Clinical Biochemistry, Military Medical Academy, Belgrade, Serbia;
[3] Institute of Nuclear Medicine, Military Medical Academy, Belgrade, Serbia;
[4] Institute of Cardiovascular Diseases, Clinical Centre of Serbia, Belgrade, Serbia;
[5] Institute of Cardiovascular Diseases, Clinical Centre of Niš, Niš, Serbia;
[6] Institute of Cardiovascular Diseases, Sremska Kamenica, Serbia;
[7] Department for Haemostasis and Thrombosis, Institute of Transfusiology, Belgrade, Serbia;
[8] Department of Molecular Medicine and Surgery/Coagulation Research, Karolinska Institute, Stockholm, Sweden;
[9] Department of Cardiology, Clinical Hospital Centre "Dr. Dragiša Mišović", Belgrade, Serbia

SUMMARY

Introduction Activation of haemostasis during physical stress or during myocardial ischemia could be an important mechanism to trigger coronary and stent thrombosis. We examined changes in haemostatic parameters and its association with myocardial ischemia during adenosine-exercise-SPECT (adeno-EX) stress test in coronary patients at least 4 months after coronary stenting.

Objective The aim of this study was to examine relationship between changes in haemostatic parameters and stress induced myocardial ischemia quantified by perfusion scintigraphy in stented coronary patients.

Methods Thirty-seven patients on dual antiplatelet therapy (26 on clopidogrel plus aspirin and 11 on aspirin only) 4-8 months after successful intracoronary stent implantation were enrolled in the study. We determined the levels of platelet aggregability (PA) on ADP (PA-ADP) and epinephrine (PA-EPI), beta-thromboglobulin, platelet factor-4, protein C (PC) and antithrombin (AT) before and 15 minutes after intravenous injection of 150 μ/kg adenosine for 4 minutes concomitant with supine ergo-bicycle exercise test for 50 W. The size of stress perfusion defect was measured 15 minutes after stress and in rest 4 hours later by ^{99m}Tc-tetrofosmin single photon emission computed tomography (SPECT) within 17 myocardial segments.

Results There were no differences between haemostatic parameters before and after stress. A significant myocardial ischemia after exercise was registered in 12 patients on combined antiaggregation therapy and in 5 patients on aspirin. In this preliminary report, because of a small number of patients in the aspirin group we did not analyse difference in the levels of haemostatic markers and their correlations with the size of perfusion defect. The only significant difference between measured haemostatic parameters in the patients with stress induced ischemia compared to the patients without it, was a lower level of AT activity after stress (81.0% vs. 87.5%; p=0.027). Antithrombin activity before stress had significant negative correlation with the size of perfusion defect in rest (R^2=0.219; p=0.016) and PC activity before stress had significant linear correlation with stress perfusion defect (R^2=0.248; p=0.010).

Conclusion Baseline activities of natural anticoagulant proteins AT and PC are associated with the size of myocardial perfusion defect during adeno-EX-SPECT test. Patients with significant stress-induced ischemia had lower levels of AT activity after stress.

Keywords: single photon emission computed tomography (SPECT); adenosine exercise stress test; platelets; protein C; antithrombin

INTRODUCTION

Both physical and mental stress can provoke myocardial ischemia in patients with coronary stenosis and even plaque rupture or erosion with subsequent intracoronary thrombus formation and the development of myocardial infarction [1]. Stress causes increase of oxygen demand and procoagulant state. Balance between coagulation and anticoagulation system can be of crucial importance for the outcome of plaque rupture or endothelial denudation caused by a stress event [2]. Elevated levels of fibrinogen and D-dimer have a prognostic value in patients with proven coronary artery disease in predicting cardiovascular death even after adjustment for conventional risk factors and C-reactive protein level [3]. Enhanced platelet reactivity and activity of several coagulation factors together with down-regulation of anticoagulant and fibrinolytic system are closely connected with several risk factors in coronary patients, which can trigger thrombus formation and myocardial infarction [4]. Furthermore, patients with acute coronary syndrome, especially myocardial infarction, have pronounced procoagulable state, with increased markers of platelet activation and thrombin generation and suppression of fibrinolytic system [5, 6].

The association of stress induced myocardial ischemia quantified by state of art perfusion scintigraphy imaging and changes in platelet function and other haemostatic parameters in coronary patients have not been established till now.

OBJECTIVE

The goal of this study was to establish if there is any relationship between changes in the platelet activity, coagulation and anticoagulation system during adenosine-exercise induced myocardial ischemia measured by perfusion scintigraphy with a ^{99m}Technecium-sestamibi (^{99m}Tc-MIBI) radiotracer in coronary patients submitted to percutaneous coronary intervention with stenting 4-6 moths before testing.

METHODS

Subjects

Thirty-seven patients who underwent adenosine-exercise myocardial perfusion scintigraphy stress test at least 3 months after coronary stenting for acute coronary syndrome were enrolled in the study. All patients were on chronic aspirin therapy 100 mg per day, and 26 of them received 75 mg of clopidogrel daily. The main characteristics of patients are presented in Table 1. Only 5 patients had mild effort angina and all others were asymptomatic.

Stress test

Single photon emission computed tomography (SPECT) was performed after 30 minutes in rest with a ^{99m}Tc-MIBI (740 MBq) radiotracer administered at the end of the second minute of the combination of adenosine (150 μg/kg) for 4-minutes intravenous bolus injection concomitant with a low level of exercise on the ergo-bicycle in supine position (50 W for 4 minutes). Imaging started 15 minutes after stress by an Orbiter Siemens gamma camera. The second imaging was performed at rest 3-4 hours after the stress with 370 MBq of ^{99m}Tc-MIBI iv. bolus (imaging started 30 minutes after administration of radiotracer).

The quantification of myocardial perfusion defect was measured using the AutoQuant Software, within 17 myocardial segments and was shown as a total percentage of perfusion defect (uptake of radiotracer less than 50%) or using the sum of four-graded scoring across the segments (1 – normal perfusion; 2 – mild; 3 – moderate; and 4 – severe decrease of radiotracer uptake). Significant ischemia was defined as the difference of both total perfusion defects by more than 5% and scoring sum by more than 5 between rest and stress imaging (stress score – rest score ≥5%).

Haemostatic parameters

Venous blood was sampled in tubes containing 3.8% sodium citrate from an antecubital vein under minimal stasis after 30 minutes of rest and just before stress test and 15 minutes after the stress (4 minutes exercise plus adenosine bolus injection). Platelet-rich plasma (PRP) was obtained by centrifugation at 150× g for 10 minutes at room temperature. The platelet aggregation response to ADP (20 μmol/l) was recorded 5 min. after addition of the agonist using an aggregometer from BCT-system (Dade-Behring, Germany). For the determination of protein C (PC) and antithrombin (AT) activity, as well as β-thromboglobulin (BTG) and platelet factor-4 (PF4) concentrations, platelet poor plasma (PPP) was obtained from the citrated samples with 2000× g centrifugation for 15 minutes at room temperature and aliqouots were frozen at -80C° till the time of final measurement. Protein C and antithrombin activity were determined by colorimetric assays (Berichrom, Dade-Behring, Germany). BTG and PF4 were assayed in plasma by enzyme immunoassay (Boehringer-Mannheim kits Asserachrom bTG and Asserachrom PF4, respectively). All procedures were performed according to the instructions from the manufacturer.

Statistics

Since this is a preliminary report with a relatively small number of participants and some variables had normal and some abnormal distribution assessed by Kolmogorov-Smirnov test, we decided to present non-parametric statistics for the description of data (median and interquartile range as 25-75 percentiles) and we used Wilcoxon's test for the comparison of haemostatic parameters before and after stress. Linear correlation was performed using variables with normal distribution. The value $p<0.05$ was considered to indicate significance.

RESULTS

The baseline characteristics of the patients are shown on Table 1. The patients on clopidogrel and aspirin therapy had increased heart rate during test from 62±11 beats/min to maximum of 116±15 beats/min at the end of test. A similar increase of heart rate was noticed in the aspirin group. Because of the small number of patients, especially in the aspirin group, in this preliminary report we did not compare demographic parameters between the two groups. There were no significant changes of haemostatic parameters after stress (Table 2).

Significant ischemia was induced in 12 patients on clopidogrel plus the aspirin group and 5 patients on aspirin only. There was no difference between platelet aggregability and markers of platelet activation between the patients with

Table 1. Baseline characteristics of study population

Parameter	Clopidogrel + Aspirin	Aspirin
Number of patients (male/female)	26 (19/7)	11 (10/1)
Mean age (years)	57 (33-76)*	55 (28-69)*
Mean BMI (kg/m^2)	26.6 (20.5-31.0)*	26.2 (23.8-29.5)*
Hypercholesterolemia (n)	19	7
Treated hypertension (n)	13	7
Active smokers (n)	4	1
Diabetes (n)	6	2
Previous infarction (n)	19	7

* range; n – number

Table 2. Haemostasis parameters before and 15 minutes after stress (median value and IQR in parentheses)

Haemostatic parameters	Clopidogrel + Aspirin		Aspirin	
	Before stress	After stress	Before stress	After stress
Platelet aggregability on ADP	37.1 (13.7-56.3)	38.2 (30.0-55.3)	64.4 (45.5-72.0)	70.5 (46.7-76.0)
Platelet aggregability on EPI	35.2 (29.0-47.4)	36.7 (31.7-43.7)	39.0 (32.3-42.9)	33.0 (29.6-39.5)
Protein C activity	110.0 (99.0-122.0)	109.5 (100.0-117.0)	113 (109.0-124.0)	114.0 (104.0-127.0)
Antithrombin activity	87.0 (84.0-89.0)	87.0 (82.0-89.0)	92.0 (86.0-96.0)	91.0 (87.0-93.0)
Platelet factor 4	50.0 (45.0-50.0)	50.5 (45.0-59.0)	48.0 (45.0-50.0)	49.0 (45.0-59.0)
Beta-thromboglobulin	100.0 (77.0-105.0)	96.0 (80.0-100.0)	100.0 (95.0-105.0)	100.0 (94.0-105.0)

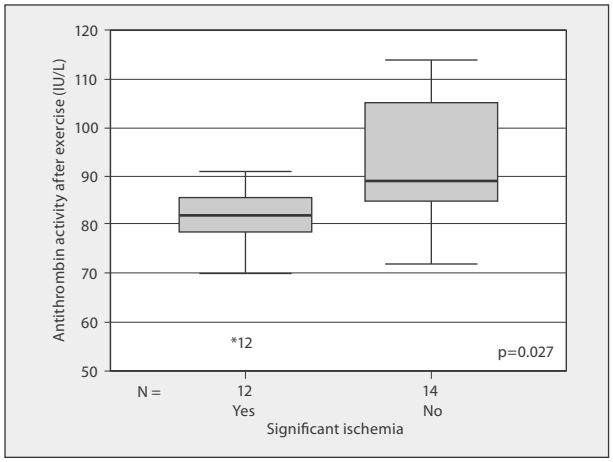
* none of the values were statistically significant

and without ischemia in both groups. In the clopidogrel plus aspirin group and in all patients antithrombin activity after stress was significantly lower in the patients with ischemia (Graphs 1 and 2).

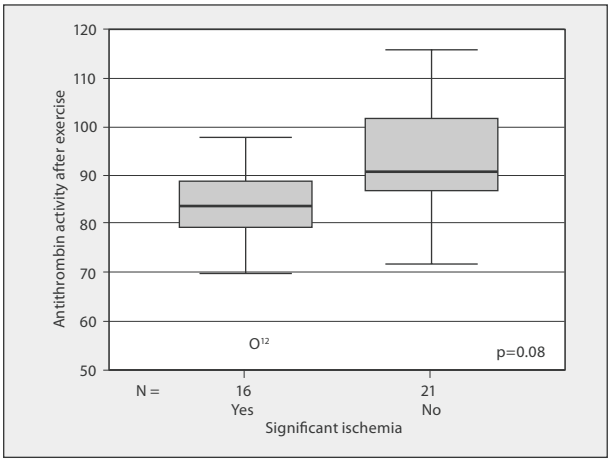
In the clopidogrel + aspirin group both PC and AT activity showed significant but opposite linear correlation with the size of perfusion defect in rest and stress, respectively. AT activity before stress had the best negative correlation with the size of rest perfusion defect (Graph 3), and PC activity before stress had the strongest positive correlation with stress perfusion defect (Graph 4). Other haemostatic parameters did not show significant linear correlation with the size of perfusion defect either in rest or stress SPECT (Table 3).

DISCUSSION

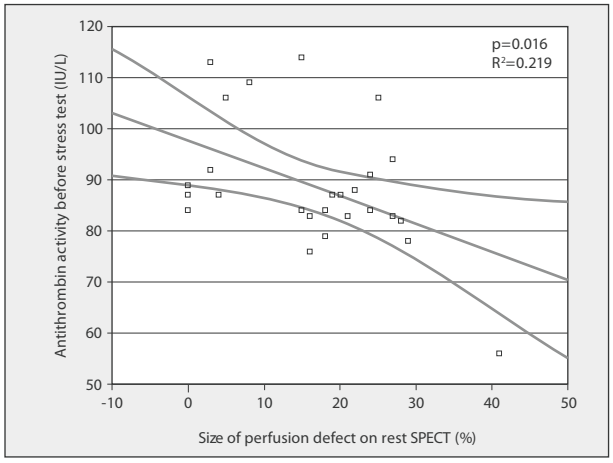
In our study pharmacological-exercise stress test with adenosine plus a concomitant mild physical exercise did not influence the measured haemostatic parameters, but induced significant myocardial ischemia in 16/37 patients. In these patients we found lower levels of antithrombin activity. Such event mimicked unstable angina as a mismatch between myocardial perfusion and needs. In this situation thrombin generation was raised during acute myocardial ischemia [7] and the activity of antithrombin lowered very probably due to the consumption of this anticoagulant protein [8] and its capture in the microcirculation of ischemic myocardium. Since there was no differ-



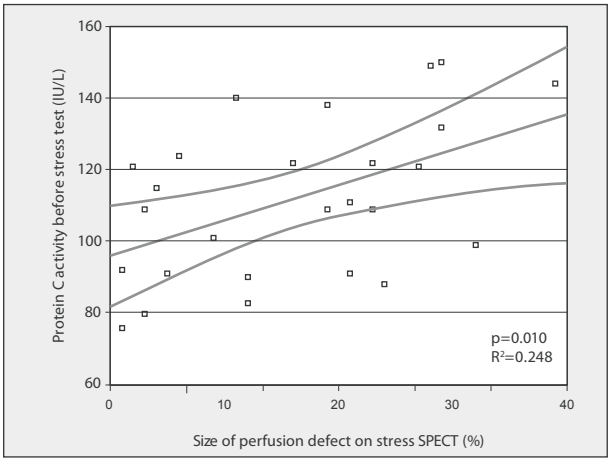
Graph 1. Antithrombin activity after exercise and ischemia on SPECT-stress test in patients on clopidogrel and aspirin therapy



Graph 2. Antithrombin activity after exercise and ischemia on SPECT-stress test in all patients



Graph 3. Linear correlation between antithrombin activity before stress and the size of perfusion defect on rest-SPECT in patients on clopidogrel and aspirin therapy



Graph 4. Linear correlation between protein C activity before stress and the size of perfusion defect on stress-SPECT in patients on clopidogrel and aspirin therapy

Table 3. Linear correlation between haemostasis parameter and the size of perfusion defect in rest and stress (SPECT)

Haemostatic parameters		Rest perfusion defect		Stress perfusion defect	
		R^2	p	R^2	p
Platelet aggregability on ADP	Before stress	0.022	NS	0.031	NS
	After stress	0.003	NS	0.007	NS
Platelet aggregability on EPI	Before stress	0.002	NS	0.001	NS
	After stress	0.053	NS	0.032	NS
Protein C activity	Before stress	0.176	0.033	0.248	0.010
	After stress	0.095	NS	0.160	0.043
Antithrombin activity	Before stress	0.219	0.016	0.172	0.035
	After stress	0.081	NS	0.049	NS
Platelet factor 4	Before stress	0.047	NS	0.066	NS
	After stress	0.089	NS	0.021	NS
Beta-thromboglobulin	Before stress	0.031	NS	0.101	NS
	After stress	0.019	NS	0.021	NS

ence between antithrombin level change during adenosine-exercise challenge in the patients with and without stress induced ischemia, our results support the hypothesis that antithrombin levels are predetermined very probably with repeated attacks of myocardial ischemia. Neither aspirin [9] nor clopidogrel [10] could inhibit the development of procoagulant state and platelet activation during exercise and this decrease of antithrombin levels probably mirrored the defensive mechanism in such situation.

Lower activity of protein C and antithrombin predict ischemic events in patients with acute coronary syndrome without ST segment elevation [11]. However, it is unknown if this parameters are somehow changed after myocardial infarction and how lower ejection fraction can influence the activity of anticoagulant proteins activity. Our results show that antithrombin and protein C levels correlate with myocardial perfusion at rest and stress. The myocardial perfusion defect at rest represents the size of irreversible myocardial damage by the pervious infarction and the defect in stress is wider as a consequence of significant ischemia. It seems that patients with larger infarctions have depressed level of antithrombin activity and up-regulation of protein C activity. We hypothesized that patients with larger infarction, somehow have up-regulation of protein C activity probably as a natural response to prothrombotic state in patients with ischemic cardiomyopathy.

CONCLUSION

The activity of natural anticoagulant system, especially antithrombin and protein C, has been neglected in the patophysiology of acute myocardial infarction and ischemia, but they may have a very important role for the fate of plaque erosion and rupture, the initial events for the coronary thrombosis. A larger study is needed together with the determination of other haemostatic parameters which can interplay with myocardial ischemia and antiplatelet drugs.

REFERENCES

1. Wallen NH, Heldf C, Rehnqvistf N, Hjemdahl P. Effects of mental and physical stress on platelet function in patients with stable angina pectoris and healthy controls. Eur Heart J. 1997; 18:807-15.
2. Tofler GH, Muller JE. Triggering of acute cardiovascular disease and potential preventive strategies. Circulation. 2006; 114:1863-72.
3. Morange PE, Bickel C, Nicaud V, Schnabel R, Rupprecht HJ, Peetz D, et al. Haemostatic factors and the risk of cardiovascular death in patients with coronary artery disease. The AtheroGene Study. Arterioscler Thromb Vasc Biol. 2006; 26:2793-9.
4. Undas A, Szułdrzynski K, Brummel-Ziedins KE, Tracz W, Zmudka K, Mann KG. Systemic blood coagulation activation in acute coronary syndromes. Blood. 2009; 113:2070-8.
5. Obradović S, Jovičić A, Djordjević D, Gligić B, Dinčić D, Stamatović D. Hemostaza i ateroskleroza. Vojnosanit Pregl. 2000; 57(2):209-16.
6. Obradović S, Mandić-Radić S, Dinčić D, Subota V, Gligić B. Hemostazni poremećaji u akutnom infarktu miokarda. Jugoslov Med Biohem. 2003; 22:109-18.
7. Merlini PA, Bauer KA, Oltrona L, Ardissino D, Cattaneo M, Belli C, et al. Persistent activation of coagulation mechanism in unstable angina and myocardial infarction. Circulation. 1994; 90(1):61-8.
8. Vaziri ND, Kennedy SC, Kennedy D, Gonzales E. Coagulation, fibrinolytic and inhibitory proteins in acute myocardial infarction and angina pectoris. Am J Med. 1992; 93:651-7.
9. Wallén NH, Held C, Rehnqvist N, Hjemdahl P. Effects of mental and physical stress on platelet function in patients with stable angina pectoris and healthy controls. Eur Heart J. 1997; 18:807-15.
10. Perneby C, Wallen NH, Hu Ha, Li N, Hjemdahl P. Prothrombotic responses to exercise are little influenced by clopidogrel treatment. Thrombosis Research. 2004; 114:235-43.
11. Pelkonen KM, Wartiovaara-Kautto U, Nieminen MS, Ahonen K, Sinisalo J. Low normal level of protein C or of antithrombin increases risk for recurrent cardiovascular events. Blood Coagul Fibrinolysis. 2005; 16(4):275-80.

Промене у тромбоцитима и активности антикоагулантних протеина током аденозинске вежбе за испитивање стреса – сцинтиграфски преглед (*adeno-EX-SPECT*)

Слободан Обрадовић[1], Весна Субота[2], Бранислав Башкот[3], Марија Допуђа[3], Небојша Антонијевић[4], Немања Ђенић[1], Снијежана Вукотић[1], Соња Шалингер[5], Александра Јовелић[6], Данијела Миковић[7], Јован Антовић[8], Александра Грденић[9], Бранко Глигић[1]

[1] Клиника за ургентну интерну медицину, Војномедицинска академија, Београд, Србија;
[2] Институт за клиничку биохемију, Војномедицинска академија, Београд, Србија;
[3] Институт за нуклеарну медицину, Војномедицинска академија, Београд, Србија;
[4] Институт за кардиоваскуларне болести, Клинички центар Србије, Београд, Србија;
[5] Институт за кардиоваскуларне болести, Клинички центар Ниш, Ниш, Србија;
[6] Институт за кардиоваскуларне болести, Сремска Каменица, Србија;
[7] Одељење за хемостазу и тромбозу, Институт за трансфузију крви Србије, Београд, Србија;
[8] Одељење за молекуларну медицину и хирургију/истраживање коагулације, Институт Каролинска, Стокхолм, Шведска;
[9] Одељење за кардиологију, Клиничко-болнички центар „Др Драгиша Мишовић", Београд, Србија

КРАТАК САДРЖАЈ

Увод Активација хемостазе током физичке активности или исхемије миокарда може бити важан механизам за настанак тромбозе стента. Испитивали смо промене параметара хемостазе и њихову везу с исхемијом миокарда током комбинованог аденозинског и физичког стрес-сцинтиграфског прегледа (*adeno-EX-SPECT*) код болесника с исхемијском болешћу срца најмање четири месеца након интракоронарне уградње стента.

Циљ рада Циљ испитивања је био да се на основу перфузионе сцинтиграфије утврди однос између промена параметара хемостазе и исхемије миокарда изазване стресом код коронарних болесника са стентом.

Методе рада У студију је укључено 37 болесника на антиагрегационој терапији – 26 на клопидогрелу и ацетилсалицилној киселини (АК) и 11 само на АК – 4-8 месеци након успешне имплантације коронарног стента. Одређивани су агрегабилност тромбоцита на аденозиндифосфат (*ADP*) и епинефрин и ниво бета-тромбоглобулина, тромбоцитног фактора 4, протеина *C* и антитромбина пре и 15 минута након четвороминутне интравенске инјекције 150 *μ/kg* аденозина уз оптерећење на ергобициклу од 50 *W*. Величина миокардне перфузије одређивана је 15 минута након оптерећења, а величина перфузије у мировању четири сата касније сцинтиграфијом с технецијумом-99м (^{99m}Tc) и тетрафозмином (енгл. *single photon emission computed tomography – SPECT*) унутар 17 сегмената.

Резултати Није било значајне разлике између параметара хемостазе пре и после оптерећења. Значајна исхемија миокарда је забележена код 12 болесника на комбинованој антиагрегационој терапији и пет који су примали АК. Због малог броја испитаника који су примали АК, у тој групи није рађена корелација показатеља хемостазе и исхемије миокарда. Једина значајна разлика између испитаника с миокардном исхемијом и оних без ње била је слабија активност антитромбина код болесника с исхемијом након теста (81,0% према 87,5%; p=0,027). Активност антитромбина и протеина *C* пре стреса имала је значајну негативну корелацију с величином перфузионог оштећења на стрес-сцинтиграфији миокарда (R^2=0,219, p=0,016; и R^2=0,248, p=0,010).

Закључак Основне активности природних антикоагулантних протеина (антитромбина и протеина *C*) повезане су с величином перфузионог оштећења изазваног стресом на сцинтиграфији миокарда. Болесници с откривеном значајном исхемијом миокарда током стреса имају мању активност антитромбина пре теста од болесника без исхемије.

Кључне речи: физички стрес-сцинтиграфски преглед (*adeno-EX-SPECT*); аденозинске вежбе за испитивање стреса; тромбоцити; протеин *C*; антитромбин

Slobodan OBRADOVIĆ
Clinic of Emergency Internal Medicine, Military Medical Academy, Crnotravska 17, 11000 Belgrade, Serbia
Email: sloba.d.obradovic@gmail.com