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Original Article

Significant Alteration of Nitrogen Species in Acute Myocardial Infarction Does Not Relate to The Site of Infarction

Marwan S.M. Al-Nimer[1], Adil H. Alhusseiny[1]

ABSTRACT

This study aimed to assess the level of nitrogen species in patients presented with acute myocardial infarction and to relate the levels of nitrogen species to the infarction site. A total number of 113 patients admitted to the intensive care unit at Diyala Teaching Hospital. The patients were presented within 6 hours of pain onset and diagnosed as acute myocardial infarction using electrocardiograph criteria and positive cardiac troponin test. The most common infarction site was anterior followed by inferior and lateral. Serum peroxynitrite level was significantly higher than healthy subjects by 100 folds and serum nitric oxide level is significantly reduced compared with healthy subjects by 50%. There were non significant differences in nitrogen species regarding the infarct sites. Associated risk factors e.g. hypertension, diabetes mellitus and smoking influenced the nitrogen species levels. It concludes that the significant alterations in nitrogen species not related to the site of acute infarction

Key words: *Acute myocardial infarction, nitric oxide, peroxynitrite*

Enfarktüsün Lokalizasyonu ile İlişkisiz Akut Miyokard İnfarktüsünde Nitrojen Türlerinin Belirgin Değişikliği

ÖZET

Bu çalışmada akut miyokard infarktüsü ile başvuran hastalarda seviyesini ve enfarktüs bölgesi ile nitrojen türlerinin ilişkisi değerlendirmek amaçlanmıştır. Diyala Eğitim Hastanesi'nde yoğun bakım ünitesine başvuran 113 hasta çalışmaya alındı. Hastalar ağrı başlangıcından ilk 6 saat içinde sunulan elektrokardiyografi kriterleri ve pozitif troponin testi ile akut miyokard infarktüsü tanısı konuldu. En yaygın enfarktüs bölgesi anteriyor, inferiyor ve lateral idi. Serum peroksinitritlerin seviyesi 100 kez sağlıklı kontrol grubuna göre daha yüksekti. Serum nitrik oksit düzeyi %50 oranında sağlıklı bireylerle karşılaştırıldığında zalmıştı. Enfarktüs bölgelerine göre nitrojen türlerinin anlamlı olmayan farklılıklar vardı. Hipertansiyon, diabetes mellitus ve sigara gibi ilişkili risk faktörleri nitrojen türlerinin düzeylerini etkilemiştir. Bu azot türlerindeki değişiklikler akut enfarktüs bölgesi ile ilgili olmadığı sonucuna varılmıştır.

Anahtar kelimeler: *Akut miyokard infarktüsü, nitrik oksit, peroksinitrit*

[1]*Department of Pharmacology College of Medicine, Al-Mustansiriya University, Baghdad, Iraq.* [2]*Department of Medicine College of Medicine, Diyala University, Diyala, Iraq.*

Correspondence: *Professor Dr. Marwan S.M. Al-Nimer*
Department of Pharmacology College of Medicine, Al-Mustansiriya University
P.O. Box 14132, Baghdad, Iraq
E mail: alnimermarwan@ymail.com

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INTRODUCTION

Acute myocardial infarction (AMI) is a significant cause of mortality and morbidity in the western world. The diagnosis of acute myocardial infarction (AMI) (1) can be made with the detection of a rise/fall of cardiac troponin and one of; symptoms of ischemia; electrocardiogram (ECG) changes of new ischemia; new pathological Q waves or; imaging evidence of new loss of viable myocardium. Both the ECG and cardiac troponin are markers of AMI. In 2000, Cardiac troponin replaced creatine kinase-MB isoenzyme as the biomarker of choice for diagnosing a myocardial infarction (2). Cardiac troponin level is dependent on infarct size (3), thus giving clinicians an idea of the prognosis following an infarction.

Biomarkers of biomechanical stress for AMI included B-type natriuretic peptide, mid regional pro-BNP, growth diffrentiation factor-15, endothelin-1 or C-terminal portion of pro-endothelin-1 (4-6). Biomarkers of plaque instability and inflammation for AMI included highly sensitive C-reactive protein and myeloperoxidase (7.8). Nitric oxide (NO) is a member of a family of labile biological mediators termed gasotransmitters (9). It is generated in mammals, including humans, by nitric oxide synthases (NOSs) and plays a prominent role in controlling blood pressure via the regulation of vascular tone. Previous studies clearly demonstrated that the deficiency of endothelial (NOS) exacerbates myocardial ischemia / reperfusion injury (10), whereas the over expression of eNOS (11, 12), the administration of NO donors, and inhaled NO gas therapy (13) all significantly protect the myocardium (14). A recent report by Kleinbongard et al. (15) demonstrates that plasma nitrite levels progressively decrease with increasing cardiovascular risk. The study aimed to assess the serum levels of nitrogen species in acute myocardial infarction and to relate these levels to the site of infarction.

MATERIALS AND METHODS

This study conducted in Department of Medicine, College of Medicine, Diyala University and The General Teaching hospital in Diyala, Iraq. The study was conducted according to the guidelines from the Declaration of Helsinki with approval from a local ethical review board. A prospective, cohort study was performed on patients with acute myocardial infarction (age: 35-80 years) who were admitted to our institute within 6 h of symptoms onset. The criteria of inclusion included ST-elevation myocardial infarction (STEMI) which defined as the presence of typical prolonged (>30 min) chest pain accompanied by typical ST segment elevation ≥0.2 mV in two or more contiguous leads on the standard 12-lead electrocardiogram (ECG) and abnormal increase of MB fraction of creatine kinase greater than twice the normal upper limit and positive troponin C test. No ST-elevation myocardial infarction (NSTMI) was defined as STEMI without ECG findings. The present study did not include patients with a history of hematological, neoplastic, renal, liver, or thyroid diseases, or patients receiving treatment with anti-inflammatory drugs. Patients with acute or chronic infections and autoimmune disease were also excluded from the study.

A total number of 113 patients (77 male and 36 female); 108 patients with STEMI; 5 patients with NSTEMI fulfill the inclusion criteria during the study were admitted in the study. Another fifteen healthy subjects without previous or current cardiovascular diseases were also admitted in this study. Demographic data, medical history and treatment were collected in the hospital. Modifiable risk factors, events or complications, and current therapy were recorded. A person who reported smoking on admission was defined as current smoker.

Peripheral venous blood was drawn immediately after admission into tubes until clot formation, then the samples were centrifuged at 2500 rpm for 10 min, and the sera were frozen and stored at -70□ until analyzed for nitric oxide (NO) and peroxynitrite (ONOO). Nitric oxide donating activity was determined as described by Newaz and co-workers (16). Briefly 0.5 ml serum was added to 200 µl HCl (6.5M) and 200 µl sulfunalic acid (37.5mM). After incubation for 10 min, 50 µl naphthylethylenediamine dihydrochloride (12.5mM) was added and incubated for further 30 min, centrifuged for 10 min at 1000g. The absorbance at 540 nm was immediately

***Table 1**. Characteristics of participants*

Gender (M:F)	77:36
Age (year)	61.9±11.05
Smoking	58(51.3%)
Alcohol	1(0.88%)
Family history of IHD	27(23.9%)
Risk factors	
Diabetes mellitus	55(48.7%)
Hypertension	62(54.9%)
Hyperlipidemia	31 (27.4%)

Table 2. Localization of cardiac infarct according to electrocardiograph records

Site of infarction	No. (%)
Anterior	48 (42.5)
Inferior	38 (33.6)
Lateral	16 (14.2)
Septal	2 (1.8)
Anterior septal	2 (1.8)
Posterior septal	1 (0.9)
Posterior lateral	1 (0.9)
NSTEMI	5 (4.4)

NSTEMI: non ST elevation myocardial infarction

Table 3. Prescribed drugs on admission

	No. (%)
Anticoagulants(heparin, clexan)	108(95.6)
Antiplatelets	108(95.6)
Lipid lowering agents	81(71.7)
Beta-adernoceptor blocking agents (metoprolol, carvedilol, atenolol)	71(62.8)
Angiotensin converting enzyme inhibitors	57(50.4)
Thrombolytics (t-plasminogen activator)	40(35.4)
Calcium entry blockers (Diltiazem, amlodipine)	18(15.9)
Antiarrhythmiacs (digoxin, amiodarone, adenosine)	15(13.3)
Angiotensin receptor blockers	0

recorded. Peroxynitrite (ONOO) mediated nitration of phenol was measured as described by others (17, 18). Briefly, 50 µl of serum was added to 5mM phenol in 50 mM sodium phosphate buffer pH 7.4 in a final volume of 3 ml. After incubation for 2 hours at 37°C, 25 µl of 0.1M NaOH was added, and the absorbance at 412 nm of the samples was immediately recorded. The yield of nitrophenol was calculated from ε = 4400 M-1.cm-1.

Statistical analysis

Data are expressed as means ± SD or percentage. Unpaired Student's t-test was used to evaluate differences in normally distributed continuous variables between the two groups. For all tests, a 2-tailed $p < 0.05$ was considered statistically significant. All calculations were made using SPSS statistical software for Windows (version 10.0).

RESULTS

Patients' characteristics are presented in Table 1. Among these study participants, 108 STEMI patients and 5 NSTEMI were recruited. The participants were more likely smokers, have positive family history of coronary artery disease and modifiable risk factors. Sixty four patients (56.6%) were complicated with heart failure and sixteen patients (14.2%) were presented with cardiac arrhythmias. Electrocardiograph records revealed that the most common type of AMI according to the infarct site was anterior AMI (42.5%) followed by inferior AMI (14.2%) (Table2). On admission different drug modalities were prescribed according to the clinical presentations (Table 3). Anticoagulants, antiplatelets, lipid lowering agents and ß-adrenoceptors agents were the most common prescribed drugs (Table 3). Thrombolytic agents (t-plasminogen activator) prescribed in 35.4% according to the clinical status of the patients taking in consideration the contraindications of these compounds. Higher percents of risk factors were reported in patients admitted with lateral AMI compared with other sites to reach significant level in association of lateral AMI with diabetes mellitus compared with anterior AMI (Table 4). Patients with inferior AMI were treated with a higher number of drugs (5.42±1.29) than corresponding means of anterior (4.87±1.26, $p<0.05$) and lateral AMI (4.69±1.74, $p>0.05$). Acute myocardial infarction patients of whatever infract site tended to have significant low serum NO and high serum ONOO levels compared with healthy subjects (Table 5). Patients presented with inferior AMI were more likely to have non-significant low serum NO and high serum ONOO levels compared with anterior or lateral AMI (Table 5). This reflected on the bioavailability of NO which represented with NO/ONOO ratio which amounted 0.173 in inferior AMI compared with 0.248 in anterior AMI) and 0.270 in lateral AMI). Further analysis revealed that AMI patients with diabetes mellitus tend-

Table 4. Frequency of modifiable risk factors according to the infarction site

Site of infarction	No. of patients	Smoking	Family history	Hypertension	Diabetes mellitus	Hyperlipidemia
Anterior	48	23 (47.9)	10 (20.8)	23 (47.9)	20 (46.5)	16 (33.3)
Inferior	38	20 (52.6)	12 (31.6)	23 (60.5)	16 (42.1)	9(23.7)
Lateral	16	9 (56.3)	6 (37.5)	12 (75)*	11 (68.8)	4 (25)

The results are expressed as number (%) of patients, * $p < 0.05$ in comparison with anterior AMI

Table 5. Serum reactive nitrogen species according to infarction site

	No. of patients	Serum No	Serum ONOO	NO/ONOO ratio
Healthy subjects	15	101.3 ± 12.2	2.95±0.52	0.338
Site of infarction				
Anterior	48	57.9 ±22.5*	233.2±171.4*	0.248
Inferior	38	49.7±19.2*	286.7±189.2*	0.173
Lateral	16	57.6±14.5*	213.1±159*	0.270

The results are expressed as mean ± SD of number of subjects, * $p < 0.05$ in comparison with healthy subjects

ed to have non-significant low serum NO and high serum ONOO compared with; hypertension, combined hypertension and diabetes mellitus, and none hypertension none diabetes patients (Table 6).

DISCUSSION

The results of this study demonstrate that the serum NO is reduced by 50% and ONOO increased by about 100 folds of that values of healthy subjects respectively. These changes in nitrogen species do not significantly related t to the infarct site. Smoking, hypertension, diabetes mellitus and hyperlipidemia that reported in the characteristics of patients are shared in disturbances of nitrogen species level. There is evidence that the association of smoking, obesity, dyslipidemia and/or metabolic syndrome with the TT genotype polymorphism of eNOS gene increased the risk of the development of PCAD (19). In ST-elevation myocardial infarction, Serum nitrite/nitrate (NO_2^-/NO_3^-) values were significantly (20). Endothelial nitric oxide synthase (eNOS) enzyme activity and nitric oxide level levels were significantly lower in acute myocardial infarction in presence or absence of macrovascular disease (21). The significant low serum nitric oxide level in patients with acute myocardial infarction could be attributed to increased activity of asymmetric dimethylarginine; an endogenous nitric oxide synthase inhibitor (22). Recently, Cavalca et al (2012) reported non significant changes in asymmetric dimethylarginine but significant increase in symmetric dimethylarginine in NSTEMI (23). In this study impaired NO production was observed in both STEMI and NSTEMI. Therefore, the endogenous nitric oxide synthase inhibitor is not the only mechanism that cause low serum nitric oxide level in myocardial infarction (23). In this study lateral myocardial infarction is associated with higher percent of risk factors while inferior myocardial infarction is associated with higher number of drug prescriptions. Even though, the serum nitric oxide did not significantly differ in between patients presented with NSTEMI or STEMI and the site of infarction. In experimental animal model utilizing occlusion of left anterior descending coronary artery of rat, the infarct size is determined by prostaglandin and nitric oxide pathways and cardiac protection can be achieved by increasing the bioavailability of nitric oxide (24). Significant high level of peroxynitrite indicated the occurrence of reperfusion injury and it also related to the infarct size. Bianchi et al demonstrated the pathogenic role of endogenously produced peroxynitrite in determining the infarct size followed ligation the left anterior descending coronary artery in pig (25). Therefore, the changes in nitrogen species levels are more likely related to infarct size rather than to the site of infarction. In experimental animal model using cardiac ischemia/reperfusion insult, the infarct size was related to high peroxynitrite and low nitric oxide level (26). The results of this study demonstrate that the changes in nitrogen species seemed to be not related to the co-morbidity or risk factors because of non-significant differences in nitric oxide or nitrogen species in between co-morbidities or risk factors. Determination of symmetric and asym-

Table 6. Serum reactive nitrogen species according to risk factors

Risk factors	No. of patients	Serum No	Serum ONOO	NO/ONOO ratio
Hypertension	28	55.5±14.3	225.2±130.7	0.246
Diabetes mellitus	21	52.6±23.3	279.5±212.1	0.188
Combined hypertension and diabetes mellitus	34	56.3±23.9	254.2±203.6	0.221
Neither hypertension nor diabetes mellitus	30	55.8±18.2	226.0±140.9	0.247

metric dimethylarginine was not done in this study and it considered as a limitation of the study.

It concludes that the significant alterations in nitrogen species that followed acute myocardial infarction do not relate to the site of infarct or to the presence of ST elevation.

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