

## THE RELATIONSHIP BETWEEN TOTAL ANTIOXIDANTS, C-REACTIVE PROTEIN AND OTHER PARAMETERS IN PATIENTS WITH ACUTE MYOCARDIAL INFARCTION

العلاقة بين مضادات الأكسدة الكلية، البروتين التفاعلي C والمشعرات الأخرى عند مرضى احتشاء العضلة القلبية الحاد

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### ملخص البحث

**هدف البحث:** تقييم العلاقة بين حالة مضادات الأكسدة، البروتين التفاعلي C، التروبونين I وأداء العضلة القلبية عند مرضى احتشاء العضلة القلبية الحاد.

**طرق البحث:** شملت هذه الدراسة 38 مريضاً تم قبولهم في وحدة العناية المشددة القلبية في مشفى ابن سينا التعليمي في مدينة الموصل خلال الفترة بين شهري آذار وأيلول من عام 2007 (33 رجال و5 نساء) مع مجموعة شاهد موافقة من ناحية العمر، الجنس، ومشعر كتلة الجسم BMI (مجموعة الشاهد 40 شخصاً، 33 رجال و7 نساء). تم جمع المعلومات السكانية مع الحصول على موافقة خطية من جميع المشتركين. تم استبعاد المدخنين ومرضى الداء السكري. تم قياس مضادات الأكسدة الكلية TAS عبر مقايصة البروكسيداز ABTs/H2O2 اللونية. تم قياس مستوى البروتين التفاعلي C بالطريقة نصف الكمية، كما قيست مستويات التروبونين I عبر تقنية الأضداد وحيدة النسيلة والمقايصة المناعية التآلفية المرتبطة بالأنزيم ELIFA. تم إجراء تصوير للقلب بالأشعة فوق الصوتية لجميع المرضى خلال 72 ساعة الأولى من بدء الألم الصدري.

**النتائج:** لوحظ أن مستويات مضادات الأكسدة الكلية TAS أخفض وبشكل هام عند مرضى احتشاء العضلة القلبية الحاد (AMI) ( $p < 0.001$ )، كما أنها ترتبط بشكل مباشر مع نسبة الكسر القذفي EF ( $p < 0.001$ ). لوحظ ارتفاع مستويات البروتين التفاعلي C لدى مجموعة المرضى ( $p < 0.001$ )، كما أظهرت مستوياته ارتباطاً مع نسبة الكسر القذفي EF، حيث ارتفعت مستوياته عندما تكون قيمة  $EF > 55$  ( $p < 0.03$ )، وتتنخفض عندما تكون  $EF < 55$  ( $p < 0.03$ ). لوحظ وجود علاقة مباشرة هامة بين مستوى التروبونين القلبي I والمستويات المصلية من مضادات الأكسدة الكلية TAS ( $p < 0.001$ ).  
**الاستنتاجات:** لوحظ انخفاض هام في مستويات مضادات الأكسدة الكلية TAS عند مرضى احتشاء العضلة القلبية الحاد، كما ترتبط مستوياتها بشكل مباشر مع نسبة السكر القذفي EF والتروبونين القلبي I.

### ABSTRACT

**Objective:** To evaluate the relationship between antioxidant status, C-reactive protein, Troponin I and cardiac performance in patients with acute myocardial infarction.

**Methods:** The study was conducted on thirty eight

patients who were admitted in the Intensive cardiac care unit (ICCU) in Ibn-Sina Teaching Hospital in Mosul city from March to September 2007, of them 33 were males and 5 were females, and control group of 40 persons nearly matched with age, sex and body mass index of them 33 were males and 7 were females. Demographic details and written informed consent were obtained for

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both groups; the diabetic and smokers were excluded. Total antioxidant status (TAS) measured by Peroxidase H<sub>2</sub>O<sub>2</sub>/ABTS Colorimetric Assay. CRP measured by semi quantitative test procedure. Troponin I assay was done by Monoclonal Antibody Technique and Enzyme Linked Immunofluorescent Assay. Echocardiography done to all patients within the first 72 hours after the onset of chest pain.

**Results:** TAS was significantly low in patients with AMI ( $p < 0.001$ ), and was directly associated with ejection fraction ( $p < 0.001$ ). CRP was significantly high in patient group ( $p < 0.001$ ). CRP and EF value showed significant association; patients with EF < 55 found to have a higher CRP level ( $p < 0.03$ ), and those with EF > 55 have a lower CRP level ( $p < 0.03$ ). Cardiac troponin I was found to have significant direct relationship with serum level of TAS ( $p < 0.001$ ).

**Conclusions:** Total antioxidant status (TAS) was significantly low in patients with AMI and was directly associated with ejection fraction and cardiac troponin I.

## INTRODUCTION

Coronary heart disease is the most common form of heart disease and it is almost always due to atheroma and its complication.<sup>1</sup> The pathogenesis of atherosclerosis involves damage to the vascular endothelium caused by various factors including oxidized low-density lipoprotein (LDL).<sup>2,3</sup>

The oxidation of low density lipoprotein (LDL) is a major factor in the process of atherogenesis mainly by causing an increased uptake of lipids by macrophages in the arterial wall contributing in the formation of atherosclerotic plaque.<sup>4</sup>

Reactive oxygen species are important for life and are involved in cell signaling, but they may contribute to the etiology and progression of many diseases.

On the other hand the enhanced formation of reactive oxygen species may affect four fundamental mechanisms that may contribute to atherogenesis, namely: oxidation of LDL, endothelial dysfunction, vascular smooth muscle growth and monocyte migration.<sup>5</sup>

C-reactive protein (CRP) has been recognized as one of the most sensitive of the acute phase reactant. It can rise dramatically after AMI, stress, trauma, infection, inflammation, surgery or neoplastic proliferation.

This rise occurs within 24 to 48 hour and the level may be 2000 times of normal and can rise up to 10,000 folds during inflammation.<sup>6</sup>

The aims of this study were to assess the total antioxidant status in acute myocardial infarction (AMI), and define the relation between antioxidant status, CRP and ejection fraction in patients with AMI. Also to evaluate the correlation of antioxidant status with serum troponin I level.

## METHODS

The study was conducted on patients suffering from acute myocardial infarction, who were admitted in the Intensive coronary care unit (ICCU) in Ibn-Sina teaching hospital in Mosul City, between March to September 2007. A case control study was adopted to achieve the aims of the study. Demographic details and written informed consent were obtained for both groups.

Thirty eight patients with acute myocardial infarction were selected, 33 were males and 5 were females with a mean  $\pm$ SD age 60.88 $\pm$ 8.16 years.

Patient with diabetes mellitus, and cigarette smoker (but those who quit smoking in the last six months were involved in this study) were excluded.

The control group consists of 40 normal persons (33 were males and 7 were females) with a mean  $\pm$ SD age 60.95 $\pm$ 10.36 years, nearly matched with age and body mass index of the patients group and all have no previous history of IHD.

A 10 ml venous blood sample were collected from all patients within the first 24 hours of onset of chest pain and from the control group, collected in a plane tubes and centrifuged and then tested for the level of total antioxidant status (TAS), CRP level and cardiac Troponin I. Serum total antioxidant status (TAS), was measured by peroxidase (H<sub>2</sub>O<sub>2</sub>) ABTS colorimetric assay,<sup>7</sup> a kit supplied by Randox Company Ltd.UK.

C-reactive protein (CRP), was measured by LABKIT CHEMELEX.S.A.Pol. Ind.Can Castells/ Industria 113, Nau J 08420 Canovelles BARCELONA.

Troponin I assay was done by Monoclonal Antibody Technique and Enzyme Linked Immunofluorescent Assay using VIDAS TROPONIN Ultra kit.REF 30 448, Bio. Merieux SA 69280 Marcy l Etoile-France.

Echocardiography done to all patients in the first 72 hours after the onset of chest pain.

**Statistical analysis:** The age of the patients measured as a mean age  $\pm$ SD. The p-value calculated by Chi square test and the relationships proved by using Pearson correlation.

### RESULTS AND DISCUSSION

To evaluate the antioxidant status in patients with acute myocardial infarction, a case-control study was carried out and revealed that the concentration of TAS is low in AMI patients and it is significantly low compared to control group ( $p < 0.001$ ), this finding is in accordance with study done by Surekha et al.<sup>5</sup> Following ischemia Reactive oxygen species (ROS) produced during reperfusion phase.<sup>8,9</sup>

		Control		Patients	
Number		40		38	
Age (year), Mean $\pm$ SD		60.88 $\pm$ 8.16		60.95 $\pm$ 10.36	
BMI (kg/m <sup>2</sup> )		27.30 $\pm$ 1.98		26.95 $\pm$ 6.51	
		No.	%	No.	%
Sex	Male	33	82.5	33	86.8
	Female	7	17.5	5	13.2
Hypertension	+ve	-	-	7	18.4
	-ve	-	-	31	81.6
Unstable angina	+ve	-	-	4	10.5
	-ve	-	-	34	89.5
Old MI	+ve	-	-	6	15.8
	-ve	-	-	32	84.2
MI Type	Anterior	-	-	22	57.9
	Inferior	-	-	16	42.1

The Mean $\pm$ SD of TAS in patients is 1.17 $\pm$ 0.21 and for the control 2.13 $\pm$ 0.3 with significant p-value of  $< 0.001$ .

**Table 1. Demographic characteristics of the studied groups.**

EF	CRP +ve		CRP -ve		p-value
	No.	%	No.	%	
<0.55	16	72.7	6	37.5	0.03
>0.55	6	27.3	10	62.5	
Total	22	100	16	100	

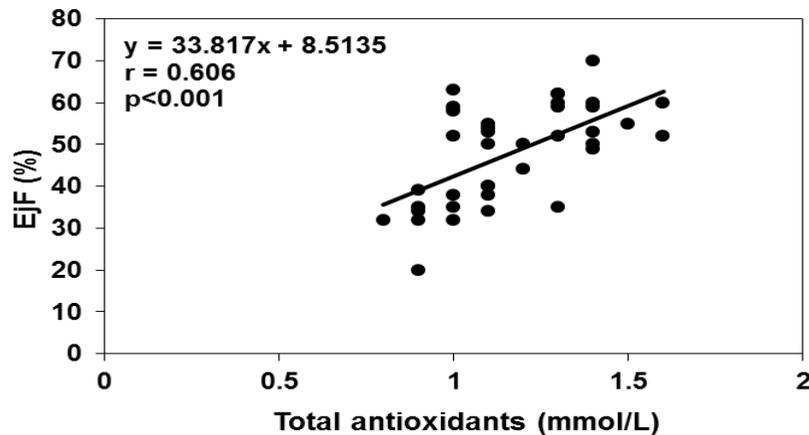
**Table 2. Relationship between CRP concentrations and EF.**

The antioxidant particularly secondary antioxidant act as scavenging the effect of the ROS and interfere with chain propagation therefore it is called (chainbreakers).<sup>10</sup> This explain the harmful effect of ROS during ischemia when theTASis low.

Other studies show that the level of thiobarbituric acid as predictor of lipid peroxidation was significantly increased and TAS was significantly decreased in AMI.<sup>11,12</sup>

CRP is an acute phase reactant that can rise markedly after AMI.<sup>13</sup> The level of CRP in this study was found to be significantly high in AMI in comparison to healthy persons ( $p < 0.001$ ). We observed a significant inverse relationship between ejection fraction and the level of CRP. Patients who have a normal ejection fraction have undetectable or minimally raised CRP ( $p < 0.03$ ). Those who have a reduced ejection fraction have raised CRP levels ( $p < 0.03$ ), Table 2. Whether the elevated serum level of CRP reflect the burden of atherosclerotic process, size of infarction or the rupture of plaque, Hon-kan et al concluded in their study that elevated serum level of CRP in AMI <6 hours may portend plaque rupture<sup>14</sup> so CRP might not only mirror the inflammatory stimulus, on the other hand other studies found that the clinical outcome both early and late significantly related to the plasma level of CRP post-infarction, therefore its level is correlated with the extent of myocardial damage.<sup>15</sup> whether CRP can be used clinically to monitor the progress of MI or to predict the severe complicated MI needs further study.

The ejection fraction; which reflect the cardiac performance; is found in this study to be directly related



The Mean±SD of CRP concentration (mg/L) in patients 13.11±9.34 where as it is 2.88±2.1 in the control group with significant p-value of <0.001.

Figure 1. Relationship between TAS and EF.

to the concentration of TAS (Figure 1), and this may be explained by the fact that free radicals and antioxidant status play a major role in both atherosclerosis and cardiac damage<sup>16</sup> and through ischemic injury can lead to alteration in cardiac performance in the form of myocardial stunning and irreversible damage.<sup>17</sup> It was found that prolonged ischemia reduces the naturally occurring defense mechanism of the heart against free oxygen radicals, particularly mitochondrial manganese, superoxide mutase and intracellular pool of glutathione and that the degree of oxidative stress was inversely correlated with the recovery of mitochondrial damage.<sup>18</sup>

Michael et al shows that heart failure subsequent to left ventricular MI is associated with antioxidant deficit, and that depressed cardiac function and heart failure may occur as a consequence of increase in oxidative stress and relative antioxidant deficiency.<sup>19</sup>

Cardiac troponin I is a part of a new generation of biochemical markers that provides an additional clinical tool for assessment of the acute coronary syndrome. Functionally troponin I is a 24 KD structural protein that interacts with troponin T, 37 KD, and troponin C, 18 KD as a part of the three troponin complex that is essential for the contraction of striated muscle in both cardiac and skeletal tissue.<sup>20</sup> Troponin I is both sensitive and specific for the diagnosis of AMI with other biomarker, with history of chest pain and characteristic ECG finding.

There was a significant inverse correlation between serum TAS concentrations and serum Troponin I levels ( $p < 0.001$ ) as shown in Figure 2. This correlation can be explained clearly depending on the function of total antioxidant and what does cTr I elevation means? Knowing the fact that cTrI is a highly specific and sensitive biomarker of cardiac injury<sup>21</sup> and it is not expressed by skeletal muscles or other tissues is considered as a marker of cardiac necrosis.<sup>22</sup>

Depending on these two facts the observed differences in the concentrations of cTrI of patients represent the differences in the amount of cardiac damage produced by myocardial infarction; since cTrI peak levels can estimate the scintigraphic infarct size if measured at 72 hour of onset of AMI.<sup>23</sup>

All serum samples of the patients in this study were collected in the first 24 hour of onset of chest pain; i.e. the period of sample collection is equal to all patients; as if putting them on a start line and allowing the difference to occur in the concentration of cTrI depending on infarct size.

On the other hand the differences in the concentrations of TAS in relation to cTrI concentrations can be explained by the fact that TAS are utilized in the oxidative process and the more concentration present means the more protection offered, applying this concern on infarct size; represented by cTrI; means the high concentration of TAS available the better is the protection provided and

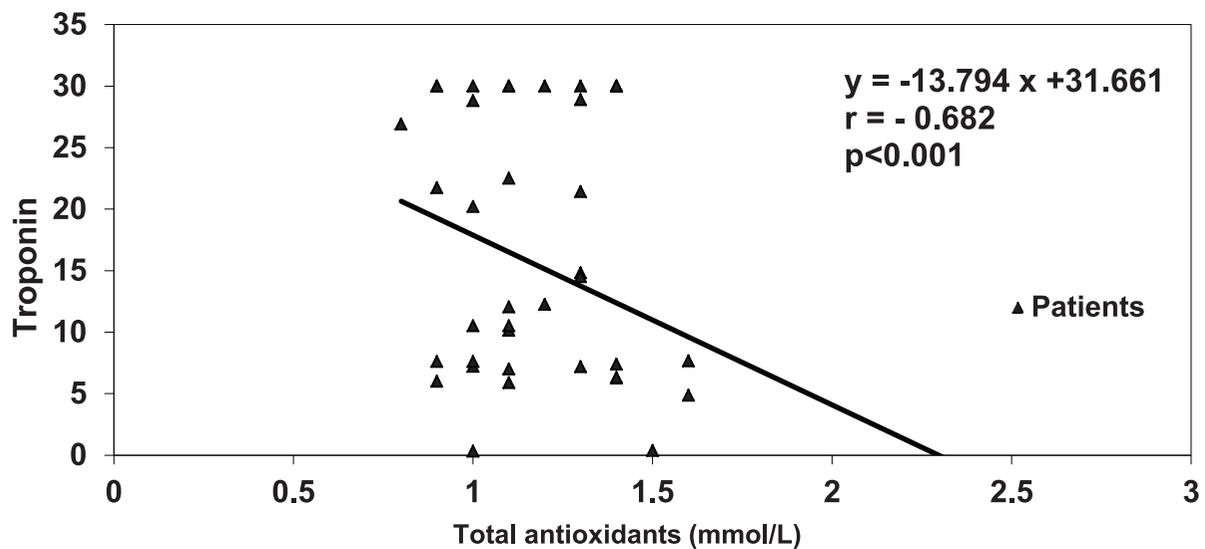


Figure 2. The relationship between the concentrations of cTr I and TAS in AMI patients.

the less cardiac damage to result. The equation is true vice versa i.e. the low TAS concentration is present the more damage is likely to occur.

## CONCLUSIONS

Total antioxidant status (TAS) was significantly low in patients with AMI and was directly associated with ejection fraction and cardiac Troponin I.

## REFERENCES

1. Newby DE, Grubb NR, Bradbury A. Cardiovascular system. In: Walker B, Niki RC, Penman ID, et al. Davidson's principles and practice of medicine 22th edition. China: Elsevier limited;2014. p. 583-90.
2. Ross R. The pathogenesis of atherosclerosis: a perspective for the 1990s. *Nature* 1993;362:801-9.
3. Shimokawa H. Primary endothelial dysfunction: atherosclerosis. *J Molec Cell Cardiol* 1999;31:23-7.
4. Alwine FM, Antti A, Jeremy DK, et al. Association between B carotene and acute myocardial infarction depends on poly unsaturated fatty acid status. *Am Heart Assoc* 1995;15:726-32.
5. Surekha RH, Sirikan BM V, Jharna P, et al. Oxidative stress and total antioxidant status in myocardial infarction. *Singapore Med J* 2007;48(2):137.
6. Shine B, deBeer FC, Pepys MB. Solid phase radioimmunoassays for C-reactive protein. *Clin Chem Acta* 1981;117:13-23.
7. Miller NJ, Rice EC, Davies MJ, et al. Total antioxidant status by colorimetric method. *Clin Sci* 1993;84:407-12.
8. Espat NJ, Helton WS. Oxygen free radicals, oxidative stress, and antioxidants in critical illness. *Support Line* 2000;22:11-20.
9. Zweier JL, Flaherty JT, Weisfeldt ML. Direct measurement of free radical generation following reperfusion of ischemic myocardium. *Proc Natl Acad Sci* 1987;84:1404-7.
10. Mayes PA. Structure and function of the lipid soluble vitamins and the pentose phosphate pathway and other pathways of hexose metabolism. In: Murray RK, Garnner DK, Mayes PA, et al (editors). *Harper biochemistry*. 25th ed. China:McGraw Hill; 2000. p. 642-82.
11. LoPresti R, Catania A, D'Amico T, et al. Oxidative stress in young subjects with acute myocardial infarction: evaluation at the initial stage and after 12 months. *Clin Appl Thromb Hemost* 2008;14:421-7.
12. Ragab M, Hassan H, Zaytoun T, et al. Evaluation of serum neopterin, high sensitivity C-reactive protein and thiobarbituric acid reactive substances in Egyptian patients with acute coronary syndromes. *Ext Clin Cardiol* 2005;10:250-5.
13. Sano T, Tanaka A, Namba A, et al. C-reactive protein and lesion morphology in patient with acute myocardial infarction. *Circulation* 2003;108:282-5.

14. Hon-Kan Yip, Chiung-Jen Wu, Hsueh-Wen Chang, et al. Levels and values of serum high-sensitivity C-reactive protein within 6 hours after the onset of acute myocardial infarction. *Chest* 2004;126:1417-22.
15. Grisille M, Herbert J, Hutchinson WL, et al. C-reactive protein and complement are important mediators of tissue damage in acute myocardial infarction. *J Exp Med* 1999;190:1733-9.
16. U.N.Das. Free radicals, cytokines and nitric oxide in cardiac failure and myocardial infarction: *Molec Cell Biochem* 2000;215:145-52.
17. Goldhaber JI, Weis JN. Oxygen free radicals and cardiac reperfusion abnormalities. *Hypertension* 1992;20:118-27.
18. Ferrari R, Ceconi C, Curello S, et al. Oxidative stress during myocardial reperfusion. *Moll Cell Biochem* 1992;111:61-9.
19. Micheal H, Pawan k. Right and left myocardial antioxidant responses during heart failure subsequent to myocardial infarction. *Circulation* 1997;96:2414-20.
20. Mair J, Dienstl F, Puschendorf B. Cardiac troponin T in the diagnosis of myocardial injury. *Crit Rev Clin Lab Sci* 1992;29:31-57.
21. Alan HBW, Yue-Jin F, Robert M, et al. Characterization of cardiac troponin sub unit release into serum after acute myocardial infarction and comparison of assays for Troponin I and T. *Clin Chem* 1998;44:1198-208.
22. Elliott MA, Milinko JT, Bruce T, et al. Cardiac specific troponin I levels to predict the risk of mortality in patients with acute coronary syndromes. *N Eng J Med* 1996;335:1342-9.
23. Babuin L, Alla SJ. Troponin: the biomarker of choice for detection of cardiac injury. *CMAJ* 2005;173:1-91.