UTILITY OF ANTIOXIDANT GAP IN ACUTE MYOCARDIAL INFARCTION

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ABSTRACT: BACKGROUND: Increased free radical production and impaired antioxidant defense system are being implicated in various disease conditions. Myocardial infarction is one such disease in which free radical mediated injury and antioxidant activity are largely studied. However there is controversy regarding the role of antioxidants in the pathogenesis and prognostication of patients following acute myocardial infarction. With these aspects in mind, this study was conducted with the objective to evaluate the utility of antioxidant gap in myocardial infarction. The serum "antioxidant gap" reflects the antioxidant activity of ascorbate, alpha-tocopherol, carotene, bilirubin and radical scavenging antioxidants other than albumin and uric acid

Aim: 1) To assess the following parameters in acute myocardial infarction patients:

a. Serum albumin
b. Serum uric acid.
c. Serum total antioxidant activity

2) To study the changes in the antioxidant gap and serum uric acid levels over a period of 3 days. Antioxidant gap will be calculated as follows:
Antioxidant gap= Total antioxidant activity (TAA)-(serum albumin + serum uric acid)
3) To associate the changes in the serum antioxidant gap with the clinical prognosis of the patient.

SETTINGS AND DESIGN: A Prospective follow up study consisting of patients with the diagnosis of ST segment Myocardial infarction (STEMI). 30 Patients who were admitted with the diagnosis of acute ST segment elevation myocardial infarction were included in the study. Serum total antioxidant activity, albumin, uric acid and plasma Malondialdehyde levels were measured on admission, and for the next 2 days of hospital stay. The clinical prognosis of the patients was assessed based on the TIMI scoring system for mortality rates of patients with STEMI. **STATISTICAL ANALYSIS:** The data was entered in Microsoft excel and analysed using SPSS 15.0, Stata 8.0, Medcalc 9.0.1 and systat 11.0 software. Repeated measures analysis of variance and student t test was used to find the significance of study parameters on different days. Significance is assessed at 5% level of significance. Chi-square test and Fisher Exact test has been used to find the significance of study parameters on categorical scale. **RESULTS AND CONCLUSIONS:** On analysis, it was found that increase in the total antioxidant gap was significantly associated with good prognosis ie, they had lower mortality rates (p=0.008) and increase in serum uric acid levels is significantly associated with higher mortality rates (bad prognosis), with a p value of 0.002. Hence it can be concluded from this study that changes in gap antioxidants levels following acute myocardial infarction may be of use in evaluating the clinical prognosis of the patient.

KEY WORDS: Antioxidants, acute myocardial infarction, free radicals.

INTRODUCTION: Acute Myocardial Infarction (AMI) is one of the major causes of mortality and morbidity in the world. The most common cause underlying an episode of acute myocardial

infraction is Coronary Artery Disease (CAD) with erosion or rupture of a plaque causing transient or partial or complete arterial occlusion[1]. Excessive production of Reactive Oxygen Species (ROS) has been implicated to play an important role in a number of cardiovascular pathologies including hypertension, atherosclerosis, myocardial infarction, ischemia/ reperfusion injury, restenosis etc. The formation of free radicals is balanced out by antioxidant defenses. Oxidative stress in cardiac and vascular cells describes the injury caused to cells resulting from increased formation of ROS and /or decreased antioxidant reserve.[2]

The increase in the generation of ROS seems to be due to impaired mitochondrial reduction of molecular oxygen, secretion of free radicals by leucocytes, autoxidation of catecholamines, as well as exposure to radiation or air pollution. On the other hand depression in the antioxidant reserve, which serves as a defense mechanism in cardiac and vascular myocytes, appears to be due to exhaustion and / or changes in gene expression.[3]

Reactive oxygen species are capable of reacting with unsaturated lipids and of initiating the self-perpetuating chain reactions of lipid per oxidation in the membranes. Free radicals can also cause oxidation of sulfhydryl groups in proteins and strand scission in nucleic acids is also possible. Myocardial antioxidants inhibit or delay the oxidative damage to subcellular proteins, carbohydrates, lipids and DNA. There is evidence that antioxidants can protect against free radicals and may thereby inhibit thrombosis, myocardial damage and arrhythmias during AMI. Total Antioxidant Capacity (TAC) is a critical tool for assessing redox status. The TAC may play an important role in protecting the organism from free radical mediated damage. [4, 5]

There is growing evidence that increase in free radical production and impaired antioxidant protection is relevant to plaque activation. In addition to traditional risk factors, oxidative stress has been regarded as one of the most important contributors to the progression of atherosclerosis. In ischemia, the ATP is drastically reduced and is converted to hypoxanthine and then to uric acid by Xanthine oxidase upon reperfusion. It has been suggested that increased lipid peroxide levels in blood of patients with acute myocardial infarction. Several antioxidants protective mechanisms exists against free radical damage and constitute a primary defensive system including vitamins like C, E, beta-carotenes, and enzymatic defenses. It is known that plasma antioxidant capacity decreases and oxidant/antioxidant balance shifts to the oxidant side in patients with coronary artery disease.[1]

During past several years there has been a growing interest in the medical implications of free radicals. The role of albumin as a free radical scavenger is well established. Albumin is a main source of thiol groups in the serum and protein thiol oxidation has been shown to be a sensitive and specific indicator of oxidant stress to the vascular compartment. Increased loss of albumin in urine is known to be an early response to myocardial infarction. Uric acid is the next most abundant serum antioxidant in terms of mass and activity. Ascorbic acid accounts for just fewer than 10% of total plasma antioxidant activity and its high hepatic and renal clearance make it unlikely that this value could be greatly raised. The remaining antioxidant activity in the plasma, up to 40% is accounted for by substances like beta-carotene, bilirubin, glutathione, ubiquinol and other phenolic and poly phenolic plasma components.[5]

The serum ˝antioxidant gap˝ reflects the antioxidant activity of ascorbate, alpha-tocopherol, carotene, bilirubin and radical scavenging antioxidants other than albumin and uric acid. Up to 40% of total plasma antioxidant activity is accounted for by substances like alpha-tocopherol, beta carotene, bilirubin, cysteine, glutathione, other phenolic and poly phenolic components.

Unfortunately, direct investigation of these free radicals in vivo is difficult because most aggressive species have an extremely short half-life and cannot easily be trapped under physiological conditions. But indirect information on the impact of free radicals may be obtained by comparisons of the antioxidant concentrations [5].

Most of the studies in myocardial infarction, premature babies, smokers, asthmatics, all conditions of oxidative stress have shown changes in serum total antioxidant activity. While some others have reported no statistically significant change in the total antioxidant activity during 48 hours post myocardial infarction.[5, 6]

Therefore there is a need for more research and further studies in this subject to know the actual status of antioxidants in myocardial infarction and also whether they have any prognostic value. Hence this study intends to find out if there is any utility of antioxidant gap and serum uric acid in the prognostication of myocardial infarction patients.

METHODS: The source of data were the patients diagnosed as acute ST segment elevation myocardial infarction using the standard guidelines of American college of cardiology/American heart association(ACC/AHA) (using ECG/cardiac enzymes) by cardiologists in the Department of Cardiology, St. John's Medical College Hospital .Written informed consent was obtained from them.

A total of 30 patients were eligible for the study who met the inclusion and exclusion criteria. Blood sample was collected from these patients on the day of admission (day 0), 12-30 hrs after collecting the first sample (day 1), and 35-54 hrs after collecting the second sample (day 2). The patients were followed upto their discharge.

Serum albumin, uric acid, total antioxidant activity was estimated from the blood samples of these patients. Antioxidant gap was calculated as: Antioxidant gap= Total antioxidant activity-(serum albumin + serum uric acid)

TAA was estimated by Spectrophotometric method of Koracevic and Koracevic. [7]

Principle: A standardized solution of Fe-EDTA complex reacts with hydrogen peroxide by a Fenton type reaction, leading to the formation of hydroxyl radicals (OH·). These reactive oxygen species degrade benzoate, resulting in the release of Thiobarbituric acid reactive substances (TBARS). Antioxidants from the added sample of human fluid cause suppression of the production of TBARS. This reaction can be measured spectrophotometrically and the inhibition of colour development defined as the Antioxidant activity.

Serum albumin was estimated by Bromocresol green dye binding method [8]

Principle: Many dyes bind to serum proteins and a more selective form of binding has been exploited for the determination of serum albumin. In this method bromocresol green (BCG) dye with a p.k value of 4.6 is used. When albumin is added to a solution, the resulting change in colour is proportional to the concentration of albumin in the given sample.

Serum uric acid was estimated by Caraway's Method [9]

Principle: Phosphotungstic acid is reduced by uric acid in presence of sodium carbonate (alkaline medium) to give a blue complex (tungsten blue) which absorbs light at 700 nm.

TIMI SCORING SYSTEM FOR STEMI[10]

- Prognosis of the patient will be assessed based on Thrombolysis in myocardial infarction risk score (TIMI) for STEMI.

Details	Points	Score
Historical: Age>or equal to 75 yrs Age:64-75 yrs.	3 2	
Diabetes or hypertension or angina	1	
EXAMINATION		
Systolic blood pressure<100mmhg	3	
Heart rate> 100bpm	2	
Killip 11-1V	2	
Weight<67kgs	1	
PRESENTATION		
Anterior STE or LBBB	1	
Time to treatment >4 hrs	1	

Risk Score	10 day mortality
0	0.8
1	1.6
2	2.2
3	4.4
4	7.3
5	12
6	16
7	23
8	27
>8	36

This scoring system can also be used to assess the prognosis of non thrombolysed STEMI patients, who will be subjects of this study.

Inclusion criteria:

A. All patients of ST segment elevation myocardial infarctions (STEMI) who are not thrombolysed.
B. Age group- 30 to 65 yrs

Exclusion criteria:

A. Cases of myocardial infarction who have or who are going to receive thrombolytic therapy of any kind
B. Patients who have or who develop renal complications / cerebral complications during the course of the study
C. Patients with gout.
D. Cases of non ST segment elevation myocardial infarction.

Ethics: the study was approved by the institutional ethics committee.

STATISTICS:

Type: prospective follow up study

Statistical Methods[11, 12, 13]: Descriptive statistical analysis has been carried out in the present study. Results on continuous measurements are presented as mean±sd (Minimum-maximum) and results on categorical measurements are presented in number (%). Significance is assessed at 5% level of significance. Repeated Measures Analysis Of Variance (RMANOVA) has been used to find the significance of study parameters between three or more group of patients, Student t test(two tailed, independent) has been used to find the significance of study parameters on continuous scale

between two groups(inter group analysis). Chi-square/ Fisher exact test has been used to find the significance of study parameters on categorical scale between two groups.90% confidence interval has been computed to find the significant features. Confidence interval with lower limit > 50% is associated with statistical significance.

The Statistical software namely SPSS 15.0, Stata 8.0, Medcalc 9.0.1 and Systat 1.0 were used for the analysis of the data. Microsoft word and Excel have been used to generate graphs, tables etc**.**

Significant figures:

Suggestive significance- p value: $0.05<p<0.10$
Significant - p value: $0.01<p<0.05$
Strongly significant - p value: $p<0.01$

RESULTS:

Table 1 shows age distribution of patients. Table 2 a shows the gender distribution of patients.
Table 3 shows the prognostication of patients. Of the 30 patients, 21 of them (70%) had a TIMI score of STEMI less than or equal to 7 (good prognosis) and 9 patients (30%) had a TIMI score for STEMI more than 7(bad prognosis)
Table 4(graph 4a) shows the mean antioxidant gap values on day0, day1 and day 2 of hospital stay of patients diagnosed as STEMI. On day 0, the mean AOG values were 0.29+0.15(0.04-0.66), day 1: 0.33+0.12(0.09-0.56) and on day 2: 0.36+0.14(0.1-0.68).The percentage change on day 1 for good prognosis was 22.2% on day 1 and 48.1% on day 2 with a p value of less than 0.001(table 5&graph 5a). 18 patients (85.7%) whose AOG values were increased compared to day 0 and day1 showed good prognosis and 6 patients (66.7%) whose AOG values were decreased showed bad prognosis with a p value of 0.008(table 6, graph 6).This study showed that increase in total antioxidant gap is significantly associated with good prognosis

Table 1: Age distribution of patients studied

Age in years	Number	%
45-50	3	10.0
51-60	18	60.0
61-70	9	30.0
Total	30	100.0
Mean ± SD	58.00±5.69	

Table 2: Gender distribution of patients studied

Gender	Number	%
Male	**23**	**76.7**
Female	**7**	**23.3**
Total	**30**	**100.0**

Table 3: Prognosis based on TIMI score

Prognosis	Number	%	90%CI
Good (≤7.0)	**21**	**70.0**	**55.06-81.63**
Bad(>7.0)	**9**	**30.0**	**18.37-44.94**
Total	**30**	**100.0**	**-**

Table 4: Evaluation of study parameters during myocardial infarction

Variables	Day 0	Day 1	Day 2	Significance
Total antioxidants gap (mmol/L)	0.29±0.15 (0.04-0.66)	0.33±0.12 (0.09-0.56)	0.36±0.14 (0.10-0.68)	F=3.745; P=0.045
Serum uric acid levels (mmol/L)	0.35±0.08 (0.22-0.51)	0.31±0.07 (0.21-0.49)	0.31±0.10 (0.20-0.55)	F=5.478; P=0.019
Total antioxidants levels (mmol/L)	1.29±0.11 (1.08-1.60)	1.23±0.08 (1.03-1.39)	1.19±0.09 (1.04-1.32)	F=11.191; P<0.001
Serum albumin levels (mmol/L)	0.65±0.10 (0.40-0.81)	0.59±0.07 (0.39-0.72)	0.52±0.08 (0.34-0.70)	F=49.082; P<0.001

Results are presented in Mean± SD (Min-Max)

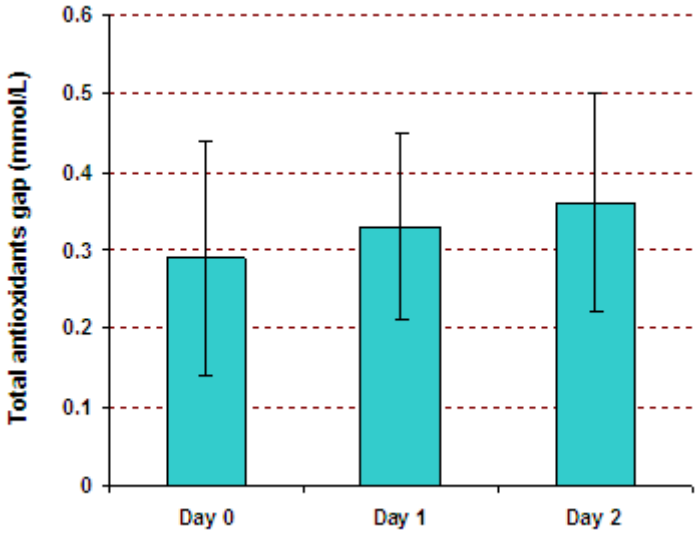


Figure 4a

Table 5: Evaluation of patients based on Total antioxidants gap (mmol/L) in relation to prognosis

Total antioxidants gap (mmol/L)	Prognosis		Significance
	Good	Bad	
Day 0	0.27±0.14	0.35±0.17	t=1.263;p=0.217
Day 1	0.33±0.12	0.32±0.14	t=0.118;p=0.906
Day 2	0.40±0.11	0.26±0.13	t=3.095;p=0.004**
% change at day 1	+22.2%	-8.6%	-
% change at day 2	+48.1%	-25.7%	-
Significance	F=13.227; P<0.001	F=2.160; P=0.148	-

Graph 5a

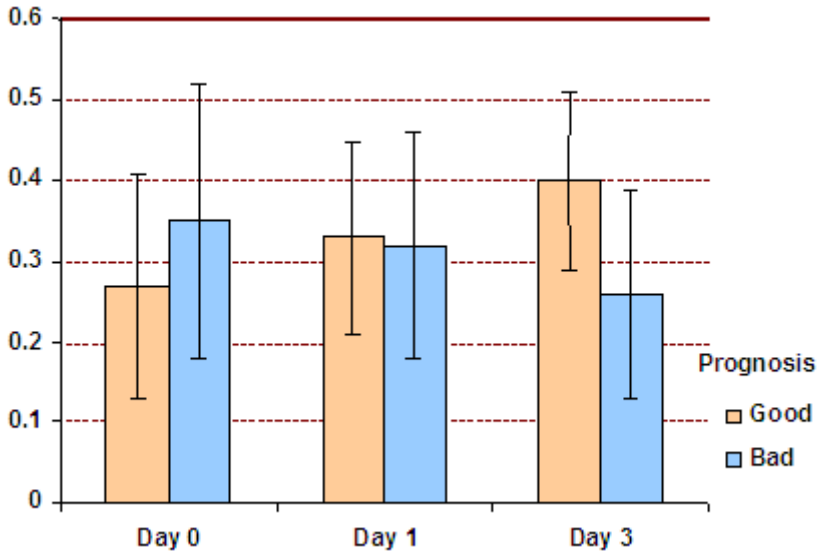
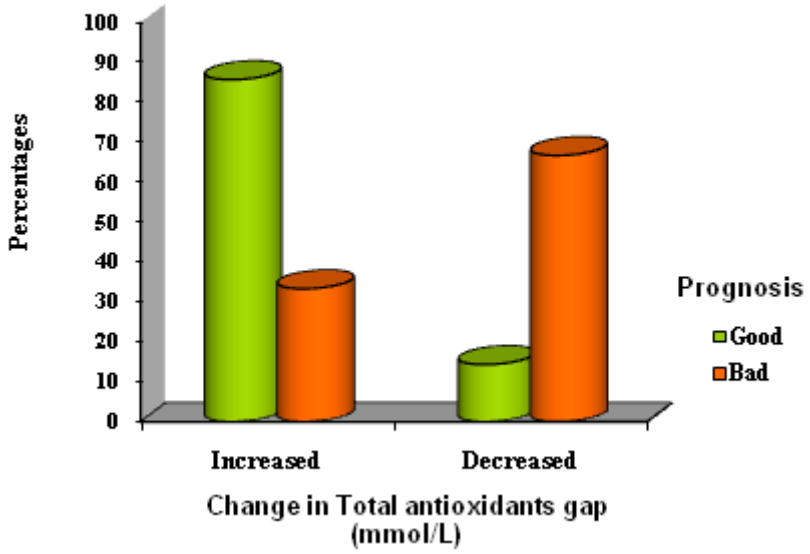


Table 6: Evaluation of patients based on change in Total antioxidants gap (mmol/L) in relation to prognosis

Change in Total antioxidants gap (mmol/L)	Prognosis	
	Good	Bad
Increased	18 (85.7%)	3(33.3%)
Decreased	3(14.3%)	6(66.7%)
Total	21(100.0%)	9(100.0%)
Inference	Increase in Total antioxidant gap is significantly associated with Good prognosis with P=0.008	

Graph 6a



DISCUSSION: The serum antioxidant gap reflects the antioxidant activity of ascorbate, alpha-tocopherol, beta-carotene and other radical scavenging antioxidants. Antioxidant Gap was derived by subtracting the Total Antioxidant Activity ascribable to albumin and uric acid from Total Antioxidant value for each sample ie., TAA-(serum albumin + serum uric acid)[5].

This study suggests that an increase in total antioxidant gap is significantly associated with good prognosis of patients with STEMI. This is in agreement with the study conducted by Miller NJ et al[5]. Their study included 61 consecutive patients suffering from AMI and antioxidant gap was calculated on day 0 (admission), day 1, the following morning and day 2, the next days. It was shown that 25% of the patients whose AOG had fallen had a higher mortality rate and thus bad prognosis after infarction whereas only 1 patient (2.8%) whose AOG had risen had a bad prognosis with a p value of 0.01 .But the study took into consideration both thrombolysed and non thrombolysed patients. It is well proven that reperfusion injury in thrombolysed patients affects the antioxidant levels due to oxidative stress[14]. Hence in the present study thrombolysed patients were excluded and only patients receiving supportive therapy for AMI were included and this study suggested that serum AOG values after AMI may be prognostic. This suggests that change in gap antioxidants are of significance in events occurring during or after AMI.

Oxidative stress in cardiac and vascular injury describes the damage caused to cells resulting from increased formation of reactive oxygen species and/or decreased antioxidant reserve[7] . Despite remarkable improvements in strategies for treatment of MI occurrence of antioxidant deficit has been reported as one of the mechanisms for developing complications and poor prognosis after MI.[15]

One of the most important mechanisms for aggravation of myocardial damage by free radical is inappropriate neutrophil activation that may participate in the exacerbation of myocardial tissue injury and for which vitamin C and alpha tocopherol may be helpful[4, 5, 6]. Antioxidants inhibit or delay the oxidative damage to subcellular proteins and nucleic acids. There is evidence that

antioxidants can protect against free radicals and may thereby reduce myocardial damage, thrombosis and arrhythmias during AMI[1]. Serum AOG values may serve as an estimate of the reserve antioxidants in a patient and this study suggests that this reserve of gap antioxidants may serve as a good defence against the oxidative stress following an event like AMI, since in this study increase in antioxidant gap was significantly associated with good prognosis.

REFERENCES:

1. Secil K, Aymelek G, Delya ES, Ismet H. "Serum cardiac markers in patients with acute myocardial infarction: oxidative stress, CRP, N Terminal pro Brain natriuretic peptide." J Clin Biochem Nutr 2007; 41(1): 50-57.
2. Levenen AL, Vanakangas E, Keponen JK, Yla Herltuala S. "Antioxidant gene therapy for cardiovascular disease: current status and future perspectives". Circulation 2008; 117(16): 2142-50.
3. Dhalla NS, Temsah RM, Netticadan T. "Role of oxidative stress in cardiovascular diseases". Hypertension 2000; 18(6) : 655-673
4. Myers ML, Roberto Bolli, Raymond FL, Hartely CJ, Robert R. "Enhancement of recovery of myocardial function by oxygen free radical scavengers after reversible regional ischemia." Circulation 1985; 72(4): 915-21
5. Miller NJ, Johnston JD, Collis CS, Rice-Evans CA. "Serum total antioxidant activity after myocardial infarction ." Ann Clin Biochem 1997; 34: 85-90.
6. Mc Murray J, Chopra M, Bridges A, Belch JJ. "Evidence of enhanced free radical activity in chronic congestive heart failure secondary to coronary artery disease." Am J Cardiol 1990; 65: 1261-2.
7. Koracevic D, Koracevic G, Djordjevic V, Andrejevic S, Coric V. Method for the measurement of antioxidant activity in human fluids. J Clin Pathol 2001; 54: 356-361
8. Janet R McMurray- Plasma proteins in Gowenlock AH, McMurray JK, Mclauchlan DM, eds. Varleys practical clinical biochemistry 6th ed. Heinmann medical books; 1988;402-405.
9. Mclauchla DM. Creatinine, urate, urea. In Gowemlock AH, McMurray JK, Mclauchlan DM, eds. Varleys practical clinical biochemistry 6th ed. Heinmann medical books; 1988:408-411
10. Morrow DA, Elliott M, Antmann MD, Charlesworth A, Cairns R, Sabina A et al. Thrombolysis in myocardial infarction 2000; 102:2031-2037.
11. Bernard Rosner Fundamentals of Biostatistics, 5th ed, Duxbury : 80-240
12. Venkataswamy Reddy. Statistics for Mental Health Care Research, NIMHANS publication 2002: 108-144.
13. Sunder Rao P S S, Richard J: An Introduction to Biostatistics, A manual for students in health sciences, New Delhi: Prentice hall of India. 86-160
14. Hamilton KL.Antioxidants and cardioprotection.Med Sci Sports Exer 2007; 39:1544-53.
15. Gupta M, Singhal KP. Higher antioxidant capacity during chronic stable heart hypertrophy. Circ Res 1989; 64: 398-406.

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