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Increased Levels of Erythrocyte Glutathione in Acute Myocardial Infarction: an Antioxidant Defence

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Abstract

Objective:
Glutathione (GSH) has a central role in the defence against oxidative damage. This study was carried out to investigate any change in erythrocyte GSH levels in a population of patients with acute myocardial infarction (AMI) and compare them with levels in normal healthy subjects.

Method:
GSH levels were determined in erythrocytes of one hundred and seventy six patients with AMI (age: 30-70 years; 131 males and 45 females) admitted to the National Institute of Cardiovascular Diseases, Karachi. These levels were compared with eryrocyte GSH levels obtained from 95 normal healthy subjects (controls).

Results:
Mean ± SD erythrocyte GSH levels in AMI patients and controls were found to be 2.34 ± 0.62 µmol/ml of packed cells and 2.08± 0.62 µmol/ml of packed cells, respectively. The two values when compared with one way ANOVA were found to be significantly different (p=0.001). Age had little effect on erythrocyte GSH levels in both AMI patients and normal healthy subjects.

Conclusion:
Increased production of reactive oxygen species is a feature of cardiovascular disease, such as AMI and cells can respond to mild oxidative stress by upregulating antioxidant defence in terms of increased production of GSH (JPMA 54:254;2004).

Introduction

Glutathione is an important endogenous antioxidant in the human body. It protects cells from toxic effects of reactive oxygen compounds.1 A number of studies have demonstrated that blood glutathione (GSH) could be a predictor of mortality and morbidity, because low blood glutathione values have been associated with a number of clinical disorders including diabetes2,3, chronic renal failure4 malignancies5, Parkinson's disease6, cataract formation7,8 and HIV infection.9,10 However, the data on the correlation between the blood levels of GSH and the ischemic process is quite controversial. Usal et al11 have reported 3.8% depressed levels of total erythrocyte GSH in patients with acute myocardial infarction (AMI) compared to normal healthy subjects whereas Mills et al12 have shown no change in GSH levels in blood of patients with cardiovascular

disease. The present study was undertaken to investigate any change in the erythrocyte GSH levels in a Pakistani population of AMI patients as compared to normal healthy subjects.

Patients & Methods

One hundred and seventy six consecutive Pakistani patients with AMI (age 30-70 years) admitted to the National Institute of Cardiovascular Diseases (NICVD), Karachi from January to June 2001, were selected for this study. They had confirmed diagnosis of AMI on the basis of clinical history, ECG and biochemical data and were not taking any vitamin supplementations during the past 6 months. Those cases who were found to be pregnant, using antiepileptics, oral contraceptives, having malabsorption syndrome, suffering from tuberculosis, liver disease, uremia, or cancer were not included in the study.

Similarly, 95 normal healthy subjects belonging to the same socio-economic class, were selected from the personnel of the Aga Khan University and Civil Hospital, Karachi for this study. Informed consent was obtained from the participants and the study was approved by the Ethical Committee of the Aga Khan University.

A stringent criteria was used for the selection of normal healthy control subjects. In addition to being matched for sex, socio-economic background, and to some extent for age they had no evidence of coronary artery disease (CAD), diabetes mellitus, hypertension, obesity and hypercholesterolemia. Those control subjects who were found to be pregnant; using antiepileptics, oral contraceptives; having malabsorption syndrome; suffering from tuberculosis, uremia or liver disease, were excluded from this study.

Determination of GSH level

Five millilitres venous blood collected from patients as well as from normal healthy subjects was transferred immediately to EDTA containing tubes. Samples were centrifuged at 1500g for 5 minutes. Plasma was carefully removed and an aliquot (10μl) of packed erythrocytes was removed and analyzed for total GSH by a modification of the method by Tietze.[13]

Briefly, 10μl of packed erythrocytes were added to 0.99 ml of cold 0.01M phosphate buffer, pH 7.5, containing 0.005M EDTA. For analysis, 25μl of the resulting hemolysate was added to a reaction mixture containing 975μl of 0.01M phosphate/0.005M EDTA buffer, pH7.5, 50μl of 5, 5´-dithiobis-(2-nitrobenzoic acid) (DNTB) and 40μl of glutathione reductase (6 units/ml). After mixing at room temperature, 50μl of NADPH (4mg/ml) was added to

Table 1. Demographic and clinical characteristics of subjects

Varible	Controls (n=95)		Patients (n=176)		
	Value (mean + SD)	Frequency (%)	Value (mean + SD)	Frequency (%)	P-value
Age (years)	45.93 + 8.6		50.96 + 8.3		0.0001
Gender					
Male		76 (80%)		131 (74.4%)	0.3
Female		19 (20%)		45 (25.6)	
BMI (kg/m2)	24.11 + 4.62		24.51 + 3.6		0.431
Glucose (mg/dl)	102.94 + 21		140 + 72		0.0001

	Controls	Patients	P Value
Cholesterol (mg/dl)	173 + 28	186 + 47	0.022
Smoking status			
Smoker	13 (13.7%)	77 (43.8%)	0.0001
Non-smoker	82 (86.3%)	99 (56.3%)	
Diet			
Vegetarian	2 (2.%)	1 (0.6%)	0.59
Non-vegetarian	93 (97.9%)	175 (99.4%)	
Parental history of IHD			
Yes	21 (22.1%)	42 (23.9%)	0.74
No	74 (77.9%)	134 (76.1%)	
Hypertensive			
Yes	0	117 (66.5%)	
No	95 (100%)	59 (33.5%)	
Diebetic			
Yes	0	71 (40.3%)	
No	95 (100%) 105 (33.5%)		

*P value compares the mean or percentage values in controls with values in patients.

the reaction and the rate of change in OD at 412 nm was monitored using a DU-70 spectrophotometer (Beckman Instruments Inc., Palo Alto., CA). A standard curve ranging 0.5nmol-2nmol GSH in 10mM perchloric acid in the above mentioned phosphate buffer was run along with the unknown samples. The standard curve was plotted using concentration of GSH and the rate of change in OD as ordinates. The value of GSH in the unknown sample was determined using this standard curve. Concentration of GSH was expressed as µmol/100ml of packed red cells.

Statistical analysis

All mean values are expressed as mean ± SD. Percentages were compared by using chi-square. Mean values of various groups were compared using one way analysis of variance (ANOVA). The analyses were performed with the statistical package (Epi Info 6, version

Table 2. Glutathione levels in erythrocytes of normal healthy subjects and AMI patients

Variable	Controls		Patients		P Value(a)
	n	GSH (umol/ml of packed cells)	n	GSH (umol/ml of packed cells)	
Male	76	2.09 + 0.65	131	2.32 + 0.62	0.01
Female	19	2.0 + 0.45	45	2.37 + 0.59	0.02
Smokers	13	2.0 + 0.58	77	2.31 + 0.63	0.01
Non-smokers	82	2.09 + 0.62	99	2.35 + 0.61	0.0005
Diabetics	0		71	2.30 + 0.6	
Non-diabetics	95	2.08 + 0.62	105	2.36 + 0.6	0.001
Hypertensive	0		117	2.23 + 0.61(b)	

Non-hypertensive	95	2.08 + 0.62	59	2.52 + 0.56b	0.0001
Age > 50 years	64	2.07 + 0.59(c)	84	2.27 + 0.6(c)	0.045
< 50 years	31	2.1 + 0.69(c)	92	2.4 + 0.63(c)	0.022

(a)	P-value compares the mean GSH levels in controls with patients using one way ANOVA.
(b)	A comparison of the mean GSH values in hypertensive patients and normotensive patients revealed a significant difference (P=0.0001)
(C)	A comparison of mean GSH values below and above 50 years within each group (patients or controls) showed no significant difference.

6.04 d, 2001, WHO). A p-value less than 0.05 was considered significant.

Results

Demographic and clinical characteristics of the patients have been listed in Table 1. Among the patients there were 131 males and 45 females, while among the normal healthy subjects 76 were males and 19 females. Mean age of AMI patients was 50.96±8.3 years, while mean age in normal controls was 45.93±8.6 years. The two values were statistically different indicating that AMI patients and normal healthy subjects were not exactly matched for age in this study. Mean body mass index (BMI) values for both patients and normal healthy subjects were nearly same (24.5±3.6 vs 24.1±4.6, respectively). Mean levels of fasting serum glucose in patients and controls were 140±72 mg/dl and 102±21 mg/dl, respectively. The two values when compared by one way ANOVA were found to be significantly different. Proportion of smokers among patients was higher than controls (p=0.0001). Serum cholesterol levels in the two groups were significantly different, while parental history of ischemic heart disease (IHD) was nearly the same in normal healthy subjects and AMI patients.

Figure shows the concentration of erythrocyte GSH in AMI patients and normal healthy subjects (controls). The levels in AMI patients and controls were found to be 2.34±0.62µmol/ml of packed red cells and 2.08±0.62µmol/ml of packed red cells, respectively. The two values when compared with one way ANOVA were found to be statistically significant (p=0.001).

Table 2 shows glutathione levels in erythrocytes of AMI patients and normal healthy subjects relative to their age, gender, smoking status, blood pressure, being diabetic or nondiabetic. With the exception of the smokers category, erythrocyte GSH values in all other listed categories (such as age, gender, diabetes and blood pressure) were significantly higher in the patients group compared to normal healthy subjects.

A comparison of the mean GSH values above and below 50 years within the patients group or within the control group revealed no statistically significant difference indicating that aging has little effect on total erythrocyte GSH levels. These results are in conformity with those reported by Samiec et al14 and Richie.15

Discussion

GSH has a central role in the defence against oxidative damage. The balance between production of free radicals and antioxidant deficiencies in the body (such as GSH) has important health implications. If there are many free radicals produced and too few antioxidants, a condition of "oxidative stress" develops which may cause damage to the tissue.

Previous studies have shown that erythrocyte GSH is decreased in diabetes2, liver disease16, Parkinson's disease6,17, myocardial infarction11 and old age.18 However, Samiec et al14 showed that in age-related pathologies oxidation of GSH was more important than a decline in pool size of GSH, while in specific pathologies such as, diabetes both oxidation and a decline in pool size might be important. Decrease in total GSH pool could occur, in part, because of

decreased synthesis of GSH due to decreased activity of gamma-glutamylcysteine synthase as has been previously reported in erythrocytes of diabetics.3 However, in certain conditions of oxidative stress, there is an increase in total GSH to boost the antioxidant defence mechanism of the body.19 Moreover, GSH has also been reported to increase the vasodilation of coronary artery when added to isolated and perfused rodent heart.20 On these observation, it is plausible that the observed increase in total GSH in our patients is in response to the oxidative stress due to AMI.

Smith et al.21 have also reported an increase in antioxidant defences in terms of increased levels of GSH in bronchiolar lavage fluid from patients with asthma. In animal model too, concentration of GSH has been found to increase in response to cigarette smoke and alcohol.22

Our results pertaining to increased levels of total GSH in AMI patients are different from those reported by Usal et al15, who have reported decreased levels of GSH in erythrocytes. This could be due to the fact that their data was on relatively small number of patients (n=21). Moreover, their maximum observed mean decrease in GSH was 11.5% on day 2 after MI and 3.8% on the following days. Our sample size was large (n=176) and we monitored GSH in patients within 24 hours after infarction. There is a possibility that with increased time interval after infarction, the levels of GSH may decline due to severe oxidative stress which can damage DNA, proteins and lipids leading to cell death by apoptotic or necrotic mechanisms.23

In conclusion, it can be said that increased production of reactive oxygen species is a feature of cardiovascular disease and cells can respond to mild oxidative stress by up-regulating antioxidant defence. The data in this study is suggestive that induction of GSH could be one of these mechanisms in AMI.

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