Integrative Biosciences 11: 161-164, 2007

Integrative
Biosciences
http://www.ibiosci.or.kr

Homocysteine and Total Antioxidant Status in Acute Myocardial Infarction Patients Among Tamil Population

T. Angeline[1,*], Rita Mary Aruna[2], K. Rama Devi[3] and Nirmala Jeyaraj[1]

[1]*PG& Research Department of Zoology & Biotechnology, Lady Doak College, Madurai, South India;* [2]*Department of Biochemistry, Penang International Dental College, Vinayaga Mission, Salem, South India;* [3]*Institute of Biochemistry, Madras Medical College,Chennai, South India*

Abstract: Oxidative stress is prerequisite for the development of atherosclerosis. Apart from the traditional risk factors that contribute to this devastating condition, in the past few decades, much attention has been focused on plasma total homocysteine mainly because of its strong association with coronary artery disease. It has been suggested that homocysteine induces oxidative stress and hence the present work was undertaken to assess the total homocysteine status and plasma total antioxidant capacity in the acute myocardial infarction (AMI) patients among Tamil population. The study subjects included only the Tamil population. Blood samples were collected from 100 AMI patients and 100 controls. Plasma was separated and the total antioxidant status was assessed as a measure of ferric reducing power of antioxidants using spectrophotometric method. Plasma total homocysteine concentrations were assessed by automated chemiluminescence method. While Total antioxidant status was significantly decreased, the plasma homocysteine concentrations were elevated in AMI patients compared to the controls. However, there was no correlation between the homocysteine levels and total antioxidant status. The findings of this study may have therapeutic implications, including food sources rich in antioxidants for all AMI patients to minimize the effect of free radicals formed during oxidative stress among Tamil population.

Key words: Oxidative stress, homocystein, antioxdant

Oxidative stress is the major cause of several diseases including cardiovascular disease, cancer, diabetes and neurodegenerative pathologies (Ozben, 2003). It refers to an unfavourable imbalance between potentially harmful oxidants and protective antioxidants. The harmful oxidants include reactive oxygen species (ROS) such as superoxide (O_2^-) hydrogen peroxide (H_2O_2) and potent hydroxyl free radical (OH^-) (Waring, 2001). Oxidative stress is prerequisite for the development of atherosclerosis. Apart from the traditional risk factors that contribute to this devastating condition, in the past few decades, much attention has been focused on plasma total homocysteine mainly because of its strong association with coronary artery disease. McCully (1969) first proposed the homocysteine theory of atherosclerosis that moderately elevated total homocysteine may be a cardiovascular risk factor in the general population.

Homocysteine is a strong oxidant and its oxidation products (H_2O_2, O_2^-, ROS) are toxic and may directly alter vascular cell function. Homocysteine may cause vascular damage due to the chemical characteristics of its thiol group (R-SH). Sulfur has lower electronegativity than oxygen. Therefore, the energy needed to release sulfur from hydrogen of the thiol group is relatively low, which makes this group more acidic ($R\text{-}S^-$) and highly reactive (Aleman *et al.*, 2001).

Homocysteine undergoes auto-oxidation, forming H_2O_2 in the presence of copper or ceruloplasmin. H_2O_2 generated from homocysteine can lyse cultured endothelial cells, and this process can be prevented by the antioxidant enzyme catalase (Starkebaum and Harlan, 1993).

In addition to increased generation of peroxide and superoxide radicals, homocysteine has recently been shown to inhibit intracellular antioxidant enzymes, including glutathione peroxidase, thus decreasing the cell's ability to neutralize oxidant radicals (Upchurch *et al.*, 1997, Outinen *et al.*, 1998). Homocysteine also decreases the intracellular glutathione, which is an endogenous anti-oxidant (Hultberg *et al.*, 1997).

Previous studies conducted among Tamilians indicated that hyperhomocysteinemia is more prevalent in AMI patients (Angeline *et al.*, 2005; 2007). As homocysteine induces oxidative stress, the present work was further undertaken to

*To whom correspondence should be addressed.
E-mail: angelanand@yahoo.co.in

assess the plasma total antioxidant capacity in the study subjects.

MATERIALS AND METHODS

Venous blood samples were drawn within 24 h after the onset of AMI from 100 hospitalized patients with the diagnosis of AMI episode. One hundred age-matched healthy male control subjects without any cardiovascular risk factors and normal coronary arteries, as examined by ECG, were also included. A detailed clinical history was sought from each patient. Information on their smoking habits, alcohol intake, consumption of drugs and vitamins and major risk factors of coronary heart disease, i.e. hypertension, diabetes, hypercholesterolemia, obesity and family history of coronary artery disease was gathered. Only individuals without any of the major conventional risk factors were included for the study. The inclusion criteria for all study participants required that they should have no cardiovascular risk factors. The objective of the study was described and informed consent was obtained. Ethical clearance was obtained from the hospital ethical committee.

Venous blood was collected from study participants after overnight fasting (≥10 h). Plasma homocysteine concentrations were assessed by automated chemiluminescence immunoassay manufactured by Bayer on an ACS Centaur, as previously mentioned (Angeline *et al.*, 2007). Blood plasma was separated and the total antioxidant status was assessed as a measure of ferric reducing power of antioxidants using spectrophotometric method (Benzie and Strain, 1999).

Mean values and standard deviations were calculated for every variable in each group and were compared between AMI patients and controls. Student's t test was used to test the significance. Correlations were assessed by Pearson correlation coefficient.

RESULTS

The mean level of plasma total antioxidant capacity (FRAP) was found to be decreased in AMI patients when compared to the age-matched healthy controls (314.48 ± 122.59 μM/L vs. 932.25 ± 231.07 μM/L). The student t-test analysis of plasma total antioxidant capacity in AMI patients and healthy controls showed high significance ($p < 0.001$; Table 1). There was a high frequency of subjects with pathologically decreased plasma total antioxidant capacity among patients (72/100), whereas this condition was completely absent in the control subjects. The plasma antioxidant capacity was also not correlated to the concentration of tHcy in both AMI patients and controls.

The mean plasma homocysteine concentrations in AMI patients and controls were 32.35 ± 10.3 μM/L and 13.62 ± 3.56 μM/L, respectively (Table 1, Fig. 1). Hyperhomocysteinemia occurred in 94% of AMI cases compared to 40% of the control group. The 't'-test showed that homocysteine concentrations were significantly higher in the patient group than in control subjects ($p < 0.01$).

Table 1. Level of Total Homocysteine and Total Antioxidants Among The Study Subjects

Parameters	AMI patients	Controls
	Mean ± SD	Mean ± SD
Homocysteine (μmol/L)	32.35 ± 10.3	13.62 ± 3.56
FRAP (μmol/L)	314.48 ± 122.59	947.14 ± 241.42

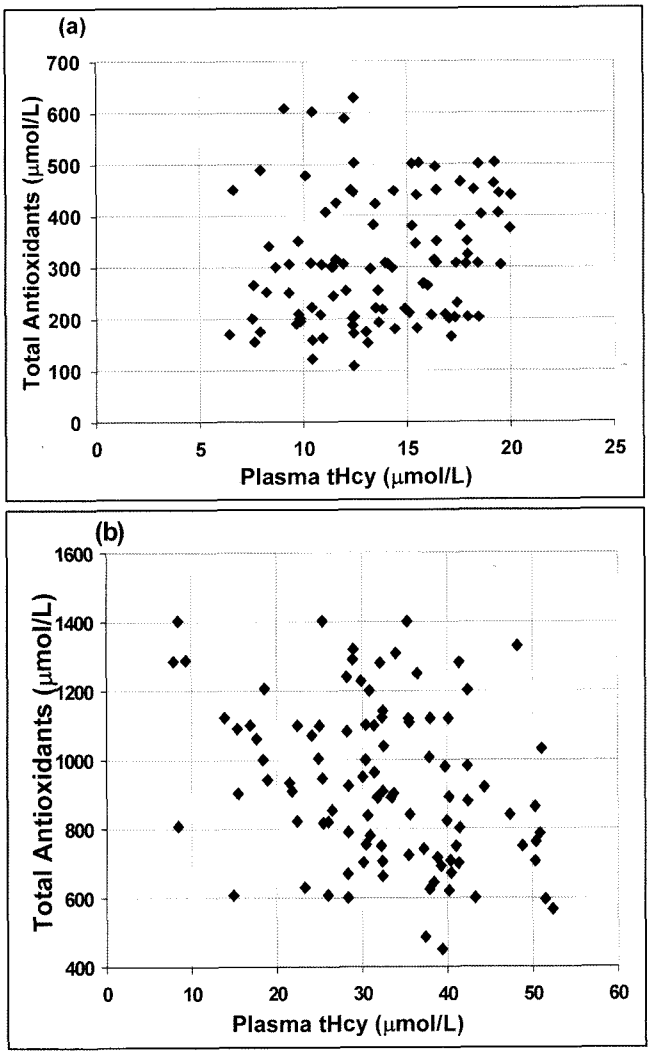

Fig. 1. Scatter diagrams showing the absence of correlation between tHcy and Tas (frap value) in AMI patients (a) and controls (b).

When plasma total antioxidant capacity was considered with respect to tHcy concentrations, the plasma antioxidant capacity in AMI patients with normal tHcy status was 305.35 ± 164.41 μmol/L among AMI patients and 947.14 ± 241.42 μmol/L among healthy individuals. In individuals with hyperhomocysteinemia, the plasma total antioxidant

capacity was 315.06 ± 120.58 μmol/L in AMI patients and 909.91 ± 215.66 μmol/L among controls. There was also no significant correlation between homocysteine and total antioxidant status between AMI patients and controls ($r = 0.0315$ and -0.0856 respectively; $p > 0.05$) (Fig. 1).

DISCUSSION

Oxidative stress was evaluated by measuring the total antioxidant status. The present study suggests that patients with AMI are under more oxidative stress than the healthy individuals. The plasma total antioxidant capacity was found to be decreased in AMI patients, when compared to the age-matched healthy controls. Another study conducted on North Indian AMI patients reported a significant decrease in plasma total antioxidant capacity (1562 ± 529 μmol/L) compared to controls (2082 ± 448 μmol/L). When compared to the total antioxidant status among North Indian men, South Indian patients in the present study have a decreased baseline plasma total antioxidant capacity. However, the total number of cases (N = 17) included in their study is a limiting factor (Srivastava *et al.*, 2003).

Puri *et al* (2003) obtained similar results in North Indian population. The tHcy concentration in the control group of their study matches that of the present study. The tHcy in CAD patients (27 ± 13.11 μmol/L) was also comparable to that obtained from AMI patients in this study (32.35 ± 10.39 μmol/L). Another recent study conducted in North India by Abraham *et al.*, (2006), reported a significant increase in the tHcy level among coronary artery disease patients (22.81 ± 13.9 μmol/L) compared to controls (7.77 ± 7.3 μmol/L).

Some studies suggested that homocysteine is also involved in generating oxygen free radicals thereby causing oxidative stress. Hence, it will be more appropriate to identify those individuals with reduced antioxidant protection, because they must be more susceptible to free radical mediated diseases including coronary artery disease. Increased cardiovascular risk is often associated with reduction in circulating antioxidants although this may result from consumption in the presence of increased oxidant activity (Waring, 2001). Thus homocysteine-induced oxidative stress may also be responsible for the decrease in plasma total antioxidant capacity in AMI patients.

Srivastava (2003) suggested that patients with coronary heart disease are under more oxidative stress than healthy individuals. In cardiovascular disease, oxidants can contribute to the causative pathology as well as perpetuate further damage. An efficient antioxidant status was suggested to prevent the oxidation of low density lipoprotein. This oxidation has been hypothesized to be an initiating step in the development of heart and vessel disease (Srivastava, 2003).

The reason for the absence of correlation between homocysteine and total antioxidant status between AMI patients and controls may be because the extent of oxidative stress might depend on individual's health conditions and other unmeasured nutritional factors including vitamin status, which may be responsible for hyperhomocysteinemia.

Homocysteine may decrease the bioavailability of NO by forming S-nitroso-Hcy. The initial response to elevated Hcy appears to be stimulation of intracellular production of glutathione, nitric oxide or S-nitroso-Hcy. However, prolonged elevation of Hcy leads to saturation of the scavenging potential of glutathione and nitric oxide. This produces toxic effect of Hcy by increased formation of ROS (Nygard, 1999).

Hcy alters endothelial and smooth muscle cell function by generating reactive oxygen species. The resulting increase in oxidative stress diminishes the antioxidative capacity, which increases the risk for atherosclerotic vessel diseases (Herrmann, 2001).

Vanden Berg *et al.*, (1995) demonstrated an impaired endothelial anticoagulant function in young patients with hyperhomocysteinemia and peripheral vascular disease. Nappo *et al.*, (1999) evaluated the effect of acute hyperhomocysteinemia with and without antioxidant vitamin pretreatment on cardiovascular risk factors and endothelial functions. Their results suggested that mild to moderate elevations of plasma homocysteine levels in healthy subjects activate coagulation, and modify the adhesive properties of endothelium. Pretreatment with the antioxidants vitamin E and ascorbic acid blocks the effects of hyperhomocysteinemia, suggesting an oxidative mechanism. Rauma and Mykkanen (2000) recommended evaluation of the total antioxidant capacity rather than the status of a single antioxidant, because their study reported that measurements of antioxidant status in vegetarians showed that a vegetarian diet maintains a high antioxidant vitamin status (vitamins C and E, β-carotene) but a variable antioxidant trace element status compared with omnivorous diet (19).

The reduced antioxidant status may occur not only because of oxidative stress due to diseased conditions or hyperhomocysteinemia, but also due to decreased intake or absorption of dietary antioxidants and due to low intake. In case of hyperhomocysteinemia, vitamin B supplementation may protect from further oxidative stress (Angeline *et al.*, 2007). Similarly, supplementing antioxidants such as vitamins E and C, Beta carotene, vitamin A, and selenium may minimize further complications of AMI and enhance health promoting effects. The findings of this study may have therapeutic implications, including food sources rich in antioxidants for all AMI patients.

ACKNOWLEDGMENTS

This project was funded by Council of Scientific and Industrial Research (CSIR),India in the form of Senior Research Fellowship (CSIR-SRF) to T.Angeline.The authors are thankful to Mr. R. Parthasarathy, Statistician, Madurai Medical College, Tamil Nadu.

REFERENCES

Abraham R, Joseph JM, Calton R and Dhanoa J (2006) Raised serum homocysteine levels in patients of coronary artery disease and the effect of vitamin B12 and folate on its concentration. *IJCB* 21: 95-100.

Aleman G, Tovar AR and Torres N (2001) Homocysteine metabolism and risk of cardiovascular diseases: Importance of the nutritional status on folic acid, vitamins B_6 and B_{12}. *Rev Invest Clin* 53: 141-151.

Angeline T, Aruna RM, Ramadevi K, Mohan G and Jeyaraj N (2005) Homocysteine status and acute myocardial infarction among Tamilians. *Ind Jour Clin Biochem* 20: 18-20.

Angeline T, Nirmala Jeyaraj and Gregory J Tsongalis (2007) MTHFR Gene polymorphisms, B-vitamins and hyperhomocystinemia in young and middle-aged acute myocardial infarction patients. *Experimental and Molecular Pathology* 82: 227-233.

Benzie IFF, and Strain JJ (1999) Ferric reducing/antioxidant power assay: direct measure of total antioxidant activity of biological fluids and modified version for simultaneous measurement of total antioxidant power and ascorbic acid concentration. In: Packer L, ed. Methods in Enzymology: Oxidants and Antioxidants Part A. 1st ed. Vol. 299. London Academic Press Limited. 15-27.

Herrmann W, Schorr H, Geisel J and Riegel W (2001) Homocysteine, cystathionine, methylmalonic acid and B-vitamins in patients with renal disease. *Clin Chem Lab Med* 39: 739-746.

Hultberg B, Andersson A and Isaksson A (1997) The cell-damaging effects of low amounts of homocysteine and copper ions in human cell line cultures are caused by oxidative stress. *Toxicol* 123: 33-40.

McCully KS (1969) Vascular pathology of homocysteinemia: Implications for the pathogenesis of arteriosclerosis. *Am J Pathol* 56: 111-128.

Nappo F, DeRosa N, Marfella R, DeLucia D, Ingrosso D, Perna A F, Farzati B and Giugliano D (1999) Impairment of endothelial functions by acute hyperhomocysteinemia and reversal by antioxidant vitamins. *JAMA* 281: 2113-2118.

Nygard O, Vollset SE, Refsum H, Brattstrom L and Ueland PM (1999) Total homocysteine and cardio vascular disease. *INT J Med* 246: 425-454.

Outinen PA, Sood SK, Liaw PC, Sarge KD, Maeda N, Hirsh J, Ribau J, Podor TJ, Weitz JI and Austin RC (1998) Characterization of the stress-inducing effects of homocysteine. *J Biol Chem* 332: 213-221.

Ozben T (2003) Effect of hemodialysis on oxidative stress and antioxidants in Type II Diabetic patients with uremia. Trends in Clinical Biochemistry and Laboratory Medicine. Publication of Association of Clinical Biochemists of India. pp 605-611.

Puri A, Gupta OK, Dwivedi RN, Bharadwaj RPS, Narain VS and Singh S (2003) Homocysteine and lipid levels in young patients with Coronary Artery Disease. *JAPI* 51: 681-686.

Rauma AL and Mykkanen H (2000) Antioxidant status in vegetarians versus omnivores. *Nutr* 16: 111-119.

Srivastava LM, Kankra M, Manocha A and Bhargava S (2003) Evaluation of antioxidant status in the critically ill with special reference to patients of cardiovascular disorders. Trends in Clinical Biochemistry and Laboratory Medicine. Publication of Association of Clinical Biochemists of India. pp 600-604.

Starkebaum G and Harlan JM (1993) Endothelial cell injury due to copper-catalyzed hydrogen peroxide generation from homocysteine. *J Clin Invest* 77: 1370-1376.

Upchurch GR, Welch GN, Fabian AJ, Freedman JE, Johnson JL, Keaney JF and Loscalzo J (1997) Homocyst(e)ine decreases bioavailable nitric oxide by a mechanism involving glutathione peroxidase. *J Biol Chem* 272: 17012-17017.

Van den Berg M, Boers GH, Franken DG, Blom HJ, Van Kamp GJ, Jakobs C, Rauwerda JA, Kluft C and Stehouwert CD (1995) Hyperhomocysteinaemia and endothelial dysfunction in young patients with peripheral arterial occlusive disease. *Eur J Clin Invest* 25: 176-181.

Waring WS (2001) Antioxidants in prevention and treatment of cardiovascular disease. *Proc R Coll Physicians Edinb* 31: 288-292.

[received July 29, 2007; accepted September 3, 2007]