DOI: https://doi.org/10.53350/pjmhs22163224

ORIGINAL ARTICLE

Role of up-regulation of oxidative stress biomarkers in thrombotic transformation of atherosclerotic plaque in Acute Coronary Syndrome (ACS) patients from a lower middle income country

AAMENAH MALIK[1], HUMA SATTAR[2], ARIF MALIK[3], RUKHSHAN KHURSHID[4], AWAIS ALTAF[2], MAIRA MAHMOOD[5], MARIAM MALIK[6], FARHAT IJAZ[7]

[1]*Department of Biochemistry, CMH Lahore Medical College and Institute of Dentistry, Lahore, Pakistan*
[2]*Institute of Molecular Biology and Biotechnology (IMBB), The University of Lahore, Pakistan*
[3]*Institute of Applied Sciences, Minhaj University, Lahore, Pakistan*
[4]*Department of Biochemistry, Shalamar Medical and Dental College, Lahore, Pakistan*
[5]*Department of Biochemistry, FMH College of Medicine and Dentistry, Lahore, Pakistan*
[6]*Department of Radiology, Tehsil Headquarter Hospital, Pasrur, Pakistan*
[7]*Department of Physiology , CMH Lahore Medical College and Institute of Dentistry, Lahore Pakistan*
Corresponding author: Dr Aamenah Malik, Email:aamenah@gmail.com, Cell: 0300-8443469

ABSTRACT

Background: In response to oxidative stress, reactive oxygen species aggravate and activate different signaling mechanism and stimulate the release of different inflammatory mediators, and provoke the generation of oxidative stress, all of which can promote the development of acute coronary syndrome.

Methodology: The study sample comprised of 76 ACS patients and 76 healthy controls. Blood samples of ACS patients and controls were collected to determine the serum concentration of antioxidants, and the serum levels of inflammatory biomarkers. Spectrophotometric method and ELISA kit method was used to measure these variables accordingly.

Results: The results indicated a significant rise in the levels of IL-1, IL-6, IL-8, TNF-α, MMP-11, ICAM-1 and VCAM-1 in ACS patients compared to controls whereas significant decrease was recorded in GSH, CAT, SOD, GRx and HDL levels in patients with ACS compared to controls.

Conclusion: The present study indicated that the increased serum concentration of inflammatory cytokines and antioxidant reduction may influence the pathogenesis of ACS.

Keywords: Acute coronary syndrome, oxidative stress , antioxidants

INTRODUCTION

Acute coronary syndrome (ACS) mainly arise from abrupt changes in lipid plaque structure secondary to rupture of plaque or plaque erosion and subsequently release thrombogenic substrates into the blood stream which in turn triggers platelets and coagulation system activation leading to acute occlusive thrombosis of a coronary artery[1,2]. In case of truncated thrombus volume and lesser plaque burden, this abrupt change in atherosclerotic plaque architecture may take place silently[3]. Additionally lipid plaque rupture is characterized by the existence of a large necrotic core with distraction of an overlying thin fibrous cap[4]. It is believed that inflammation play a pivotal preliminary and disseminating role in initiation, development and thrombotic transformation of atherosclerotic plaque[5]. Likewise inflammation is evidently pervasive in coronary circulation of patients with unstable angina[6]. Previous studies demonstrate that putative mechanism leading to vulnerability and thrombogenicity of atherosclerotic plaque implicate an interactive cascade of inflammatory cells, cytokines, chemokines and adhesion molecules. On the basis of 12-lead electrocardiogram ACS divided into two major categories involving ST-elevation myocardial infarction (STEMI) which present ST-elevation on the ECG and non ST-elevation acute coronary syndrome (NSTEACS) having T wave changes, ST-segment depression or no ECG abnormalities[7,8]. The later one embraces both unstable angina (UA) and ST- elevation myocardial infarction as well as encompasses an increasing number of ACS patients and is evolving a foremost health problem in Asia, Western countries and other developing countries[9]. To identify subgroups of ACS patients who are at increased risk for consequent cardiovascular event various potential biomarkers are being used, among them inflammatory biomarkers has focused mainly because multiple inflammatory mechanisms comprising migration of leucocyte, endothelial dysfunction, platelet activation and extracellular matrix degradation leads to rupture of atherosclerotic plaque or plaque erosion[10]. Rupture of plaque or plaque erosion results in exposure of subendothelial space to circulating platelets and further stimulate the existing proinflammatory and prothrombotic state. Pathological studies of plaque shown that there is an abundance of T cells and macrophages in the environs of rupture site in patients with ACS. Culprit coronary plaques are believed to be characterized by lipid content, angiogenesis, macrophages count, apoptosis and dilation of internal elastic lamina[11]. Macrophage provoke vascular cell adhesion proteins (VCAMs) to regulate leukocyte attachment and transendothelial migration. In addition to that activated macrophages stimulate the production of tumor necrosis factor α which in turn trigger the release of interleukin-6 by vascular smooth muscle cells. Numerous cells including macrophages, T cells, monocytes, vascular smooth muscle cells and endothelial cells at nuclear level express two established activator of inflammatory gene such as nuclear factor kappa B (NFKβ) and TLR4 (toll like receptor-4)[12]. Various stimulus including interleukins (IL-1, IL-6, IL-17), TNF-α and lipopolysaccharide (LPS) regulate the expression of genes associated with inflammation and atherogenesis comprising IL-1, IL-6, IL-8, TNF-α, IFNγ, ICAM, VCAM via modulating I-kB and PPARs(act as a transcription factor complex) which leads to NFKβ nuclear translocation. Previous studies have documented the increased NFKβ activation in patients with ACS[13]. To degrade collagen matrix of atherosclerotic plaque T cells trigger the stimulation of macrophages which leads to production of matrix metalloproteinases. Additionally T cells are believed to produce IFNγ which is responsible for the inhibition of smooth muscle cell's ability to produce collagen[10,14]. Destabilization of plaque is apparently associated with apoptosis as increased cellular apoptosis has been observed in carotid arteries of patients with ACS. Proinflammatory genes activation and immunological properties of apoptotic cell can perpetuate the process of inflammation in atherosclerotic plaque. Compromised reparative process of fibrous cap results from increased apoptosis of smooth muscle cells. Certainly ruptured plaque have thin and delirious caps because of the increased apoptotic loss of smooth muscle cell which have the ability to produce collagen[15].

Oxidative stress is associated with the development of reactive oxygen species or free radicals that may damage cell and tissue. The exact mechanism of action of reactive oxygen species and its relationship with the pathogenesis of atherosclerosis remains uncertain, nevertheless the association of reactive oxygen species and free radicals with acute coronary syndromes is

exceedingly credible. Reactive oxygen species and free radicals are capable to bind to lipids, proteins, lipoproteins, enzymes and nucleic acids[16] Objective of current study was to evaluate the pathophysiological role of inflammatory biomarkers in ACS that may offer distinctive evidence to the clinician as well as to support their prognostic importance.

METHODS

The study sample contained 152 subjects who were distributed into two groups: patients having acute coronary syndrome and healthy individuals as control. Control group consisted of 76 subjects and patient group comprised of 76 subjects. Patients with acute coronary syndrome were selected from patients hospitalized at Punjab Institute of Cardiology, Lahore. Written informed consent forms were signed by all subjects approving their participation in the study. No subject from study population had the history of any other disease or was on medication. Current study was conducted following sanction by the Institutional Research and Ethics Committee at the University of Lahore. Peripheral blood samples (5ml) were collected and centrifuged for ten minutes, plasma was separated and were maintained at -70°C until further analysis.

Laboratory parametres:

Inflammatory biomarkers: The plasma concentration levels of inflammatory markers such as IL-1, IL-6, IL-8, TNF-α, MMP-11, VCAM-1 and ICAM-1 were measured by enzyme-linked immunosorbent assay using commercially available ELISA kit.

Antioxidants biomarkers: Activity of SOD was quantified by spectrophotometric method as explained by Kakkar, 1972[17]. GSH levels were measured using spectrophotometric method described by Moron *et al.,* 1979[18]. Catalase activity was determined according to the spectrophotometric method of Aebi, 1974[19]. The GRx and GPx activity was quantified by the method of David and Richard, 1983[20]. Vitamin E was evaluated by the Emmerie-Engel reaction as described by Rosenberg (1992)[21].

Statistical analysis: Data management and statistical analysis were performed by using Statistical Package for the Sociological Sciences (SPSS, version 17.0). Depending on data distribution Independent t-test was applied to check the comparison among ACS patients group and control group. Difference were considered statistically significant when the p value is <0.05. To determine statistically significant correlation between variables Pearson correlation was done.

RESULTS

Table 1 presents the levels of inflammatory biomarkers analysed in ACS patients and healthy controls. The IL-1, IL-6, IL-8 and TNF-α levels were higher in patient group when compared to the control group (7.59±1.59 Vs. 4.59±0.441 pg/ml), (14.08±2.58 Vs. 5.03±1.09 pg/ml), (21.28±3.29Vs. 7.65±1.88 pg/ml) and (47.59±4.29 vs 23.25±5.26 pg/ml). Figure 3 depicts the inflammatory markers including IL-1, IL-6, IL-8 and TNF-α showed significant (p=0.017), (p=0.025), (p=0.039) and (p=0.023) difference between the ACS patients and normal healthy individuals respectively. Additionally MMP-11, ICAM-1 and VCAM-1 showed similar results in two study groups. As MMP-11 (102.26±8.59 Vs56.35±8.49 ng/ml.), sICAM-1(238.64±21.29 Vs. 75.177±12.29 ng/ml) and sVCAM (1012.115±35.29 Vs. 2107.648±16.35 ng/ml) had an increasing trend in ACS patients than in healthy controls. Furthermore the MMP-11, ICAM-1 and VCAM-1 level was significantly higher than that in controls (p=0.014), (p=0.000) and (p=0.019) respectively. Table 2 depicts the antioxidants levels measured in patient group and healthy controls, where the oxidative stress capacity to overcome has been evaluated. Figure 4 shows that the levels of SOD, GSH, CAT, Vit-E, GPx and GPr were as follows: patients with ACS (0.19±0.0012 µg/ml), (6.35±0.045 µg/ml), (2.015±0.013µmol/mol), (1.58±0.88 µg/ml), (2.59±0.053 µmol/ml) and (4.59±1.59 µmol/ml) and healthy controls (0.562±0.008 µg/ml), (8.29±2.19 µg/ml), (3.29±0.005 µmol/mol), (2.99±0.58 µg/ml), (1.22±0.021µmol/ml) and (8.596±2.15 µmol/ml) respectively. As the results of current study indicate, the concentrations of SOD, GSH, CAT, Vit-E, GPx and GPr in ACS patients were significantly lower than in healthy subjects (p=0.025), (p=0.036), (p=0.041), (p=0.025), (p=0.023) and (p=0.014).

Table 1. Serum levels of inflammatory biomarkers in ACS patients versus controls

BIOMARKERS	CONTROL n=76	PATIENTS n=76	P VALUE (P<0.05)
IL-1 (pg/ml)	4.59±0.441	7.59±1.59	0.017
IL-6 (pg/ml)	5.03±1.09	14.08±2.58	0.025
IL-8 (pg/ml)	7.65±1.88	21.28±3.29	0.039
TNF- α (pg/ml)	23.25±5.26	47.59±4.29	0.023
MMP-11 (ng/ml)	56.35±8.49	102.26±8.59	0.014
sICAM-1 (ng/ml)	75.177±12.29	238.64±21.29	0.000
sVCAM-1 (ng/ml)	1012.115±35.29	2107.648±16.35	0.019

Table 2. Serum levels of anti-oxidative biomarkers in ACS patients and controls

BIOMARKERS	CONTROL n=76	PATIENTS n=76	P VALUE (P<0.05)
SOD (µg/ml)	0.562±0.008	0.19±0.0012	0.025
GLUTATHIONE (µg/ml)	8.29±2.10	5.35±0.45	0.036
CATALASE (µmol/mol)	3.29±0.07	2.015±0.13	0.041
VITAMIN E (µg/ml)	2.99±0.58	1.58±0.44	0.025
GPx (µmol/ml)	1.22±0.021	2.59±0.053	0.0231
GRx (µmol/ml)	8.596±2.15	4.59±1.59	0.014

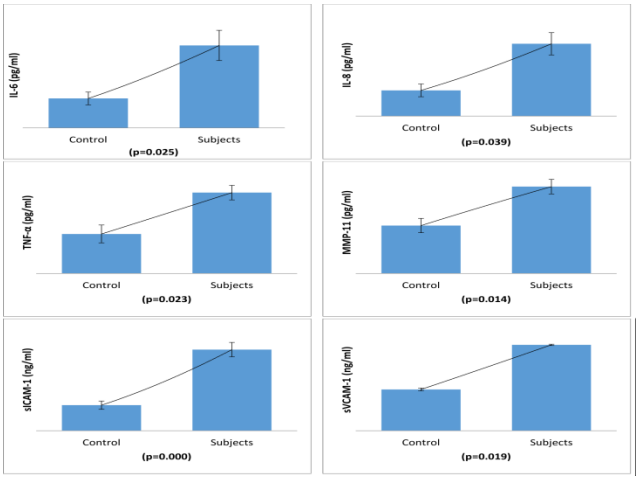

Figure 3. Comparison of oxidative stress markers amongst patients with ACS and controls

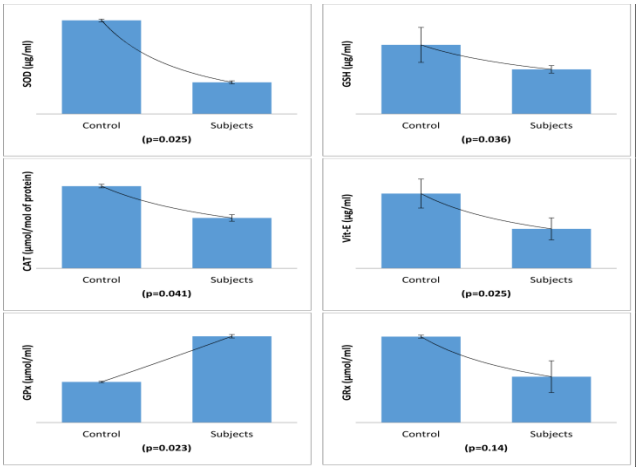

Fig.4 Comparison of serum levels of antioxidants in ACS patients and controls

DISCUSSION

Several studies point out the fundamental role of inflammation in the acceleration of acute coronary syndrome. The pathological mechanism underlying ACS remain unclear, however there is growing evidence that inflammation and oxidative stress are associated with the incidence of ACS. Reactive oxygen species are produced in low amount as by-product during various physiological and biochemical processes in human such as by mitochondrial oxidative phosphorylation, by NADPH/NADP oxidase in neutrophils, endothelial cells and vascular smooth muscle cells, in endothelium by xanthine oxidase, cyclooxygenase/lipoxygenase signalling pathway, cytochrome P450 and by the process of autoxidation and regulate the expression of different genes, posttranslational modification of proteins and act as signalling molecule in cell differentiation, homeostasis, mitosis and apoptosis[22,23]. Excessive amount of free radical production disturb the fine balance between antioxidants and reactive oxygen species, under this condition free radicals attack DNA, proteins and lipids hence, play an important role in heart pathophysiology. ROS trigger increased production of TNF-α and IL-6 leading to the dysregulation of calcium which ultimately leads to increase in intracellular calcium concentration. Cytosolic increase in calcium concentration trigger the activation of calcium dependent proteases and phospholipases and results in cell disruption, cytoskeletal damage and myofibrillar hypercontracture. Calcium overload in mitochondria leads to necrosis by affecting the production and utilization of ATP[24]. In addition to direct effect on biomolecules, excessive production of ROS stimulate multiple regulatory chain reactions and are involved in the control of apoptosis via caspase cascade and TNF-α receptor signalling pathway and activate transcriptional factor cascade, stimulate the production of proinflammatory cytokines (IL-1, IL-6, IL-8 and TNF-α), expression of adhesion molecules (VCAM-1, ICAM-1, MCP-1 and MCSF) and results in recruitment of inflammatory cells such as neutrophils that penetrate to the vessel endothelium of effected area and excessively express NADPH oxidase which give rise to increase production of free radicals as well as to a vicious cycle[25]. Apart from neutrophils T-lymphocytes, smooth muscle cells and fibroblasts also stimulate the generation of ROS. Activation and recruitment of leukocyte can cause thrombi development in micro-vessels and microvascular cell dysfunction and edema, increased pellets aggregation and results in aggravation of ischemia reperfusion[26,27].

Previous studies suggested the antioxidant consumption during myocardial infarction particularly in myocardial reperfusion injury[28]. In precise different enzymatic antioxidants (GSH, CAT, SOD) as well as non-enzymatic antioxidant (Vit-E) have been found markedly dropped in ACS patients[29]. Thus results of current study demonstrated antioxidant system damage in patients with ACS as compared to normal controls as dropped serum levels of SOD, GSH, CAT, GPx, GRx and Vit-E were recorded in patient group of current study. Some workers have reported that oxidative stress induced production of reactive oxygen species and reactive nitrogen species are the major cause of apprehension in acute coronary syndrome. Increased levels of oxidative stress biomarkers and lipid-peroxidation biomarkers has been established in the pathogenesis of acute coronary syndrome[30]. Hence current study assessed the levels of various biomarkers such as oxidative stress marker, activity of different anti-oxidants, in acute coronary syndrome patients.

CONCLUSION

Current study suggested that excessive lipid peroxidation owing to enhanced oxidative stress coupled with antioxidants reduction might be involved directly and actively to aggravate the amount of oxidative stress that may either exaggerate inflammatory action or co-affect with inflammatory mediators to accelerate acute coronary syndrome. These findings suggest the fundamental role of these variables in the onset and progression of ACS and may provide helpful tools to assess risk stratification as well as diagnosis and prevention of ACS.

Conflict of interest: Authors declares no conflict of interest

REFERENCES

1. Sugiyama, T., Yamamoto, E., Fracassi, F., Lee, H., Yonetsu, T., Kakuta, T., Takano, M. Calcified plaques in patients with acute coronary syndromes. *JACC: Cardiovascular Interventions*. 2019, *12*(6), 531-540.
2. Fracassi, F., Crea, F., Sugiyama, T., Yamamoto, E., Uemura, S., Vergallo, R., Jang, I. K. Healed culprit plaques in patients with acute coronary syndromes. Journal of the American College of Cardiology. 2019, 73(18), 2253-2263.
3. Finn AV, Nakano M, Narula J, Kolodgie FD, Virmani R. Concept of vulnerable/unstable plaque. Arterioscler Thromb Vasc Biol. 2010, 30, 1282-92.
4. Virmani R, Burke AP, Farb A, Kolodgie FD. Pathology of the vulnerable plaque. J Am Coll Cardiol. 2006, 47, 13-8.
5. Keaney JF Jr, Vita JA: The value of inflammation for predicting unstable angina. N Engl J Med. 2002, 347, 55-57.
6. Casscells W, Naghavi M, Willerson JT: Vulnerable atherosclerotic plaque: a multifocal disease. Circulation. 2003, 107, 2072-2075.
7. Morrow DA, Cannon CP, Jesse RL, Newby LK, Ravkilde J, Storrow AB, et al. National Academy of Clinical Biochemistry Laboratory Medicine Practice Guidelines: clinical characteristics and utilization of biochemical markers in acute coronary syndromes. Clin Chem. 2007, 53, 552-74.
8. Wiviott SD, Braunwald E. Unstable angina and non-ST-segment elevation myocardial infarction: part I. Initial evaluation and management, and hospital care. Am Fam Physician 2004, 70, 525-32.
9. Moe, K. T., & Wong, P. Current trends in diagnostic biomarkers of acute coronary syndrome. Ann Acad Med Singapore. 2010, 39(3), 210-5.
10. Libby P. Inflammation in atherosclerosis.Nature. 2002, 420, 868-874.
11. Burke AP, Kolodgie FD, Farb A, Weber D, Virmani R: Morphological predictors of arterial remodeling in coronary atherosclerosis. Circulation. 2002. 105, 297-303.
12. Kiechl S, Wiedermann CJ, Willeit J: Toll-like receptor 4 and atherogenesis. Ann Med. 2003, 35, 164-171.
13. Alam, S. E., Nasser, S. S., Fernainy, K. E., Habib, A. A., Badr, K. F. Cytokine imbalance in acute coronary syndrome. Current opinion in pharmacology. 2004, 4(2),166-170.
14. Libby P, Ridker PM, Maseri A. Inflammation and atherosclerosis. Circulation. 2002, 105,1135-1143.
15. Mallat Z, Tedgui A. Current perspective on the role of apoptosis in atherothrombotic disease. Circ Res. 2001, 88, 998-1003.
16. Elahi MM, Matata BM. Free radicals in blood: evolving concepts in the mechanism of ischemic heart disease. Arch Biochem Biophys. 2006, 450, 78-88.
17. Moshage H, B Kok, JR Huizenga, PL Jansen. Nitrite and nitrate determinations in plasma: a critical evaluation. Clinical Chemistary. 1995, 41, 892-896.
18. Kakkar PB, P Das, PN Viswanathan. A modified spectrophotometer assay of superoxide dismutase.Ind J Biochem Bio. 1984, 21, 130-132
19. Moron MS, JW Depierre, B Mannervik. Levels of glutathione reductase and glutathione S-transferase in rat lung and liver.BiochemBiophysicActa. 1979, 582, 67-68.
20. Aebi H. Methods in enzymatic analysis. 3rd Ed. New York Academic Press. 1974, 674-684.
21. David M and JS Richard. In: methods of enzymatic analysis, Bergmeyer, J and Grab M. (Eds), Verlag Chemie Wenhein Deer Field. Beach Floride. 1983, 2, 358.
22. Volz, H. C., Laohachewin, D., Seidel, C., Lasitschka, F., Keilbach, K., Wienbrandt, A. R., Andrassy, M. S100A8/A9 aggravates post-ischemic heart failure through activation of RAGE-dependent NF-κB signaling. Basic research in cardiology. 2012, 107(2), 250.
23. Zweier JL, Talukder MA. The role of oxidants and free radicals in reperfusion injury. Cardiovasc Res. 2006, 70, 181-90.
24. Vichova, T., Motovska. Oxidative stress: Predictive marker for coronary artery disease. Experimental & Clinical Cardiology. 2013, 18(2), 88.
25. Varadarajan SG, An J, Novalija E, et al. Changes in [Na(+)](i), compartmental [Ca(2+)], and NADH with dysfunction after global ischemia in intact hearts. Am J Physiol Heart Circ Physiol. 2001, 280, 280-293.
26. Sun Y. Myocardial repair/remodelling following infarction: Roles of local factors. Cardiovasc Res. 2009, 81, 482-90.
27. Hori M, Nishida K. Oxidative stress and left ventricular remodelling after myocardial infarction. Cardiovasc Res. 2009, 81, 457-64.
28. Rodrigo R., Libuy M., Feliú F., Hasson D. Oxidative stress-related biomarkers in essential hypertension and ischemia-reperfusion myocardial damage. Dis. Markers. 2013, 35, 773-790.
29. Lubrano, V., Pingitore, A., Traghella, I., Storti, S., Parri, S., Berti, S., Vassalle, C. Emerging biomarkers of oxidative stress in acute and stable coronary artery disease: levels and determinants. Antioxidants, 2019, 8(5), 115.
30. Gheddouchi, S., Mokhtari-Soulimane, N., Merzouk, H., Bekhti, F., Soulimane, F., Guermouche, B., Narce, M. Low SOD activity is associated with overproduction of peroxynitrite and nitric oxide in patients with acute coronary syndrome. Nitric Oxide. 2015, 49, 40-46.