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Evolution of protein carbonyl content as a marker of AMI

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Abstract: *Reactive oxygen species modifies amino acid side chains of proteins such as arginine, glutamyl, lysine, threonine and proline to form protein carbonyls. Oxidative modification reaction leads to carbonylated proteins which appear as aldehyde or ketones in proteins. Protein carbonyl groups are rigorously related with cardiovascular diseases, as a sensitive marker of an acute inflammatory state and as an important index of oxidative stress. Measuring protein carbonyl associated with CVDs as oxidative protein damage is the primary aim of our study. This indicates carbonyl group formation and evidence of free radical modification of proteins. Thirty patients with AMI participated in this study. The present study was carried out to evaluate carbonyl stress in acute myocardial infarction and correlations of protein carbonyl as risk measures like lipid profile and cardiac markers (Aspartate transaminase, Creatine Kinase-MB, Lactate dehydrogenase & Troponin-I) to assess their involvement in development of AMI in cardiovascular diseases.*

Keywords: *AMI: Acute myocardial infarction, CVDs: Cardiovascular diseases, PC: Protein carbonyl, ROS: Reactive oxygen species*

I. Introduction

AMI occurs when there is an imbalance between supply and demand for oxygen in the heart muscle (myocardium) resulting in injury to and eventual death of muscle cells [1]. Various risk factors for acute myocardial infarction are elevated like serum cholesterol, diabetes, and hypertension. The manifestations of AMI are chest pain, epigastic pain, breathlessness, nausea and vomiting [2]. Prolonged ischemia can leads to myocardial cell death because of the varied presentation and associated high mortality, the early diagnosis is rare and thus critical for the patient management and has learning on the prognosis. AMI is diagnosed by Aspartate transaminase, Creatine Kinase-MB, Lactate dehydrogenase & Troponin-I along with ECG changes and imaging modalities [3, 4].

AMI is a clinical model of oxidative stress by ischemia reperfusion. ROS are major initiators of myocardial damage during ischemia. Imbalance between ROS production and antioxidant defenses leads to oxidative stress. The disturbance in the normal redox status of tissues causes toxic effects by production of peroxides and free radicals that damages all cellular components [5, 6].

Proteins are the main targets for ROS, alters the primary to quaternary structures. Increased ROS generation by vascular and inflammatory cells occurs in CVD, and also elevated levels of oxidative biomarkers such as protein carbonyls and lipid peroxidation markers. Patients with AMI assayed within 24-96 hr after the acute event, elevated levels of protein carbonyl have been observed [7- 9].

Protein carbonyl is commonly used biomarker of oxidative stress; it is irreversible modification that develops when reactive aldehydes or ketones are added to amino acid residues [10]. PC is generated by oxidative modification of peptide by alpha amidation pathway or oxidation of glutamyl side chains this leads to formation of a peptide in which the N-terminal amino acid is blocked by alpha keto acyl derivatives [11, 12]. The present study was carried out to evaluate carbonyl stress in acute myocardial infarction and correlations of protein carbonyl as risk measures like standard laboratory lipid profile and cardiac markers.

II. Materials And Methods

Study was conducted in the Biochemistry Department of Shridevi Institute Of Medical Sciences & Research Hospital, Tumkur. Serum samples collected from thirty Acute Myocardial Infarction patients who presented with symptoms of chest pain, epigastic pain or arm discomfort, breathlessness, nausea, and vomiting were included in the study. Patients having history of diabetes and thyroid dysfunction were excluded in the study. Screening with fasting blood sugar was performed and abnormal were excluded from the study. Thirty healthy, age and sex matched subjects were selected as control

2.1 Biochemical analysis

Fasting blood samples were collected in tubes and centrifuged at 2000×g for 10 min. Samples were analyzed for Fasting blood glucose by routine GOD-POD method, Lipid Profile (Total Cholesterol by CHOD-PAP, Triglycerides by Trinder, HDL by HDL-Precipitation & LDL by Direct method), cardiac profile (SGOT by IFCC, CKMB by Immuno assay & LDH by DG-KC methods) using ERBA Chem – 5V2 semi auto analyzer and Troponin-I assay by cTnI rapid quantitative method using Finecare^{TM} analyzer. Protein carbonyl assay by spectrophotometric DNPH method (Reznick & packer method)

2.2 Protein carbonyl group assessment

Carbonyl groups in proteins reacts with 2, 4- dinitrophenylhyrdazine (DNPH) to form a stable dinitrophenyl (DNP) hydrazones product. This can be assayed spectrophotometrically at an absorbance of 355 nm [13].

2.3 Statistical analysis

All results were shown as mean ± SD. The results were evaluated using Student's t-test. P-value <0.05 was considered statistically significant. Statistical analysis was performed using SPSS software

III. Results And Discussion

Serum lipids profile in both healthy and in acute myocardial infarction patients groups is illustrated in (Table 1). In MI patients with the mean values of cholesterol (256.4 ± 38.61), triglycerides (211.3 ± 47.87) level were increased in the study group, when compared with control group cholesterol levels (190.0 ± 21.2) & triglycerides levels (147.1 ± 26.53).

In our study serum triglyceride levels significantly increase in AMI patients when compared with control group. The mechanism of increase in triglycerides after MI may be due to elevated fatty acids and impaired removal of VLDL from the plasma [14].

Table 1 Mean ± S.D of Lipid profile in healthy individuals & MI Groups

Clinical Parameters	Control (n=30) (Mean ± SD)	MI (n=30) (Mean ± SD)	P- value
Cholesterol (mg/dl)	190.0 ± 21.2	256.4 ± 38.61	< 0.001
Triglycerides (mg/dl)	147.1± 26.53	211.3 ± 47.87	< 0.001
Serum HDL (mg/dl)	40.7 ± 2.452	38.1 ± 4.444	0.007
Serum LDL (mg/dl)	114 ± 13.47	161.5 ± 38.96	< 0.001

The Serum HDL levels in the study group (38.1 ± 4.444) are decreased compared with controls (40.7 ± 2.452) and serum LDL levels in study group (161.5 ± 38.96) increased compared with controls (114 ± 13.47) the mean difference was statistically significant (Table 1). LDL cholesterol was found to be elevated significantly when compared with controls. LDL carries most of the cholesterol in the plasma raised LDL depends on increased levels of total cholesterol. Reduced HDL cholesterol is shown to be associated with higher prevalence and incidence of coronary artery diseases. Several studies have supported that the ratio of LDL cholesterol & HDL cholesterol shows the atherosclerotic injuries of the wall of the vessels [15, 4].

In this study the cardiac markers levels significantly is increased in AMI patients, mean values of CPK-MB (179.61 ± 143.72), LDH (193.5 ± 28.72) and SGOT (180 ± 103.9) compared with control groups of mean values 22.65 ± 3.76, 71.93 ± 22.52 & 23.56 ± 4.204 respectively (Table 2). CK-MB, LDH & AST the most common biochemical markers of coronary artery diseases increased. Prolonged ischemia can lead to myocardial cell death and is a pre-condition to infarction. Therefore, identification of Myocardial Ischemia at the early stage is a must to prevent the devastating consequences of the disease [16, 17]

In this study increased Troponin-I values is observed in MI patient (4.238 ± 5.13) when compared with control group (0.156 ±0.072) significantly (Table 2). Troponin is a contractile protein that normally is not found in serum, elevated levels indicates cardiac muscle cell death as the molecule is released into the blood upon injury to the myocardium. The cardiac Troponin is sensitive and specific markers of myocyte necrosis and it is the marker of choice for the diagnosis of MI [18]. Troponin levels have been shown to be more powerful prognosticators than CKMB levels [19, 20].

Table 2 Mean ± S.D of Cardiac profile and Protein carbonyl in healthy individuals & MI Groups

Clinical Parameters	Control (n=30) (Mean ± SD)	MI (n=30) (Mean ± SD)	P- value
CPK-MB (IU/L)	22.65 ± 3.76	179.61 ± 143.72	< 0.001
LDH (U/L)	71.93 ± 22.52	193.5 ± 28.72	< 0.001
AST (IU/L)	23.56 ± 4.204	180 ± 103.9	< 0.001
Troponin –I (ng/ml)	0.156 ±0.072	4.238 ± 5.13	< 0.001
Protein carbonyl (nmole/ml)	17.03 ± 1.829	22.39 ± 3.285	< 0.001

The Protein carbonyl levels in the study group (22.39 ± 3.285 nmole/ml) is increased compared with controls level (17.03 ± 1.829 nmole/ml) (Table 2). Accumulation of plasma protein carbonyls is called as "carbonyl stress". Protein carbonylation can modify the function of the affected proteins; it can induce protein degradation and can modulate signaling pathways. It also plays a central role in the pathogenesis of numerous disorders, e.g. heart failure, renal damage and diabetic complications [21].

IV. Conclusion

Myocardial infarction is a manifestation of major life style modifications in the modern days. It is associated with oxidative damage currently acknowledged as components of molecular and cellular damage mechanism involved in vascular dysfunctions. When proteins exposed to ROS causes major physical changes in protein structures leading to proteolysis and increased susceptibility for aggregation of cells [20]. Protein carbonyl content is a marker of protein oxidation, generally elevated levels found in pathological condition at the early stages, appears for a long period when compared with other parameters [22, 23].

In AMI elevated levels of PC is observed in early stages, if detected it can be a non-invasive tests. When PC levels are high along with altered lipid profile and cardiac profile levels in MI, it is an important diagnosis and also gives an idea of prognosis. Further studies are needed to define the mechanisms of protein carbonyl releasing due to AMI.

Increased formation of protein carbonyl, a marker of oxidative stress produced under hypercholesterolemia and hyper lipoproteinemias may be one of the probable cause for evolution of acute myocardial infarction in cardiovascular diseases. To conclude that elevated carbonyl protein in serum in the patients of AMI along with lipid profile, cardiac profile level maybe an important diagnostic tool.

References

[1]. Ahmed IA, Myocardial-infraction based on intelligent techniques, Am J Applied Sci, 7, 2010, 349-351.
[2]. I. Peluso, G. Morabito, L. Urban, F. Ioannone, and M. Serafini, "Oxidative stress in atherosclerosis development: the central role of LDL and oxidative burst," Endocrine Metabolic & Immune Disorders Drug Targets, 12(4), 2012, pp. 351–360.
[3]. Isabella Dalle-Donne,a,, Ranieri Rossib, Daniela Giustarinib, Aldo Milzania, Roberto Colomboa, Protein carbonyl groups as biomarkers of oxidative stress, Clinica Chimica Acta, 329(1–2), 2003, Pages 23–38.
[4]. Santhosh Kumar, Mohammad Anwar, Balu Mahendran. K, Kalaivanam. K N, Significance of lipid profile estimation in patient with acute myocardial infarction, IJABPT, 4(2) 2013, 248-250.
[5]. Ramón Rodrigo,Matías Libuy, Felipe Feliú, and Daniel Hasson, Oxidative Stress-Related Biomarkers in Essential Hypertension and Ischemia-Reperfusion Myocardial Damage, Hindawi Publishing Corporation Disease Markers, 35(6),2013,773–790.
[6]. Chen CL, Cardiovascular prevention in the cancer survivor, Curr Atheroscler Rep, 17, 2015, 484.
[7]. M. M. Elahi, Y. X. Kong, and B. M. Matata, "Oxidative stress as a mediator of cardiovascular disease," Oxidative Medicine and Cellular Longevity, 2 (5), 2009, 259–269.
[8]. Garcia-Garcia A1, Rodriguez-Rocha H, Madayiputhiya N, Pappa A, Panayiotidis MI, Franco R, Biomarkers of protein oxidation in human disease, Curr Mol Med, 12(6), 2012, 681-97.
[9]. Matteo Becatti, Rossella Marcucci, Giulia Bruschi, Niccolò, Oxidative Modification of Fibrinogen Is Associated With Altered Function and Structure in the Sub acute Phase of Myocardial Infarction, Arterioscler Thromb Vasc Biol, 34, 2014, 355-1361.
[10]. Dayanand C. D Pradeep Kumar Vegi, A. V. M Kutty, Protein carbonyl content as a stable Oxidative stress marker in Type II Diabetes, Int J Biol Med Res, 3(4), 2012, 2362-2365.
[11]. Seema L Jawalekar, Ujjwala J. Kulkarni, Vasant T. Surve, Y. A. Deshmukh, Status of Lipid profile, MDA and protein carbonyl in patients with cardiovascular diseases, Archives of Applied Science Research, 2 (6), 2010, 8-14.
[12]. Hami Asadi1, Ali Akbar Abolfathi1, Reza Badalzadeh2, Maryam Majidinia3, Alireza Yaghoubi2, Maryam Asadi, Bahman Yousefi, Effects of Ramadan Fasting on Serum Amyloid A and Protein Carbonyl Group Levels in Patients With Cardiovascular Diseases, J Cardiovasc Thorac Res, 7(2), 2015, 55-59.
[13]. Reznick, A. Z and Packer, L, Oxidative damage to proteins: Spectrophotometric method for carbonyl assay, Methods Enzymol, 233, 1994, 357–363.
[14]. P.K. Nigam, V.S. Narain and M. Hasan, Serum lipid profile in patients with acute myocardial infarction, Indian Journal of Clinical Biochemistry, 19 (1), 2004, 67-70.
[15]. Ahmad Shirafkan**,** Abdoljalal Marjani and Farhad Zaker, Serum lipid profiles in acute myocardial infarction patients in Gorgan, Biomedical Research, 23(1), 2012, 119-124.
[16]. Yousefi B, Faghfoori Z, Samadi N, Karami H, Ahmadi Y, Badalzadeh R, et al, The effects of Ramadan fasting on endothelial function in patients with cardiovascular diseases, Eur J Clin Nutr, 68, 2014, 835-9.
[17]. Hema Chandan Kumar Dussa, Deepak Parchwani, Pankaj Maheria, Status of lipid profile in active smokers, Int J Res Med, 1(1), 2012, 17-20.
[18]. Christenson E, Christenson RH, Characteristics of cardiac Troponin measurements, Coron artery Dis, 24(8), 2013, 698-704
[19]. Mueller C, use of high-sensetive Troponin for the diagnosis of acute myocardial infarction, coron Artery Dis, 24(8), 2013, 710-712.
[20]. ChanD, Ng LL, Biomarkers in Acute MI, BMC Med, 7(8), 2010, 34.
[21]. Eric J.Topol, Robert M. Califf. Ischemic syndromes – Early diagnosis (Textbook of cardiovascular medicine. Lippincott Williams & wilkins. 2007) 255-56.
[22]. Kalaivanam. K N, Santhosh Kumar.N, Bheemasen, R, Balu Mahendran. K, Role of protein oxidation and lipid peroxidation markers Status in NIDDM patients, WJPPS, 3 (6), 2014, 1510-1520.
[23]. Nyström T, Role of oxidative carbonylation in protein quality control and senescence, EMBO J, 6(24), 2005, 1311-7.