Analysis of cardiovascular risk factors in normolipidemic acute myocardial infarct patients on admission based on aging - A case controlled study from South Asia

A Kumar, S Nagtilak, R Sivakanesan

Citation

A Kumar, S Nagtilak, R Sivakanesan. *Analysis of cardiovascular risk factors in normolipidemic acute myocardial infarct patients on admission based on aging - A case controlled study from South Asia*. The Internet Journal of Alternative Medicine. 2008 Volume 8 Number 1.

Abstract

The goal of the present study was to address the various risk factors associated in normolipidemic acute myocardial infarction (AMI) patients admitted to the intensive coronary care unit (ICCU). The study compared serum lipid profiles, lipid peroxidation markers, antioxidants and inflammatory markers in acute myocardial infarction (AMI) patients and age/sex-matched controls. The risk variables were analysed age wise with patients <60 years and >60 years of age.A lipid profile, lipid peroxidation, enzyme antioxidants, endogenous antioxidants, ischemia modified-albumin (IscMA), ceruloplasmin, C-reactive protein (CRP), fibrinogen, lipoprotein (a) and paraoxonase-1 activities were analyzed in 165 acute myocardial infarction (AMI) patients of which 45 patients were <60 years of age.Lower superoxide dismutase (p=0.002), catalase (p=0.002) and arylesterase activity (p= 0.005) were observed and was statistically significant in >60 years age of acute myocardial infarction patients. Higher levels of C-reactive protein (p=0.003) were observed among the >60 years age patients compared to <60 years. Other biochemical findings were not statistically significant.During the process of aging the risk factors vary as lowering of antioxidants is due to decreased free radical scavenging properties.

INTRODUCTION

Coronary heart disease (CHD) is increasing progressively in developing countries and it is presumed to be the largest killer in India by 2020 1, 2). Traditional risk factors like smoking, hypertension, diabetes, hypertriglyceridaemia, truncal obesity, low levels of HDL-C, high levels of LDL-C, low levels of antioxidants, sedentary lifestyle in totality accounts only for 50% of the prevalence and severity of the disease 3, 4). During the last twenty years the fight against CHD to reduce its incidence, prevalence and outcomes has led to the discovery of the newly emerging risk factors. Now it is well established that CHD is multifactorial disease. Though there are evidences that even the subjects who are completely free from the traditional risk factors are also susceptible to coronary heart disease. Despite of our study reported earlier 5, 6, 7, 8) currently we did risk factor analysis based on age group on the cardiovascular risk factors in >60 years and <60 years of age irrespective of gender on acute myocardial infarct patients and tried to rule out whether all the emerging risk factors are similar in both young and elderly acute myocardial infarct patients.

Earlier studies conducted on young acute myocardial infarct patients of acute myocardial infarction (AMI) established some risk factors but do these similar risk factors exists in elderly AMI patients was the main objective of the current study. Literature search reveals no such study based on normolipidemia is reported so far, so the current study will highlight the risk factors associated with acute myocardial infarction in elderly patients.

SETTING DESIGN AND PATIENTS

The study consisted of 165 patients (123 men and 42 women) with AMI, admitted to the Intensive Coronary Care Unit, Sharda Hospital, India. The diagnosis of AMI was established according to diagnostic criteria: chest pain lasting for ≤3 hours, electrocardiographic (ECG) changes (ST elevation ≥ 2 mm in at least two leads) and elevation in enzymatic activities of serum creatine phosphokinase and aspartate aminotransferase. The design of this study was pre-approved by the institutional ethical committee. Informed consent was taken from the subjects or from their relatives who participated in the study.

Inclusion criteria were patients with a diagnosis of AMI with

normal lipid profile. Patients with diabetes mellitus, renal insufficiency, current and past smokers, hepatic disease or taking lipid lowering drugs or antioxidant vitamin supplements were excluded from the study.

Normolipidemic status was judged by the following criteria: LDL≤160 mg/dl; HDL, ≥35 mg/dl; total cholesterol (TC), <200 mg/dl; and triglycerides (TG), <150 mg/dl 9). For biochemical studies, 10 ml of blood was collected from the patients soon after admission to intensive care unit and the time period of collection of blood sample from the patient after experiencing the symptoms of chest pain was around three hours.

Lipid Profile TC, TG and HDL-cholesterol were analyzed enzymatically using kit obtained from Randox Laboratories Limited, Crumlin, UK. Plasma LDL-cholesterol was determined from the values of total cholesterol and HDL-cholesterol using friedwalds formula 10):

LDL-cholesterol = TC – (TG/5) – HDL-cholesterol (mg/dl)

Serum albumin Serum albumin was measured by Bromocresol green binding method 11).

Other assays- All chemicals of analytical grade were obtained from Sigma-Aldrich Company, New Delhi. Serum uric acid was estimated by the method of Brown based on the development of a blue color due to tungsten blue as phosphotungstic acid is reduced by uric acid in alkaline medium.12) Serum ascorbic acid was estimated by method of Roe and Kuether.13) Serum total bilirubin was estimated by the method of Jendrassik and Grof 14). Serum aryl esterase activity was determined by cleavage of phenyl acetate resulting in phenol formation. Aryl esterase activity is measured by the rate of enzymatic hydrolysis of 4 mM phenyl acetate to phenol in 1 mM $CaCl_2$ in 20 mM Tris/HCl (pH 8.0). The amount of phenol generated is monitored with a continuously recording spectrophotometer by the increase in absorbance at 270 nm and 25ºC. The amount of phenol generated was calculated from molar absorbitivity at pH 8.0. One unit of aryl esterase activity caused the formation of 1 mol of phenol/min at ph 8.0 at 25ºC.

Serum caeruloplasmin concentration was determined by Ravin 15) erythrocyte superoxide dismutase activity was determined by INT dye binding method and erythrocyte glutathione peroxidase was determined by Paglia and Valentine method 16)

Erythrocyte catalase activity was determined by Beers and Sizer method. 17) Plasma fibrinogen was estimated by TE Clot Fib Kit 10 (TECO GmbH, Dieselstr, 1, 84088 Neufahrn NB Germany). Serum C-reactive protein was determined by ELISA. Lipoprotein (a) was determined by Latex enhanced turbidimetric method.

Malondialdehyde (MDA) derived from lipid peroxides was determined as a thiobarbituric acid (TBA)-reactive substance.18) Conjugated dienes (CD) were measured according to the method of Recknagel and Glende. 19)

Statistical analysis: The data from patients and controls were compared by Student's t-test. Values are expressed as mean ± standard deviation (SD). Microsoft Excel for Windows 2003 was used for statistical analysis. P-value <0.05 was considered to indicate statistical significance.

RESULTS

Lipid profile of acute myocardial infarction (AMI) patients below and above sixty years is statistically not significant (Table 1). The other biochemical variables are shown in table 2. Significant difference in superoxide dismutase (p=0.002), catalase (p=0.002), arylesterase activity (p= 0.005) and C-reactive protein (p=0.003) were observed among the two groups of patients.

Figure 1

Table 1. Lipid variables in AMI patients below and above sixty years of age

Lipid variables	Age <60 years (n= 45)	Age >60 years (n=120)	*P values*
Total cholesterol (mg/dl)	186.77 ± 13.51	186.31 ± 14.16	0.425
HDL-cholesterol (mg/dl)	41.57 ± 4.91	41.15 ± 4.52	0.306
Triacylglycerol (mg/dl)	129.97 ± 12.68	128.58 ± 12.04	0.258
LDL-cholesterol (mg/dl)	119.20 ± 14.05	119.44 ± 14.11	0.462
LDL-C/ HDL-C ratio	2.91 ± 0.53	2.94 ± 0.51	0.373
TG/HDL-C ratio	3.17 ± 0.52	3.16 ± 0.48	0.441

All the parameters are statistically not significant

Figure 2

Table 2. Biochemical parameters in AMI patients below and above sixty years of age

Biochemical variables	Age <60 years (n=45)	Age >60 years (n=120)	*P values*
Albumin (g/dl)	4.25 ± 0.40	4.23 ± 0.32	0.338
Uric acid (mg/dl)	4.38 ± 0.90	4.30 ± 0.90	0.298
Ascorbic Acid (mg/dl)	2.90 ± 0.78	2.78 ± 0.69	0.178
Bilirubin –total (mg/dl)	0.68 ± 0.22	0.66 ± 0.19	0.273
Superoxide dismutase (U/gHb)	889.46 ± 185.81	785.64 ± 210.85	0.002
Glutathione Peroxidase (U/gHb)	41.31 ± 6.57	43.03 ± 6.25	0.060
Catalase (k/gHb)	205.77 ± 38.56	188.30 ± 33.75	0.002
Arylesterase activity (kU/L)	72.86 ± 10.18	68.45 ± 9.89	0.005
Caeruloplasmin (mg/dl)	52.18 ± 2.61	51.29 ± 2.22	0.016
Plasma fibrinogen (mg/dl)	358.91 ± 24.50	357.38 ± 22.76	0.353
Ischemia modified albumin (U/ml)	94.93 ± 10.95	98.46 ± 11.89	0.042
C-reactive protein (mg/l)	2.59 ± 1.02	3.12 ± 1.11	0.003
Lp(a) (mg/l)	11.11 ± 2.17	10.78 ± 2.24	0.200
Malondialdehyde (nmol/l)	14.76 ± 1.33	14.82 ± 1.78	0.414
Conjugated diene(µmol/l)	48.52 ± 5.74	48.20 ± 5.41	0.370

DISCUSSION

The current study analyzed the risk variables in normolipidemic acute myocardial infarct patients. The study observed several emerging risk factors (as shown in table 1 and 2) in the patients at the time of admission (≈ 3 hours after chest pain) in hospital.

We analyzed the risk variables in two groups of patients depending on their age and evaluated major risk factors in elderly patients (>60 years) compared to other patients (<60 years).

Of the risk variables observed in both group of patients, serum lipid profile, serum ascorbic acid, serum uric acid, serum total bilirubin, erythrocyte glutathione peroxidase, plasma fibrinogen, serum ischemia modified albumin, lipoprotein (a), malondialdehyde and conjugated diene were not significantly altered. The risk variables which were significantly altered among the two groups were superoxide dismutase (p=0.002), catalase (p=0.002), aryl esterase (p=0.005) and C-reactive protein (p=0.003).

Involvement of free radicals in the pathophysiology of inflammation, ischemia and in reperfusion damage in a number of organs and tissues have been reported earlier 20,21) Indirect evidence of free radical generation in AMI patients is validated by the presence of lipid peroxidation products, such as malondialdehyde (MDA) and conjugated diene (CD). The serum concentration of MDA and CD are reported to be higher in AMI patients but not significantly differed among the two groups.

It is believed that free radicals are generated particularly in early stage of AMI and antioxidants are involved in the reduction of free radicals, resulting in a decrease in their activities during that period 22, 23). In the present study, low activities of superoxide dismutase, glutathione peroxidase and catalase were observed in elderly (>60 years) AMI patients as compared to those with younger age group (<60 years).

Thus, the present study is suggestive of imbalance between oxidant and antioxidants in AMI patients which is mainly due to increased oxidative stress, but it is prominent in elderly patients.

The serum antioxidants, mainly superoxide dismutase and catalase are significantly decreased in elderly patients compared to the younger ones (Table 2).

The current study also observed reduction in albumin, uric acid, total bilirubin, ascorbic acid in AMI patients but the observations were prominent in elderly patients compared to younger ones with not statistically significant differences among the two groups.

The present study is in good agreement with those conducted by Verma et al 24) and Kharb 25) where they demonstrated a significant drop in serum antioxidants as observed in the current study, more over the difference was significant in antioxidants status among the two groups of patients.

In the present study, we observed a significant association between high baseline levels of serum caeruloplasmin and the subsequent risk of myocardial infarction in both groups and was statistically significant but the younger patients (<60 years) has more risk compared to those of elderly patients.

This indicates that the antioxidant system combating oxidative stress and inflammation is severely impaired in AMI patients. The findings of the present study indicate that the existence of an abnormal balance between the oxidative and protective mechanisms in patients can be a causative factor for the occurrence of AMI.

The elevated levels of C-reactive protein in AMI patients suggests inflammation leading to an acute clinical event by the induction of plaque rupture. Atherosclerosis seems to be a chronic inflammatory condition that can be converted to an acute clinical event by the induction of plaque rupture, which in turn leads to thrombosis. Hence inflammation occurs during all phases of atherosclerosis, although it must smolder for decades before resulting in a clinical event such as AMI.26) The elevated C-reactive protein in AMI patients justifies the above facts as observed in our study.

The levels of IscMA in AMI patients was significantly higher than in controls as observed in the present study is similar to previous reports 27, 28, 29) Even though determination of IscMA is promising for the prediction of AMI, its use should be limited until further studies reveal its validity as a biomarker for AMI and usefulness in constructing treatment plans in acute coronary syndrome patients. An extensive study with increased number of patients would be required to compare IscMA with other markers such as troponins and myoglobin. Whether IscMA can be an additional parameter along with troponins to boost the confidence of clinicians in ruling out cardiac ischemia would be of particular interest.

LIMITATIONS OF THE CURRENT STUDY

The samples for lipid profile and other variables were collected within 3 hours at the time of admission of patients admitted in intensive coronary care unit, which does not obey the normal criteria of standardized sampling pattern of 12 hours fasting for sample analysed for lipid profile studies. As the patients were very vulnerable and were at high risk at the time of admission so we didn't follow the standard criteria of sample collection.

CONCLUSION

AMI is a multi factorial disease that can arise even in normolipidemic subjects. Hence, earlier concepts of maintaining lipid profile within normal limits to prevent MI may be overruled. The present study suggests that measuring serum antioxidants and IscMA in normolipidemic patients will aid better prognosis and management of patients with acute coronary syndromes. Oxidative stress appears an etiological factor for MI as a consequence of free radical scavengers namely antioxidants which tends to be lower in AMI patients.

Future research including measurement of parameters of oxidative stress and inflammatory markers should be carried out as the role of inflammatory markers like C-reactive proteins, Caeruloplasmin are emerging which could be possibly be a causative factor for atherosclerosis.

References

1. Gupte,M.D., Ramachandran Vidya., Mutatkar, R.K. Epidemiological profile of India: Historical and contemporary perspectives. Journal of Bioscience, 2001; 26(4) Suppl: 437-464.
2. Singh, S.P., Sen, P. Coronary Heart Disease: The Changing Scenario. Indian Journal of Preventive and Social Medicine, 2003; 34: 74-80.
3. Reddy KS. Rising burden of cardiovascular disease in India. In: Sethi KK (ed). Coronary artery disease in Indians: a global perspective. Mumbai, Cardiological-Society-of-India 1998; Dec 10–13: 63-72.
4. Rissam, H.S., Kishore, S. & Trehan, N. Coronary Artery Disease in Young Indians-The Missing Link. Journal of Indian Academy of Clinical Medicine, 2001; 2 (3): 128-132.
5. Kumar, A., Sivakanesan, R. Singh, S. Oxidative stress, Endogenous Antioxidant and Ischemia modified albumin in Normolipidemic Acute Myocardial Infarction Patients. Journal of Health Science, 2008; 54(4):482-487.
6. Kumar, A., Sivakanesan, R. Serum Caeruloplasmin as a Coronary Risk Factor in patients with Acute Myocardial Infarction with normal lipid profile. Journal of Health Science, 2008; 54(5):567-570.
7. Kumar, A., Nagtilak, S., Sivakanesan, R., Gunasekera,S. Cardiovascular risk factors in elderly normolipidemic acute myocardial infarct patients: A Case Controlled study from India. The South East Asian Journal of Tropical Medicine and Public Health, 2009; 40(3); 581.
8. Kumar, A., Sivakanesan, R. Does plasma fibrinogens and C - reactive protein predicts the incidence of myocardial infarction in patients with normal lipids profile? Pakistan Journal of Medical Sciences, 2008; 24(2): 336-339.
9. Executive Summary of the Third Report of The National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel III). Expert Panel of Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults. JAMA 2001; 285(19):2486-97.
10. Friedewalds, W.T., Levy, R.I. and Fredrickson, D.S. Estimation of the concentration of low density lipoprotein cholesterol in plasma without the use of preparative ultracentrifuge. Clinical Chemistry; 1972; 18: 499-502.
11. Perry, B.W & Doumas, B.T. Effect of heparin on albumin determination by use of bromocresol green and bromocresol purple. Clinical Chemistry, 1979; 25:1520-1522.
12. Brown H. The determination of uric acid in human blood. Journal of Biological Chemistry, 1945; 158:601-608.
13. Roe, J.H. & Kuether, C.A. The determination of ascorbic acid in whole blood and urine through the 2,4-dinitrophenylhydrazine derivative of dehydroascorbic acid. Journal of Biological Chemistry, 1943; 147:399-407.
14. Jendrassik, J. & Grof P.Vereinfachte photometrische Methoden Zur Beetimmung des Blutbilirubin. Biochem. Z., 1938;297; 81-89.
15. Ravin, H.A. An improved colorimetric enzymatic assay of caeruloplasmin. Journal of Laboratory Medicine,1961;58:161-168.
16. Paglia, D.E., & Valentine, W.N. Studies on the quantitative and qualitative characterization of erythrocyte glutathione peroxidase. Journal of Laboratory and Clinical Medicine, 1967; 70:158-169.
17. Beers, R. F., & Sizer, I. W. A spectrophotometric method for measuring the breakdown of H2O2 by catalase. Journal of Biological Chemistry, 1952; 195:133-140.
18. Bernheim, S., Bernheim, M.L.C. & Wilbur, K.M. The reaction between thiobarbituric acid and the oxidant product of certain lipids. Journal of Biological Chemistry, 1948; 174: 257-264.
19. Recknagel, R.O & Glende, E.A. Spectrophotometric detection of lipid conjugated dienes. Methods of Enzymology, 1984; 105:331-337.
20. Frei B, Stocker R, Ames BN. Antioxidant defenses and lipid peroxidation in human blood plasma. Proc Natl Acad Sci USA 1988; 85: 9748-9752.
21. Beutler E, Duron O, Kelly B. An improved method for

the determination of blood glutathione. J Lab Clin Med 1963; 61: 822-826.
22. Recknagel RO, Glende EA. Spectrophotometric detection of lipid conjugated dienes. Methods Enzymol 1984; 105:331-337.
23. Uhling S, Wendel A. The physiological consequences of glutathione variations. Life Sci 1992; 51: 1083-94.
24. Verma V.K., Ramesh, V., Tewari,S., Gupta, R.K., Sinha,N.& Pandey,C.M. Role of Bilirubin , Vitamin C and Ceruloplasmin as antioxidants I Coronary Artery Disease (CAD). Indian J Clin Biochem 2005 20(2): 68-74.
25. Kharb S. Low blood glutathione levels in acute myocardial infarction. Indian J Med Sci 2003; 57 (8): 335-7.
26. Libby P. Vascular biology of atherosclerosis: Overview and state of art. Am J Cardiol 2003;91(Suppl):3A-6A.
27. Sinha, M.K., Roy, D., Gaze, D.C. Collinson, P.O., Kaski, J.C. Role of Ischemia Modified Albumin", a new biochemical marker of myocardial Ischaemia, in the early diagnosis of acute coronary syndromes. Emerg Med J 2004; 21:29-34.
28. Collinson,P.O., Gaze,D.C., Bainbridge, K., Morris, F., Morris,B., Price,A. and Goodacre, S. Utility of admission cardiac troponin and "Ischemia Modified Albumin" measurements for rapid evaluation and rule out of suspected acute myocardial infarction in the emergency department. Emerg Med J 2006; 23: 256-261.
29. Bar-Or, D., Lau, E., Rao, N., Bampos, N., Winkler, J.V. and Curtis, C.G. Reduction in the cobalt binding capacity of human albumin with myocardial ischemia. Ann Emerg Med 1999; 34:556.

Author Information

Arun Kumar
Assistant Professor, Department of Biochemistry, Manipal College of Medical Sciences Phulbari Campus, Pokhara, Nepal

Suryakant Nagtilak
Professor and Head, Saraswati Institute of Medical Sciences, Hapur, Uttar Pradesh, India

Ramiah Sivakanesan
Professor and Head, Biochemistry Department Faculty of Medicine, University of Peradeniya, Sri lanka